γ-Glutamylamine cyclotransferase
An enzyme involved in the catabolism of ε-(γ-glutamyl)lysine and other γ-glutamylamines

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Summary

γ-Glutamylamine cyclotransferase, an enzyme found in a number of animal tissues and cells, catalyzes the conversion of ε-(L-γ-glutamyl)-L-lysine to free lysine and 5-oxo-L-proline as well as the release of free amines and the formation of 5-oxo-L-proline from a variety of other L-γ-glutamylamines. Among its substrates are both the mono- and di-γ-glutamyl derivatives of putrescine, spermidine and spermine, and a derivative of ε-(L-γ-glutamyl)-L-lysine in which both the α-amino group and the carboxyl group of the lysine moiety are blocked. The enzyme does not act on most γ-glutamyl-α-amino acids, nor is it active toward the ε-lysyl derivatives of L-aspartic acid or D-glutamic acid. Derivatives of ε-(L-γ-glutamyl)-L-lysine in which the α-amino or the α-carboxyl function of the glutamyl moiety is blocked also do not serve as substrates. The specificity of γ-glutamylamine cyclotransferase is in accordance with the proposal that it functions biologically in the latter stages of the catabolism of products of the action of transglutaminases. Some suggestions as to the manner in which γ-glutamylamine cyclotransferase serves this function are made based on present knowledge of protein degradation.

I. Introduction

γ-Glutamylamine cyclotransferase catalyzes the release of free amines from γ-L-glutamylamines with the concomitant cyclization of the glutamic acid moiety to 5-oxo-L-proline (synonyms: pyroglutamic acid, 5-pyrrolidone-2-carboxylic acid) in accordance with the following reaction:

\[ \begin{array}{c}
\text{H}_2\text{N}-\text{CHR} \\
\text{C}=\text{NHR} \\
\text{CH}_2 \\
\text{H}-\text{C}=\text{NH}_2 \\
\text{CO}_2\text{H}
\end{array} \rightarrow 
\begin{array}{c}
\text{H}_2\text{N}-\text{CHR} \\
\text{C}=\text{NHR} \\
\text{CH}_2 \\
\text{H}-\text{C}=\text{NH}_2 \\
\text{CO}_2\text{H}
\end{array} \]

The recent discovery of this enzyme (1) was a consequence of our ongoing interest in the metabolic fate of ε-(γ-glutamyl)lysine crosslinks and of other protein-bound γ-glutamylamine products of transglutaminase action. Stimulus was supplied by the known resistance of the crosslink to digestion by proteases (2, 3) together with reports that the isopeptide, ε-(γ-glutamyl)lysine, can sustain the growth of rats and chicks on lysine-deficient diets (4, 5). These reports suggested the presence of an activity capable of releasing lysine from ε-(γ-glutamyl)lysine and a search for the enzyme responsible for the disassembly of this dipeptide was undertaken. The finding of the widely distributed enzyme, γ-glutamylamine cyclotransferase, which catalyzes not only the breakdown of ε-(γ-glutamyl)lysine, but that of a variety of other γ-glutamylamines provides evidence for a catabolic pathway for products of the transglutaminase reaction.
II. The \( \varepsilon-(\gamma\text{-glutamyl})\)lysine crosslink and polypeptide \( \gamma\text{-glutamylamine} \) conjugates: products of the transglutaminase reaction

Covalent crosslinking through \( \varepsilon-(\gamma\text{-glutamyl})\)-lysine bonds is essential to the functional or structural integrity of a number of mammalian proteins (for review, see ref. 6). Among these are fibrin, coagulated seminal vesicular proteins and various keratins. Identification of the isopeptide, \( \varepsilon-(\gamma\text{-glutamyl})\)lysine, after exhaustive proteolytic digestion of various tissues, cells and whole organisms provides evidence for crosslinking, albeit in many cases the proteins involved have not been identified (7–11). Similar evidence for \( \gamma\text{-glutamylpolyamine} \) conjugates in both cells and extracellular fluid has been obtained (12).

\( \varepsilon-(\gamma\text{-Glutamyl})\)lysine crosslinks and other monosubstituted \( \gamma \)-amides of peptide-bound glutamic acid are formed by the catalytic action of members of a widely distributed group of enzymes called transglutaminases. Several reviews covering the properties and distribution of the various enzymes have appeared (6, 13, 14). The transglutaminase reaction (reaction 2) occurs by way of a \( \text{Ca}^{2+} \)-dependent acyl transfer mechanism in which carboxamide groups of peptide-bound glutamine residues serve as acyl donors and in which amino groups in a wide variety of primary amines can act as acyl acceptors. Included are many alkyl amines such as methylamine and ethylamine (15–17) aromatic amines such as phenethylamine (15) diamines (15, 18) the polyamines, spermine and spermidine (12, 15, 18) and the biogenic amine, histamine (15). The fluorescent amine, monodansylcadaverine (\( \text{N-(5-aminopentyl)-5-dimethylamino} \)-aphthalene-1-sulfonamide) is an especially sensitive acceptor substrate for transglutaminases and possesses the additional property of being easily detectable (19). Several amino acid esters and amides serve as acceptor substrates (15, 20); free amino acids do not (15). The product of amine incorporation in each case is a peptide-bound \( \gamma \)-glutamylamine (\( \text{Glu} \text{-NHR} \) in reaction 2). Participation of \( \varepsilon \)-amino groups of peptide-bound lysine residues as acyl acceptors, leads to formation of \( \varepsilon-(\gamma\text{-glutamyl})\)lysine crosslinks (reaction 3). Crosslinking can also occur as a result of diamine or polyamine incorporation (reaction 4) (12, 18).

\[
\begin{align*}
\text{(2)}: & \quad \text{GLU} + \text{H}_2\text{NR} \longrightarrow \text{GLU} + \text{NH}_3 \\
\text{(3)}: & \quad \text{GLU} + \text{Lys} \longrightarrow \text{GLU} + \text{NH}_3 \\
\text{(4)}: & \quad 2 \text{GLU} + \text{H}_2\text{R} - \text{NH}_2 \longrightarrow \text{GLU} + \text{GLU} + 2 \text{NH}_3
\end{align*}
\]

III. \( \gamma \)-Glutamylamine cyclotransferase

A. Discovery and isolation

Despite an accumulation of information on the production and distribution of \( \varepsilon-(\gamma\text{-glutamyl})\)lysine crosslinks and protein-bound \( \gamma \)-glutamylamines, there has been little attention directed toward the

\* Only the un-ionized forms of amines are reactive as acceptor substrates for transglutaminases (15, 23). When one estimates the proportion of various amines in reactive form at physiological pH levels and temperature it becomes evident that a considerably larger fraction of the primary amino groups of polyamines are in a reactive form than are the \( \varepsilon \)-amino groups of lysine residues (e.g., spermine, \( pK_a \) 7.92 and 8.81 at 37°; \( \text{l-lysine} \) \( pK_a \) \( \sim 10.625 \)). Thus, the concentration dependency of the reactive forms of amines as substrates for transglutaminases must be taken into account when considering the relative effectiveness of biological amines as substrates.