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Glucosamine-6-phosphate synthase from Escherichia coli: determination of the mechanism of inactivation by N3-fumaroyl-L-2,3-diaminopropionic derivatives.

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Abstract

A mechanistic investigation of the inactivation of Escherichia coli glucosamine-6-phosphate synthase by N3-(4-methoxyfumaroyl)-L-2,3-diaminopropionate (FMDP) was undertaken. On the basis of the known participation of the N-terminal cysteine residue in this process [Chmara et al. (1986) Biochim. Biophys. Acta 870, 357; Badet et al. (1988) Biochemistry 27, 2282], the model reactions between FMDP and L-cysteine and between FMDP and the synthetic decapeptide Cys-Gly-Ile-Val-Gly-Ala-Ile-Ala-Gln-Arg, corresponding to the amino-terminal protein sequence, were studied. The results allowed us to propose a pathway that is in perfect agreement with the biochemical results: enzyme inactivation arose from Michael addition of glutamine binding site cysteine-1 on the fumaroyl double bond at the beta-position of the ester group. Upon denaturation under slightly alkaline conditions, this adduct underwent cyclization to a transient succinimide adduct, which rearranged into the stable 2-substituted 1,4-thiazin-3-one-5-carboxylate involving participation of the cysteine amino group. The tryptic radiolabeled peptides purified from [3H]FMDP-treated enzyme and resistant to Edman degradation coeluted with the products resulting from the model reaction between the synthetic decapeptide and the inhibitor.

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