

RESEARCH MONOGRAPH . COMPOUND PROFILE

Crystagen

A synthetic tripeptide (L-Glu-L-Asp-L-Pro; EDP) derived from structural analysis of the thymic polypeptide complex Thymalin, developed at the Saint Petersburg Institute of Bioregulation and Gerontology as an ultrashort peptide bioregulator with immunomodulatory, cytoprotective, and epigenetic chromatin-regulatory activity targeting thymic epithelial cells, T-lymphocyte subpopulations, and heat-shock protein gene expression.

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ABSTRACT

Crystagen (L-glutamyl-L-aspartyl-L-proline; EDP tripeptide; molecular formula $C_{14}H_{21}N_3O_8$; molecular weight 359.33 g/mol) is a synthetic tripeptide bioregulator developed by Vladimir Khavinson and colleagues at the Saint Petersburg Institute of Bioregulation and Gerontology as one of the principal short-peptide bioactive components of Thymalin, a bovine thymus polypeptide extract approved in the Soviet Union and Russian Federation since 1982 for clinical immunocorrection [1, 2]. The compound belongs to the Khavinson class of ultrashort (two to seven residue) peptide bioregulators, a family of synthetic sequences modeled on tissue-specific peptide fragments isolated from organ extracts by acid-pepsin hydrolysis and ultrafiltration, and is designated as the thymic immune system bioregulator within this peptide family. Crystagen shares the Glu-Asp dipeptide core with the bronchial bioregulator Chonluten (Glu-Asp-Gly) and the cortical bioregulator Cortagen (Ala-Glu-Asp-Pro), differing from these compounds by the third-position proline residue and the absence of the N-terminal alanine extension, respectively; the single amino acid substitution at the third position determines tissue specificity within the Khavinson classification [3, 4]. In the Khavinson laboratory internal code system, the compound is designated T-36 [5]. The principal molecular mechanism of Crystagen, characterized through molecular modeling, cell culture, and organotypic tissue studies, is epigenetic regulation of gene expression through direct interaction of the tripeptide with double-stranded DNA in gene promoter regions and with histone proteins (H1, H2B, H3, H4), producing chromatin decondensation and reactivation of age-repressed transcriptional programs in

immune cells [6, 7, 8]. The immunomodulatory activity, characterized in thymic cell cultures, splenic organotypic cultures, and in the THP-1 monocyte/macrophage cell line, includes stimulation of T-lymphocyte differentiation with increased CD3⁺ and CD4⁺ cell populations, normalization of the CD4⁺/CD8⁺ ratio, activation of B-cell immunity in the spleen, suppression of thymocyte apoptosis through p53 downregulation and Ki-67 upregulation, enhancement of normal lymphocyte proliferation with concurrent inhibition of K-562 tumor cell proliferation, and modulation of cytokine expression including interleukin-6 normalization [9, 10, 11, 12]. The cytoprotective profile includes a pronounced upregulation of heat-shock protein gene HSPA1A (encoding HSP-70), with expression increasing approximately 2.2-fold relative to baseline in a study of highly trained female artistic gymnasts receiving Crystagen in combination with other peptide bioregulators, an effect accompanied by reduced incidence of acute respiratory infections during epidemic conditions [13, 14]. In a clinical observational study of elderly patients, oral Crystagen administration normalized immunogram parameters in 82 percent of treated individuals compared to 56 percent in the control group [15]. The geroprotective context for Crystagen derives from the parent compound Thymalin, which in a 266-patient, 6-to-8-year prospective clinical study conducted at the Saint Petersburg Institute of Bioregulation and Gerontology and the Institute of Gerontology of the Ukrainian Academy of Medical Sciences produced a 2.0-to-2.1-fold reduction in mortality rate relative to control, and a 4.1-fold mortality reduction when combined with the pineal peptide Epithalamin [16]. No formal pharmacokinetic studies have been published for Crystagen as the isolated synthetic EDP tripeptide. As a linear tripeptide with unprotected termini, the compound is expected to undergo rapid

proteolytic degradation by aminopeptidases and carboxypeptidases in plasma and gastrointestinal fluid; however, molecular modeling studies have demonstrated that ultrashort peptides including EDP are substrates of the proton-coupled oligopeptide transporter (POT) family carriers PEPT1 and PEPT2, supporting intestinal absorption and cellular uptake through active transport mechanisms [17, 18]. The compound is not approved by the United States Food and Drug Administration, the European Medicines Agency, or any major Western regulatory authority. It is not registered as a pharmaceutical product outside the Russian Federation. Crystagen is commercially available in Russia as a dietary supplement in capsule and sublingual formulations and is supplied internationally as a research-grade lyophilized peptide by multiple peptide synthesis vendors at greater than 95 percent purity by high-performance liquid chromatography. This monograph reviews the chemistry, synthesis, and structural characterization of Crystagen; the discovery and development history within the Khavinson bioregulatory peptide program; the molecular pharmacology including peptide-DNA binding, histone interaction, and gene expression modulation; the pharmacokinetic considerations for ultrashort peptides; the preclinical pharmacology across immune, inflammatory, and aging cell models; the clinical evidence base (observational); sourcing and quality verification; reconstitution and handling; stack interactions and combinations; adverse events and safety signal; and a comparative assessment of five immunomodulatory peptide candidates (Thymogen, Vilon, Thymosin alpha-1, Thymulin, Epithalon) against Crystagen on five competency standards.

1. Compound Identification

1.1 Names, synonyms, and registry identifiers

International Nonproprietary Name: Not assigned. The compound has not received an INN designation from the World Health Organization.

Designated name: Crystagen. The name is a proprietary designation within the Khavinson peptide bioregulator nomenclature system and is used across the Russian-language and international research literature to identify the EDP tripeptide in its immune-system bioregulator application.

One-letter amino acid code: EDP (Glu-Asp-Pro).

Three-letter amino acid code: L-Glu-L-Asp-L-Pro.

Full chemical name: L-glutamyl-L-aspartyl-L-proline. Alternate designations in the peptide chemistry literature include H-Glu-Asp-Pro-OH (indicating unprotected N-terminal alpha-amino and C-terminal carboxyl groups) and gamma-L-glutamyl-L-alpha-aspartyl-L-proline.

Internal research code: T-36 (Khavinson laboratory peptide screening designation) [5].

Chemical Abstracts Service registry number: Not assigned. The tripeptide Glu-Asp-Pro is a defined sequence within the general peptide nomenclature but has not received a unique CAS number as a pharmaceutical entity. Investigators should reference the compound by its sequence (H-Glu-Asp-Pro-OH or EDP) and by its molecular formula for unambiguous identification.

PubChem Compound Identifier: Not assigned as a unique entry at the time of monograph preparation. The component amino acids (L-glutamic acid CAS 56-86-0, L-aspartic acid CAS 56-84-8, L-proline CAS 147-85-3) have independent CAS and PubChem entries.

1.2 Structural class and chemistry

Crystagen is an ultrashort linear tripeptide composed of three alpha-amino acid residues joined by standard alpha-peptide bonds. The structural class places it within the Khavinson ultrashort peptide bioregulator family, a series of synthetic two-to-seven-residue peptides designed as minimal pharmacophore units of tissue-specific polypeptide complexes isolated from mammalian organs [3, 4]. Crystagen is distinguished within this family by the combination of two acidic residues (glutamic acid and aspartic acid) with a cyclic imino acid (proline), a sequence that confers conformational constraint through the pyrrolidine ring of proline and net negative charge at physiological pH through the ionized carboxylate side chains of glutamic and aspartic acid.

Molecular formula: C₁₄H₂₁N₃O₈.

Molecular weight: 359.33 g/mol (monoisotopic mass 359.133 g/mol).

Isoelectric point: Approximately 3.0 to 3.2 (calculated), reflecting the predominance of acidic residues. At physiological pH (7.4), the compound carries a net negative charge of approximately minus 2 to minus 3, depending on the protonation state of the terminal alpha-amino group and the two carboxylate side chains.

Physical properties: White to off-white lyophilized powder or crystalline solid. Freely soluble in water, phosphate-buffered saline, and aqueous buffers at neutral to slightly alkaline pH. Sparingly soluble in organic solvents. Hygroscopic. Stable as a lyophilized solid at minus 20 degrees Celsius for at least 24 months when protected from moisture.

Stereochemistry: All three residues are in the L-configuration. No chiral inversion is expected during standard solid-phase or solution-phase peptide synthesis. Investigators should confirm L-stereochemistry by chiral amino acid analysis after acid hydrolysis or by comparison of chromatographic retention time to synthetic standards.

1.3 Synthesis

Crystagen is synthesized by standard solid-phase peptide synthesis (Fmoc chemistry) or by solution-phase peptide synthesis. The solid-phase approach proceeds from a proline-preloaded resin (Wang resin or 2-chlorotrityl chloride resin), with sequential coupling of Fmoc-Asp(OtBu)-OH and Fmoc-Glu(OtBu)-OH using standard

activating reagents (HBTU, HATU, or DIC/HOBt). Deprotection of the Fmoc group at each step is carried out with 20 percent piperidine in dimethylformamide. Final cleavage from the resin and simultaneous removal of side-chain protecting groups is accomplished with trifluoroacetic acid in the presence of scavengers (triisopropylsilane, water, ethanedithiol). The crude peptide is purified by reversed-phase high-performance liquid chromatography (C18 column, water-acetonitrile gradient with 0.1 percent trifluoroacetic acid) to greater than 95 percent purity. Lyophilization of the purified fraction yields the final product as the trifluoroacetate salt or, after counterion exchange, as the acetate salt. The solution-phase synthesis proceeds by sequential condensation of the three amino acids using carbodiimide or active ester chemistry. The synthesis is straightforward; the three-residue chain length and the absence of sterically demanding residues (other than the moderate steric demand of the proline pyrrolidine) result in high coupling efficiencies and overall yields typically exceeding 60 to 70 percent after purification [19].

2. Abstract

Crystagen (H-Glu-Asp-Pro-OH; EDP; T-36) is a synthetic tripeptide bioregulator developed at the Saint Petersburg Institute of Bioregulation and Gerontology by Vladimir Khavinson and colleagues as one of the principal bioactive ultrashort peptide components of Thymalin, a bovine thymus polypeptide complex approved in the Soviet Union in 1982 and used in Russian clinical practice for over four decades as an immunocorrective agent [1, 2, 16]. Crystagen represents the Khavinson laboratory's identification of the minimal immune-regulatory peptide sequence within the thymic polypeptide complex: a three-residue peptide that retains tissue-specific immunomodulatory activity despite its minimal chain length.

The compound operates through a multi-level molecular mechanism. At the epigenetic level, Crystagen and structurally related Khavinson ultrashort peptides have been demonstrated to penetrate cell nuclei and interact directly with double-stranded DNA in gene promoter regions and with histone proteins, producing chromatin decondensation (deheterochromatinization) in aging immune cells and consequent reactivation of transcriptional programs that are progressively silenced during immunosenescence [6, 7, 8]. This mechanism, characterized by molecular docking, molecular dynamics simulation, and cellular assay across the Khavinson peptide family, is proposed as the molecular basis for the immunorestorative activity observed in elderly populations, where age-related heterochromatin condensation progressively restricts access to immune-regulatory gene loci.

At the cellular level, Crystagen stimulates T-lymphocyte differentiation with preferential expansion of CD3+ and CD4+ populations, normalizes the CD4+/CD8+ ratio, activates B-cell immunity in splenic tissue, suppresses thymocyte apoptosis through reduction of p53 expression and upregulation of Ki-67 (a marker of active cell division), enhances proliferation of normal lymphocytes while simultaneously inhibiting proliferation of K-562 erythroleukemia cells, and upregulates expression of heat-shock protein 70 (HSP-70) through the HSPA1A gene by approximately 2.2-fold [9, 10, 11, 12, 13]. The anti-inflammatory component includes normalization of interleukin-6 expression, modulation of tumor necrosis factor alpha signaling in monocyte/macrophage lineage cells, and induction of TNF tolerance in monocytes [12, 20].

The clinical evidence base, drawn predominantly from Russian observational studies and dietary supplement surveillance rather than from randomized placebo-controlled registration trials, includes immunogram normalization in 82 percent of elderly patients (versus 56 percent control) and reduced incidence of acute respiratory infections in elite athletes [13, 15]. The broader geroprotective context is established by the parent compound Thymalin, which produced a 2.0-to-2.1-fold mortality reduction over 6 to 8 years in a 266-patient elderly cohort and a 4.1-fold mortality reduction when combined with the pineal bioregulator Epithalamin [16].

No formal pharmacokinetic studies of the isolated EDP tripeptide have been published. Molecular modeling studies support active intestinal absorption through proton-coupled oligopeptide transporters (PEPT1, PEPT2) of the POT family, providing a mechanistic basis for oral bioavailability despite the expected rapid proteolytic degradation of the unprotected tripeptide in plasma [17, 18]. The compound is not approved by the United States Food and Drug Administration or by any Western regulatory authority. It is supplied commercially in Russia as a dietary supplement and internationally as a research-grade lyophilized peptide.

3. Discovery and Development History

3.1 Thymalin and the bioregulatory peptide program

The discovery of Crystagen is inseparable from the broader history of the Khavinson bioregulatory peptide program, which originated in the 1970s at the Military Medical Academy in Leningrad (now Saint Petersburg) under the direction of Vladimir Khavinson and Vyacheslav Morozov. The foundational observation was that polypeptide complexes extracted from animal organs by acid-pepsin hydrolysis and ultrafiltration could restore age-declining organ function when administered to aged animals and humans. The first such preparation was Thymalin, a low-molecular-weight polypeptide complex isolated from bovine thymus tissue, which demonstrated immunomodulatory activity in aged and immunocompromised animals and was approved in the Soviet Union in 1982 for clinical immunocorrection [1, 2, 16].

Thymalin became the most extensively clinically studied preparation in the Khavinson bioregulatory peptide program. Its clinical applications in the Russian Federation included postoperative immune reconstitution, chronic infection management, adjunctive oncology support during chemotherapy and radiotherapy, geriatric immune support, and general geroprotection. Over four decades of clinical use, Thymalin accumulated a substantial observational evidence base in the Russian medical literature, with reported benefits including normalization of T-lymphocyte subpopulations, restoration of cell-mediated immunity, reduction in infectious disease incidence, and decreased mortality in elderly populations [1, 16].

3.2 Identification of active peptide components

The Khavinson laboratory subsequently undertook the identification of the minimal bioactive peptide sequences within the Thymalin polypeptide complex. This work, conducted through sequential fractionation, sequence analysis, and bioassay-guided isolation, identified several short peptide fragments with immunomodulatory activity, including the dipeptides Thymogen (Glu-Trp; EW) and Vilon (Lys-Glu; KE), and the tripeptide Crystagen (Glu-Asp-Pro; EDP) [2, 3]. Thymogen was developed independently as a registered immunomodulatory pharmaceutical in the

Russian Federation. Vilon was developed as the minimal (dipeptide) thymic bioregulator. Crystagen was identified as a distinct tripeptide with immunomodulatory properties that overlapped with but were not identical to those of Thymogen and Vilon, characterized by a stronger effect on the T-cell component of immunity (CD3+ and CD4+ cells) and a less pronounced effect on B lymphocytes than Thymogen [9].

3.3 Relationship to Cortagen and Chonluten

The EDP sequence of Crystagen shares the Glu-Asp core with two other Khavinson tripeptides: Chonluten (Glu-Asp-Gly; EDG), a bronchial mucosal bioregulator, and the C-terminal tripeptide fragment of Cortagen (Ala-Glu-Asp-Pro; AEDP), a cortical bioregulator tetrapeptide [3, 4]. Crystagen can be understood as either the C-terminal tripeptide of Cortagen (after removal of the N-terminal alanine) or as Chonluten with the C-terminal glycine replaced by proline. The substitution of proline for glycine introduces conformational constraint through the pyrrolidine ring, which restricts the phi backbone torsion angle and favors beta-turn and polyproline II helix secondary structure elements. This single amino acid substitution is the structural determinant of tissue specificity within the Khavinson classification: EDP (Crystagen) targets thymic and immune cell populations, EDG (Chonluten) targets bronchial mucosa, and EDR (Pinealon) targets central nervous system neurons [4, 21].

3.4 Product development

Crystagen has been developed commercially in the Russian Federation as a dietary supplement under the Crystagen brand name (manufactured by the Research Institute of Bioregulation and Gerontology and distributed through Russian pharmaceutical channels). Formulations include oral capsules (typically 200 micrograms per capsule in a lactose carrier) and sublingual solutions. The compound is classified as a dietary supplement (biologically active additive, or BAD) rather than as a registered pharmaceutical in Russia. It has not been submitted for regulatory approval in any Western jurisdiction. International availability is principally through peptide research suppliers and through Russian dietary supplement importers.

4. Mechanism of Action

Crystagen operates through a multi-level molecular mechanism that integrates epigenetic gene regulation, immune cell signaling, and cytoprotective stress-response pathways. The mechanisms described below have been characterized across the broader Khavinson ultrashort peptide family with specific data for the EDP tripeptide where available.

4.1 Peptide-DNA interaction and epigenetic gene regulation

The foundational mechanism proposed for all Khavinson ultrashort peptides, including Crystagen, is direct interaction of the peptide with double-stranded DNA in gene promoter regions. This mechanism has been characterized by molecular docking, molecular dynamics simulation, and experimental validation in the Khavinson laboratory over approximately two decades [6, 7, 8].

Short peptides consisting of 2 to 7 amino acid residues have been demonstrated to penetrate cell nuclei and nucleoli and to interact with nucleosomes, histone proteins, and both single-stranded and double-stranded DNA [6]. The interaction with double-stranded DNA involves sequence-specific binding in the minor groove, where the peptide backbone and side-chain functional groups form hydrogen bonds, electrostatic contacts, and van der Waals interactions with nucleotide bases and the sugar-phosphate backbone. The acidic residues of Crystagen (glutamic acid and aspartic acid) are proposed to weaken hydrogen bonds between DNA strands at specific recognition motifs, producing local strand separation and facilitating transcription factor access to promoter elements [7].

The functional consequence of this peptide-DNA interaction is chromatin decondensation (deheterochromatinization) in aging cells. During immunosenescence, progressive condensation of facultative heterochromatin restricts transcriptional access to gene loci encoding immune-regulatory proteins, cytokines, heat-shock proteins, and cell-cycle regulators. The binding of Crystagen to specific promoter-region DNA sequences is proposed to reverse this age-related heterochromatin condensation, reactivating gene expression programs that support immune cell proliferation, differentiation, and function [6, 7, 8].

The chromatin-decondensation mechanism has been directly demonstrated for the structurally related Khavinson peptides Vilon (KE), Epithalon (AEDG), and Livagen (KEDA) in lymphocytes from elderly donors aged 75 to 88 years, where these peptides produced measurable increases in euchromatin content and reactivation of ribosomal genes [22, 23]. While the specific chromatin-decondensation data for Crystagen (EDP) in this experimental system have not been independently published, the shared Glu-Asp core and the demonstrated activity of the closely related Cortagen (AEDP) support the mechanistic extrapolation [4].

4.2 Histone protein interaction

In addition to direct DNA binding, Khavinson ultrashort peptides have been demonstrated to interact with histone proteins H1, H2B, H3, and H4 at N-terminal peptide-binding motifs [7, 8]. This histone-level interaction establishes a chromatin-level epigenetic mechanism complementary to the direct DNA interaction: by modifying histone-DNA contacts, the peptide can alter nucleosome stability and accessibility without requiring direct DNA sequence recognition. The composite peptide-DNA and peptide-histone mechanism provides a dual-pathway basis for the gene-regulatory activity observed in aging immune cells.

4.3 Immunomodulatory activity

The immunomodulatory activity of Crystagen has been characterized in multiple experimental systems:

Thymic cell cultures. Studies of cortical thymocytes in culture demonstrated that the EDP-related peptides amplify thymocyte differentiation toward regulatory T cells, increase proliferative activity, and decrease apoptosis [11]. The anti-apoptotic mechanism involves reduction of proapoptotic protein p53 expression by approximately 2-fold and upregulation of the anti-apoptotic protein Mcl-1 and the proliferation marker Ki-67 in CD4+CD8+ double-positive thymocytes [11].

Splenic organotypic cultures. In organotypic cultures of spleen tissue from young and aged animals, Crystagen activated the B-cell component of immunity but did not significantly affect the processes of cellular renewal during aging, distinguishing it from Vilon (which inhibited apoptosis) and R-1 peptide (which stimu-

lated proliferation and differentiation) [9, 10]. The B-cell activation represents a complementary immunomodulatory profile to the T-cell-dominant effects observed in thymic culture.

T-lymphocyte subpopulation effects. Crystagen administration preferentially increases CD3+ and CD4+ T-lymphocyte populations and normalizes the CD4+/CD8+ ratio, with a more pronounced effect on T-cell immunity than on B lymphocytes [9, 15]. This T-cell-dominant profile is consistent with the thymic tissue specificity of the EDP sequence and with the derivation of Crystagen from the thymic polypeptide complex Thymalin.

Lymphocyte-tumor cell proliferation. Crystagen enhances normal lymphocyte proliferation while simultaneously inhibiting proliferation of K-562 erythroleukemia cells, demonstrating a selective pro-proliferative effect that discriminates between normal and transformed cell populations [12].

4.4 Heat-shock protein regulation and cytoprotection

A distinctive feature of Crystagen within the Khavinson peptide family is its pronounced upregulation of heat-shock protein gene expression. In a study of elite female artistic gymnasts receiving Crystagen in combination with other peptide bioregulators (Vezugen, Pinealon, Pancragen), expression of the HSPA1A gene (encoding the inducible 70-kDa heat-shock protein HSP-70) increased by an average of 2.2-fold relative to baseline [13, 14]. HSP-70 is a molecular chaperone that participates in protein folding, prevention of protein aggregation, translocation of polypeptides across membranes, and cellular stress resistance. The upregulation of HSPA1A by Crystagen is proposed as a principal molecular basis for the enhanced cellular stress tolerance and reduced infectious disease susceptibility observed in treated subjects.

4.5 Anti-inflammatory activity

The anti-inflammatory component of Crystagen activity includes normalization of interleukin-6 expression (which is typically elevated in chronic low-grade inflammation associated with aging, termed "inflammaging") and modulation of tumor necrosis factor alpha signaling [12, 13, 20]. Studies of Khavinson peptides in the THP-1 monocyte/macrophage cell line demonstrated that the peptide bioregulators act as natural inducers of TNF tolerance in monocytes and as anti-inflammatory

molecules during inflammatory and microbial-mediated macrophage activation, increasing tyrosine phosphorylation of mitogen-activated cytoplasmic kinases while suppressing excessive proinflammatory cytokine release [20].

5. Pharmacokinetics and Routes of Administration

5.1 Absorption

No formal pharmacokinetic studies of the isolated Crystagen EDP tripeptide have been published in the peer-reviewed literature. The pharmacokinetic considerations described below are derived from general principles of ultrashort peptide pharmacology and from molecular modeling studies conducted within the Khavinson laboratory.

Crystagen, as a linear tripeptide with unprotected N-terminal alpha-amino and C-terminal carboxyl groups, is expected to undergo rapid proteolytic degradation by aminopeptidases (attacking the N-terminal glutamic acid) and carboxypeptidases (attacking the C-terminal proline) in the gastrointestinal lumen and in plasma. The C-terminal proline may confer partial resistance to carboxypeptidase cleavage owing to the cyclic imino acid structure, which is recognized less efficiently by some exopeptidases than are standard alpha-amino acid C-termini [17].

Molecular modeling studies by the Khavinson laboratory have demonstrated that ultrashort peptides, including di- and tripeptides, are substrates of the proton-coupled oligopeptide transporter (POT) family, specifically the intestinal transporter PEPT1 (SLC15A1) and the renal and epithelial transporter PEPT2 (SLC15A2) [17, 18]. These transporters mediate active uptake of di- and tripeptides across epithelial membranes in the small intestine, providing a mechanistic basis for oral bioavailability that bypasses the requirement for passive diffusion of the intact peptide. Additional modeling studies have demonstrated that some ultrashort peptides are substrates of the L-type amino acid transporter (LAT) family, which may contribute to cellular uptake and tissue distribution [18].

The oral formulations of Crystagen used in Russian dietary supplement practice (capsules, sublingual solutions) are predicated on the assumption of PEPT1-mediated intestinal absorption. The sublingual route may provide additional absorptive surface through the oral mucosa, bypassing first-pass hepatic metabolism.

5.2 Distribution

No tissue-distribution data have been published for Crystagen. By analogy with other ultrashort peptides in the Khavinson family, the compound is expected to distribute broadly in aqueous compartments owing to its high water solubility and low molecular weight. Nuclear penetration has been demonstrated for Khavinson ultrashort peptides as a class, with accumulation in cell nuclei and nucleoli confirmed by fluorescent labeling studies [6, 7].

5.3 Metabolism

The principal metabolic pathway for Crystagen is expected to be peptide-bond hydrolysis by ubiquitous aminopeptidases, carboxypeptidases, and endopeptidases in the gastrointestinal tract, plasma, and tissue compartments. The metabolic products are the constituent amino acids (L-glutamic acid, L-aspartic acid, L-proline), all of which are endogenous, proteinogenic amino acids with established metabolic fates. The compound does not undergo cytochrome P450-mediated metabolism.

5.4 Elimination

The elimination products of Crystagen metabolism are endogenous amino acids that enter standard amino acid catabolic pathways (transamination, oxidative deamination, urea cycle). Intact peptide elimination in urine or bile has not been characterized but is expected to represent a minor fraction of the administered dose.

5.5 Routes of administration

Three routes are described in the commercial and research literature:

Oral: Capsule formulations, typically 200 micrograms per capsule, administered 1 to 2 capsules daily for 10 to 30 days. This is the principal route for dietary supplement use in Russia.

Sublingual: Liquid formulations applied sublingually, with absorption through the oral mucosa. Dosing is typically 6 to 8 drops (approximately 100 to 200 micrograms per dose) twice daily.

Subcutaneous or intramuscular injection: Research-grade lyophilized peptide reconstituted in bacteriostatic water or sterile saline. This route bypasses gastrointestinal degradation and is used in investigational settings.

5.6 Special populations

No specific pharmacokinetic data exist for pediatric, geriatric, renal-impaired, or hepatic-impaired populations. The geriatric population is the principal target for Crystagen administration on the basis of the immunosenescence rationale; no dose adjustment considerations specific to age have been published.

6. Preclinical Pharmacology

6.1 Thymalin geroprotective studies in animals

The parent compound Thymalin has been studied in multiple animal geroprotective models. In CBA mice, chronic administration of Thymalin and Epithalamin (the pineal bioregulator preparation) from 6 months of age produced increases in mean lifespan of approximately 20 to 40 percent relative to untreated controls, with decreased incidence of spontaneous neoplasms [24, 25]. These studies established the geroprotective framework within which Crystagen was subsequently characterized as a synthetic analog of the Thymalin active component.

6.2 Splenic immunomodulation

Chervyakova, Linkova, Chalisova, and colleagues (2014) studied the immunomodulatory activity of four short peptides (Vilon, Thymogen, Crystagen, and R-1) in organotypic cultures of spleen tissue from young (3-month) and old (24-month) rats [9, 10]. Crystagen activated B-cell immunity in splenic tissue, assessed by increased expression of B-lymphocyte markers, but did not significantly affect the processes of cellular renewal (proliferation, apoptosis balance) in the spleen during aging. This contrasted with Vilon (which inhibited apoptosis), R-1 (which stimulated proliferation and differentiation), and Thymogen (which activated B-lymphocytes and promoted cell proliferation). The authors interpreted these findings as evidence of peptide-specific immunomodulatory profiles within the Khavinson ultrashort peptide family, with Crystagen preferentially activating humoral (B-cell) immunity in the spleen while exerting its T-cell-dominant effects principally in thymic tissue [9].

6.3 Cortical thymocyte differentiation and apoptosis

Studies of the related peptides T-32 (Glu-Asp-Ala) and T-38 (Lys-Glu-Asp) in human cortical thymocyte cultures demonstrated that peptides sharing the Glu-Asp core sequence amplify thymocyte differentiation toward regulatory T cells (CD4+CD25+FoxP3+ Treg cells), increase proliferative activity, and decrease apoptosis [11]. The anti-apoptotic mechanism involves approximately 2-fold reduction in p53 expression and concomitant increase in anti-apoptotic protein expression in

CD4+CD8+ double-positive thymocytes. Although these specific experiments used T-32 and T-38 rather than Crystagen (T-36), the shared Glu-Asp core and the comparable biological activities across the T-32/T-36/T-38 peptide series support the mechanistic extrapolation to Crystagen [5, 11].

6.4 Organotypic tissue culture proliferation

Khavinson, Chalisova, and colleagues studied the effects of the synthesized tripeptides T-32 (Glu-Asp-Ala), T-33 (Glu-Asp-Arg), T-34 (Glu-Asp-Gly), T-36 (Glu-Asp-Pro, Crystagen), and T-38 (Lys-Glu-Asp) on organotypic skin cultures of young and old rats [5]. In skin explants from young rats, all peptides except T-34 (Glu-Asp-Gly) produced a stimulatory effect on cell proliferation, providing direct evidence of the proliferative activity of T-36 (Crystagen) in tissue explant culture and demonstrating age-dependent and sequence-dependent variation in the biological activity of the Glu-Asp-containing peptide series.

6.5 Lymphocyte and tumor cell proliferation

Crystagen has been reported to enhance normal lymphocyte proliferation while inhibiting proliferation of K-562 chronic myelogenous leukemia cells in vitro [12]. This differential effect on normal versus transformed cell populations is consistent with the broader Khavinson peptide hypothesis that bioregulatory peptides restore normal cellular function rather than producing nonspecific mitogenic stimulation, and is mechanistically attributed to the epigenetic normalization of gene expression programs that are differently dysregulated in normal aging cells versus malignant cells.

7. Clinical Evidence Base

7.1 Elderly immunogram normalization

In a clinical observational study of elderly patients receiving Crystagen orally, immunogram parameters (T-lymphocyte subpopulation counts, CD4+/CD8+ ratio, natural killer cell activity, immunoglobulin levels) were normalized in 82 percent of treated individuals compared to 56 percent in the control group not receiving peptide supplementation [15]. This study, conducted within the Russian dietary supplement surveillance framework, was not a randomized placebo-controlled trial and does not meet Western regulatory evidence standards. The magnitude of the reported effect (26 percentage point difference in normalization rates) is nevertheless substantial and consistent with the immunomodulatory mechanisms characterized in preclinical studies.

7.2 Athletic performance and immune resilience

A study of 20 highly skilled female artistic gymnasts divided into a treatment group receiving personalized peptide bioregulators (Crystagen, Vezugen, Pinealon, Pancragen) and a control group undergoing identical training regimens demonstrated several immunological and clinical effects [13, 14]. Under the influence of the peptide bioregulators, expression of the HSPA1A gene (encoding HSP-70) increased by an average of 2.2-fold relative to baseline, interleukin-6 expression was normalized, and the incidence of acute respiratory infections was substantially reduced: during a concurrent influenza epidemic, 80 percent of athletes in the treatment group did not contract respiratory infections. The peptide bioregulators were well tolerated without observed side effects, complications, or drug dependence [13, 14]. The study was not designed to isolate the contribution of Crystagen from the other peptide bioregulators administered concurrently.

7.3 Thymalin clinical context

The clinical context for Crystagen is substantially informed by the extensive clinical record of its parent compound Thymalin, which has been used in Russian medical practice since 1982. The seminal clinical study was the prospective 266-patient,

6-to-8-year trial conducted by Khavinson and Morozov at the Saint Petersburg Institute of Bioregulation and Gerontology and the Institute of Gerontology of the Ukrainian Academy of Medical Sciences [16]. Elderly patients were treated with Thymalin (10 mg intramuscularly daily for 10 days per course), Epithalamin, or both, and compared to age-matched untreated controls over 6 to 8 years. Results demonstrated normalization of cardiovascular, endocrine, immune, and nervous system function indices; 2.0-to-2.4-fold reduction in acute respiratory disease incidence; reduced clinical manifestations of ischemic heart disease, hypertension, osteoarthritis, and osteoporosis; and decreased mortality rate: 2.0-to-2.1-fold in the Thymalin group, 1.6-to-1.8-fold in the Epithalamin group, and 4.1-fold in the combined Thymalin plus Epithalamin group treated annually for 6 years [16].

Crystagen, as the synthetic minimal peptide component of Thymalin, is proposed to account for a portion of the immunomodulatory and geroprotective activity observed in the Thymalin clinical studies. However, the relative contribution of Crystagen versus the other Thymalin peptide components (Thymogen, Vilon, and additional sequences) has not been formally established in clinical comparative studies.

7.4 Limitations of the clinical evidence base

The clinical evidence base for Crystagen as an isolated synthetic tripeptide is limited to observational studies, dietary supplement surveillance, and open-label cohort reports, all conducted within the Russian bioregulatory peptide research tradition. No randomized, double-blind, placebo-controlled trials of the isolated Crystagen peptide have been registered on ClinicalTrials.gov or on international trial registries. The clinical data do not meet the evidentiary standards of the United States Food and Drug Administration, the European Medicines Agency, or other Western regulatory authorities for pharmaceutical registration. Investigators evaluating the clinical evidence should weight the consistent mechanistic and pre-clinical data alongside the methodological limitations of the available clinical reports.

8. Sourcing and Quality Verification

Crystagen is available from two principal supply categories:

Russian dietary supplement formulations. Crystagen is commercially available in the Russian Federation as a dietary supplement (biologically active additive) in capsule form (typically 60 capsules per package, 200 micrograms per capsule) and as sublingual drops. These formulations are manufactured under Russian dietary supplement quality standards and are distributed through Russian pharmacy chains and international importers. The dietary supplement formulations are not pharmaceutical-grade preparations and have not undergone the quality assurance processes required for Western pharmaceutical registration.

Research-grade synthetic peptide. Research-grade Crystagen (H-Glu-Asp-Pro-OH) is supplied by multiple international peptide synthesis vendors at greater than 95 percent purity by high-performance liquid chromatography. Supply is in milligram to gram quantities as the lyophilized trifluoroacetate or acetate salt. Pricing is moderate, in the range of \$30 to \$150 per 20 milligrams of research-grade material at the time of monograph preparation.

Investigators should confirm compound identity and purity on every research-grade lot through:

- High-performance liquid chromatography area-percent purity, ideally greater than 95 percent (greater than 98 percent preferred for dose-response studies)
- Mass spectrometry confirmation of molecular weight (360.34 plus or minus 0.5 for the protonated molecular ion $[M+H]^+$ of the free peptide)
- Amino acid analysis after acid hydrolysis (confirming equimolar Glu, Asp, and Pro residues)
- Peptide content determination by quantitative amino acid analysis or by nitrogen analysis, to distinguish peptide mass from salt, water, and counterion content in the lyophilized preparation
- Endotoxin testing (Limulus amebocyte lysate or recombinant Factor C assay) for preparations intended for cell culture or parenteral research applications
- Residual solvent analysis if the synthetic process involves non-aqueous solvents

Third-party analytical laboratories (Janoshik Analytical, Anabolic Lab, others) can provide independent confirmation of vendor certificates of analysis. The research-grade Crystagen supply chain is smaller than that for more established peptides, and lot-to-lot variability in peptide content and counterion composition is to be expected; on-receipt analytical confirmation is appropriate for any quantitative research application.

9. Reconstitution and Handling

Crystagen is supplied as a lyophilized powder that is freely soluble in water at room temperature. Stock solutions are typically prepared at 1 to 10 mg/mL in sterile water for injection, bacteriostatic water (0.9 percent benzyl alcohol), or phosphate-buffered saline (pH 7.4). The peptide dissolves rapidly without sonication or heating.

Storage of lyophilized powder: Minus 20 degrees Celsius, desiccated, protected from light. Stable for at least 24 months under these conditions. Room-temperature storage is acceptable for short periods (days to weeks) but accelerates hydrolysis of the peptide bonds and is not recommended for long-term storage.

Storage of reconstituted solutions: Refrigerated (2 to 8 degrees Celsius) for aqueous solutions used within 14 to 30 days. Bacteriostatic water preparations may be stored refrigerated for up to 30 days consistent with standard bacteriostatic reconstitution practices. Sterile water preparations without preservative should be used within 24 to 48 hours or aliquoted and frozen at minus 20 degrees Celsius for single-use applications.

Freeze-thaw stability: Limited data. As a small, hydrophilic peptide without disulfide bonds or complex tertiary structure, Crystagen is expected to tolerate multiple freeze-thaw cycles without significant degradation. Nevertheless, aliquoting for single-use is recommended to minimize cumulative peptide-bond hydrolysis during repeated temperature transitions.

Compatibility: The peptide is compatible with standard laboratory plastics (polypropylene microcentrifuge tubes, polystyrene culture plates) and with glass. Adsorption to container surfaces is minimal owing to the net negative charge and high aqueous solubility. No incompatibilities with standard diluents (saline, phosphate-buffered saline, dimethyl sulfoxide at concentrations below 10 percent) have been reported.

Light sensitivity: The peptide does not contain aromatic amino acids (tryptophan, tyrosine, phenylalanine) and is not expected to be photolabile. Light protection during storage is reasonable practice but is not critical.

10. Stack Interaction Considerations

Crystagen interacts with other compounds principally through its immunomodulatory and cytoprotective mechanisms rather than through conventional pharmacokinetic drug-drug interactions. The absence of cytochrome P450-mediated metabolism eliminates the CYP-inhibitor and CYP-inducer interaction categories that dominate conventional drug interaction pharmacology. Investigators using Crystagen in combination with other research compounds should consider the following:

Other Khavinson peptide bioregulators: Crystagen is frequently administered in combination with other Khavinson peptides in the Russian bioregulatory peptide tradition. The athletic study used Crystagen with Vezugen (vascular), Pinealon (neural), and Pancragen (pancreatic) concurrently [13, 14]. This combination approach is predicated on the tissue-specificity hypothesis: each peptide targets a different organ system, and concurrent administration produces complementary multi-system support. No adverse interactions have been reported with these combinations. Specifically, the combination of Crystagen with Epithalon (the pineal tetrapeptide bioregulator) replicates at the synthetic peptide level the Thymalin-plus-Epithalamin combination that produced the 4.1-fold mortality reduction in the Khavinson and Morozov elderly cohort [16].

Immunosuppressive agents: Crystagen stimulates immune cell proliferation and differentiation. Concurrent administration with immunosuppressive agents (cyclosporine, tacrolimus, mycophenolate, glucocorticoids at immunosuppressive doses) could produce pharmacodynamic antagonism. Investigators studying Crystagen in immunosuppressed models should consider this interaction.

Immunostimulatory agents: Concurrent administration with other immunostimulatory compounds (thymosin alpha-1, interferon alpha, interleukin-2, granulocyte colony-stimulating factor) could produce additive or synergistic immune stimulation that exceeds the desired physiological range. The combination has not been studied and should be approached with appropriate monitoring.

Chemotherapy and radiotherapy: The parent compound Thymalin has been used adjunctively during chemotherapy and radiotherapy in Russian clinical practice to support immune reconstitution [1, 2]. This adjunctive application is rational

given the immunoprotective mechanism, but no formal drug-interaction studies between Crystagen and specific chemotherapeutic agents have been published.

Conventional pharmaceuticals: No cytochrome P450 interactions are expected. No protein-binding displacement interactions are expected at the microgram dosing range used for Crystagen. The principal interaction consideration is the pharmacodynamic effect on immune function, which should be considered in the context of any concurrent immunomodulatory therapy.

11. Adverse Events and Safety

11.1 Clinical safety profile

The adverse-event profile of Crystagen is characterized by the absence of reported clinically significant adverse events across the available literature. The peptide bioregulators Pinealon, Vezugen, Pancragen, and Crystagen were reported to be well tolerated by athletes without observed side effects, complications, or drug dependence [13, 14]. The elderly patient observational studies similarly reported no consistent adverse events attributable to Crystagen administration [15].

11.2 Theoretical safety considerations

The following theoretical safety considerations apply to Crystagen and to the Khavinson ultrashort peptide class generally:

Immune stimulation in autoimmune conditions. Crystagen stimulates T-lymphocyte proliferation and differentiation. In individuals with autoimmune conditions (rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, type 1 diabetes), immune stimulation could theoretically exacerbate autoimmune activity. This theoretical concern has not been confirmed in clinical reports, but investigators should exercise caution in autoimmune populations.

Immune stimulation in lymphoproliferative disorders. Crystagen enhances lymphocyte proliferation. In individuals with lymphoproliferative disorders (chronic lymphocytic leukemia, lymphoma), immune stimulation could theoretically promote disease progression. The concurrent finding that Crystagen inhibits K-562 erythroleukemia cell proliferation partially mitigates this concern, but the selectivity of the anti-proliferative effect across the full spectrum of hematological malignancies has not been characterized.

Peptide allergenicity. Tripeptides are generally below the molecular weight threshold for independent immunogenicity (approximately 1000 to 5000 daltons for hapten-carrier complex formation). Allergic reactions to Crystagen have not been reported but cannot be excluded, particularly with repeated parenteral administration.

11.3 Toxicological data

No formal acute, subacute, or chronic toxicological studies of the isolated Crystagen peptide have been published in the peer-reviewed literature accessible to this monograph review. The parent compound Thymalin has an extensive safety record from four decades of clinical use in the Russian Federation, with the reported adverse-event profile limited to rare injection-site reactions (pain, erythema) with intramuscular administration [1, 2, 16].

11.4 Pregnancy and lactation

No data on Crystagen use in pregnancy or lactation are available. The compound is not recommended for use in pregnancy or lactation in the absence of safety data.

12. Comparable Compounds Assessment

This section presents a structured comparative assessment of five immunomodulatory peptide candidates against Crystagen, evaluated against five competency standards: novelty, effect size, promising potential (current clinical state and trajectory), side-effect profile (clinical or preclinical), and overall validation. The five candidates were selected as the principal immunomodulatory peptides with relevance to the thymic-immune bioregulation space in which Crystagen operates.

12.1 Thymogen (Glu-Trp; EW dipeptide)

Mechanism: Synthetic immunomodulatory dipeptide derived from the same Thymalin polypeptide complex as Crystagen. Thymogen principally activates B-lymphocytes and promotes splenic cell proliferation, with additional effects on T-cell subpopulations and cytokine regulation [2, 9].

Development trajectory: Thymogen is registered as a pharmaceutical product in the Russian Federation for immunocorrection, with approved indications including immunodeficiency states of various etiologies, adjunctive therapy during chemotherapy and radiotherapy, and prevention of infectious complications. It has the most advanced regulatory status of any Thymalin-derived synthetic peptide.

Novelty: Low. The mechanism is conventional within the Khavinson bioregulatory peptide framework and is well characterized.

Effect size: Moderate. The immunomodulatory effects in preclinical and clinical studies are consistent and reproducible within the Russian research literature. Thymogen was found to activate B-lymphocytes and promote spleen cell proliferation more effectively than Crystagen in the splenic organotypic culture system [9].

Promising potential: Moderate. The compound has pharmaceutical registration in Russia and an established commercial market. Western regulatory registration has not been pursued.

Side-effect profile: Favorable. Four decades of clinical use in Russia with no significant adverse events reported.

Overall validation: Moderate. The compound has the advantage of pharmaceutical registration and the most extensive clinical use history among the Thymalin-derived synthetic peptides, but validation is limited to the Russian medical literature.

Comparison to Crystagen: Thymogen (EW) and Crystagen (EDP) derive from the same parent compound but differ in tissue specificity and immunomodulatory profile. Thymogen has stronger B-cell and splenic effects; Crystagen has stronger T-cell and thymic effects. Thymogen has the advantage of pharmaceutical registration; Crystagen has the advantage of a more T-cell-directed immunomodulatory profile and the demonstrated HSP-70 upregulation that Thymogen does not share.

12.2 Vilon (Lys-Glu; KE dipeptide)

Mechanism: Synthetic immunomodulatory dipeptide bioregulator of thymic origin. Vilon is the minimal (dipeptide) Khavinson thymic bioregulator. Its principal mechanisms include sequence-specific binding to the TCGA tetranucleotide motif in gene promoters, chromatin decondensation in aging lymphocytes, stimulation of CD4 and CD5 T-lymphocyte differentiation, and inhibition of apoptosis in splenic cells [22, 23].

Development trajectory: Vilon is used in investigational and observational settings in the Russian Federation. It has not been registered as a pharmaceutical product but has an extensive preclinical and observational clinical research record.

Novelty: Low. The mechanism shares the core Khavinson peptide-DNA interaction framework with Crystagen and the other thymic peptides.

Effect size: Moderate. Vilon produced the most potent comitogenic effect on thymocyte proliferation among the Khavinson thymic peptides tested in the sphingomyelin pathway study, and demonstrated the strongest stimulatory effect on sphingomyelinase activity in thymocyte membranes [26]. In splenic cultures, Vilon inhibited apoptosis, a distinct effect from Crystagen's B-cell activation [9].

Promising potential: Low to moderate. No pharmaceutical registration. The extensive preclinical and epigenetic research record supports continued investigational use.

Side-effect profile: Favorable. No significant adverse events reported in the available literature.

Overall validation: Moderate. The compound has the strongest mechanistic validation of the epigenetic chromatin-decondensation mechanism through the direct demonstration in aged human lymphocytes [22, 23].

Comparison to Crystagen: Vilon and Crystagen are both thymic bioregulators derived from Thymalin but operate through partially distinct mechanisms. Vilon has stronger direct evidence for the chromatin-decondensation mechanism (demonstrated in human lymphocytes from elderly donors). Crystagen has the advantage of the tripeptide structure (which may provide greater conformational specificity through the proline constraint) and the demonstrated HSP-70 upregulation.

12.3 Thymosin alpha-1 (Thymalfasin)

Mechanism: A 28-amino-acid polypeptide originally isolated from thymic tissue (Thymosin Fraction 5) by Allan Goldstein at the George Washington University in the 1970s. Thymosin alpha-1 activates dendritic cell maturation, promotes T-cell differentiation (particularly CD4+ and CD8+ subsets), enhances natural killer cell cytotoxicity, modulates Toll-like receptor signaling, and stimulates interferon-alpha and interferon-gamma production [27, 28].

Development trajectory: Thymosin alpha-1 is the most extensively developed thymic peptide in Western medicine. It is approved in over 35 countries (principally in Asia, South America, and Eastern Europe) for the treatment of chronic hepatitis B and C, as an immunoadjuvant in cancer therapy, and for immune reconstitution. It is manufactured as Zadaxin (SciClone Pharmaceuticals, now Fang Holdings/Lee's Pharmaceutical). It has not been approved by the United States FDA, though it has received orphan drug designation for several indications.

Novelty: Low. The mechanism is well characterized and represents conventional thymic peptide immunomodulation.

Effect size: Moderate to high. Clinical trials in hepatitis B and C have demonstrated clinically meaningful viral suppression and seroconversion rates. The compound has the largest clinical evidence base of any thymic peptide [27, 28].

Promising potential: Moderate. The compound has established commercial markets in Asia and broad clinical acceptance in hepatology and oncology adjunctive therapy. Active clinical trials continue in immunodeficiency and oncology settings.

Side-effect profile: Favorable. The compound is well tolerated at clinical doses (1.6 mg subcutaneously, typically twice weekly). Injection-site reactions are the principal reported adverse event.

Overall validation: High. The most extensively validated thymic peptide in Western clinical medicine, with randomized controlled trials, regulatory approvals in multiple jurisdictions, and a decades-long clinical use record.

Comparison to Crystagen: Thymosin alpha-1 is a substantially larger molecule (28 amino acids versus 3) with a well-established Western clinical evidence base and multi-country regulatory approval. Crystagen has the advantages of lower cost, simpler synthesis, oral bioavailability through POT-mediated absorption, and the epigenetic chromatin-decondensation mechanism that is not characterized for Thymosin alpha-1. The two compounds represent fundamentally different approaches to thymic immunomodulation: Thymosin alpha-1 as a direct immunostimulatory polypeptide and Crystagen as an epigenetic gene-regulatory ultrashort peptide. Thymosin alpha-1 is the clear choice for investigators requiring regulatory-grade clinical evidence; Crystagen may be the more informative choice for investigators studying epigenetic mechanisms of immunosenescence.

12.4 Thymulin (FTS; Facteur Thymique Serique)

Mechanism: A nonapeptide (nine amino acids) metallopeptide that requires zinc for biological activity, secreted by thymic epithelial cells and circulating in serum at concentrations that decline with age and with thymic involution. Thymulin promotes T-cell differentiation, modulates cytokine production, and participates in the neuroendocrine-immune network [29].

Development trajectory: Thymulin was characterized in the 1970s by the Bach laboratory in France. It has been studied primarily as a biomarker of thymic function and as an investigational therapeutic agent in animal models of aging, autoimmunity, and neuroinflammation. It has not been developed as a commercial pharmaceutical product.

Novelty: Moderate. The zinc-dependent metallopeptide mechanism is unique among thymic peptides and provides a distinct pharmacological approach.

Effect size: Moderate in preclinical models. Clinical evidence is limited to small investigational studies.

Promising potential: Low. No commercial development. Research interest persists principally in the neuroendocrine-immune interface and in zinc biology.

Side-effect profile: Favorable in preclinical studies. Limited human safety data.

Overall validation: Low to moderate. Well characterized as a thymic biomarker but not validated as a therapeutic agent through clinical trials.

Comparison to Crystagen: Thymulin and Crystagen share the thymic immunomodulatory space but differ fundamentally in structure (nonapeptide metallopeptide versus ultrashort tripeptide), mechanism (zinc-dependent T-cell differentiation versus epigenetic gene regulation), and development status (investigational biomarker versus dietary supplement with observational clinical data). Crystagen has the advantages of simpler synthesis, no zinc requirement, and more practical commercial availability.

12.5 Epithalon (Ala-Glu-Asp-Gly; AEDG tetrapeptide)

Mechanism: Synthetic tetrapeptide bioregulator derived from the pineal polypeptide complex Epithalamin. Epithalon activates telomerase in human somatic cells, modulates melatonin production, produces chromatin decondensation in aging cells, and has demonstrated geroprotective activity in animal lifespan studies [24, 25, 30].

Development trajectory: Epithalon is the most studied Khavinson peptide for geroprotective applications. It has not been registered as a pharmaceutical product in any jurisdiction. It is used in the Russian Federation in investigational and dietary supplement settings and is widely available internationally as a research-grade peptide.

Novelty: Moderate. The telomerase-activation mechanism is distinctive within the Khavinson peptide family and has attracted substantial international research attention.

Effect size: Moderate. Animal lifespan extension of 20 to 40 percent has been reported in CBA mice. The Khavinson and Morozov elderly cohort study demonstrated a 1.6-to-1.8-fold mortality reduction for Epithalamin alone and a 4.1-fold re-

duction when combined with Thymalin [16].

Promising potential: Moderate. The geroprotective and telomerase-activation data have generated substantial research interest. No pharmaceutical registration or large-scale clinical trials.

Side-effect profile: Favorable. No significant adverse events reported in the available literature.

Overall validation: Moderate. The animal lifespan data and the elderly cohort mortality data provide a stronger evidence base than most Khavinson peptides, though the evidence remains below Western regulatory standards.

Comparison to Crystagen: Epithalon and Crystagen are complementary Khavinson peptides targeting different organ systems (pineal gland versus thymus) with overlapping epigenetic mechanisms but distinct primary targets (telomerase versus immune gene expression). The combination of Crystagen with Epithalon at the synthetic peptide level replicates the Thymalin-plus-Epithalamin combination that produced the strongest geroprotective effect in the Khavinson elderly cohort study. Investigators studying geroprotective peptide combinations should consider this pairing as the synthetic equivalent of the most validated Khavinson peptide combination.

12.6 Comparator summary

The five comparator compounds occupy distinct positions in the thymic-immune peptide landscape. **Thymogen** is the most clinically established Thymalin-derived synthetic peptide, with pharmaceutical registration in Russia and a B-cell-dominant immunomodulatory profile. **Vilon** is the minimal Khavinson thymic bioregulator with the strongest evidence for the chromatin-decondensation mechanism. **Thymosin alpha-1** is the most extensively validated thymic peptide in Western clinical medicine, with regulatory approvals across 35 countries and a large clinical trial database. **Thymulin** is a mechanistically distinctive zinc-dependent thymic metallopeptide that has not been commercially developed. **Epithalon** is the principal Khavinson geroprotective peptide, complementary to Crystagen through the pineal-thymic axis.

Crystagen occupies a unique position in this comparator landscape: it is the only compound in the panel that combines the T-cell-dominant immunomodulatory profile, the HSP-70 (HSPA1A) gene upregulation, the epigenetic chromatin-decon-

densation mechanism, and the Thymalin derivation that links it to the most extensive clinical geroprotective dataset in the Khavinson peptide family. Its principal limitations relative to the comparators are the absence of pharmaceutical registration (unlike Thymogen and Thymosin alpha-1), the absence of randomized controlled clinical trials (unlike Thymosin alpha-1), and the absence of formal pharmacokinetic data (shared with most Khavinson peptides). Investigators selecting among these compounds should match the selection to the experimental question: Thymosin alpha-1 for regulated clinical applications requiring Western-standard evidence, Crystagen or Vilon for mechanistic studies of epigenetic immunomodulation in aging, Epithalon for telomerase and geroprotective studies, and the Crystagen-plus-Epithalon combination for studies modeling the Thymalin-plus-Epithalamin geroprotective protocol at the synthetic peptide level.

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in the Russian Federation and as a research-grade peptide from international suppliers; investigators should consult their local regulatory authority before any research application.