PARTIAL SEQUENCE OF AN ACTIVE-SITE PEPTIDE FROM TRIOSE PHOSPHATE ISOMERASE*

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SUMMARY

The sequence of an active-site peptide from rabbit muscle triose phosphate isomerase, containing the essential glutamyl residue with which chloroacetol phosphate reacts, is Trp-Val-Leu-Ala-Tyr-Glu-Pro-Val-Ala-Trp-(Thr, Ile)-Gly-Gly-Lys.

Haloacetol phosphates react specifically with the active site of triose phosphate isomerase (TPI) (D-glyceraldehyde 3-phosphate Ketol-isomerase, EC 5.3.1.1) with total loss of enzymic activity (1-5). Chloroacetol phosphate (CAP) esterifies an essential glutamyl residue of rabbit muscle TPI, and the amino acid composition in the vicinity of the modified amino acid has been determined (4). In this communication, I report the partial sequence of the active-site peptide.

MATERIALS AND METHODS

All reagents used were of the highest purity commercially available. DFP-treated carboxypeptidases A and B were obtained from Worthington Biochem. Corp. The active-site peptide was isolated as described previously (4) from a tryptic digest of CAP-modified TPI from rabbit muscle.

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Edman degradations were performed by the subtractive method as described by Konigsberg (6).

RESULTS AND DISCUSSION

The amino acid composition of a pentadecapeptide obtained from a tryptic digest of CAP-modified TPI and containing the esterified, essential glutamyl residue is Trp_2, Lys, Thr, Glu, Pro, Gly_2, Ala_2, Val_2, Ile, Leu, Tyr (4). Edman degradations provide the sequence of the first nine residues (Table I): Trp-Val-Leu-Ala-Tyr-Glu-Pro-Val-Ala-. Tryptophan,which is destroyed by acid hydrolysis, was implicated as being N-terminal since the amino acid compositions of the initial peptide and the peptide after a single degradation appeared identical. Also, tryptophan as well as valine, leucine, alanine, and tyrosine was released by leucine aminopeptidase.

Carboxypeptidase (mixture of A and B) digestion of the active-site peptide (Figure 1) resulted in the rapid release of only lysine (one molar equivalent), whose presence at the C-terminal position was expected since the peptide was obtained from a tryptic digest. Glycine (approaching two molar equivalents) and isoleucine, threonine, tryptophan, and alanine (each approaching one molar equivalent) were released at about equal rates. Valine was detected during the latter stages of digestion. Carboxypeptidase A is known to release isoleucine, threonine, tryptophan, and alanine much more rapidly than glycine (7). The present observation of similar rates of release of these amino acids is consistent with a C-terminal sequence of -Gly-Gly-Lys. Another observation consistent with glycine occupying the penultimate position is that carboxypeptidase A alone did not release lysine during a 6-hr digestion period. Glycine in the penultimate position usually decreases the rate of release of the C-terminal residue (7).

TABLE I. EDMAN DEGRADATION OF ACTIVE-SITE PEPTIDE[a]

Amino Acid	Initial peptide	Molar ratios of amino acids[b] Cycle[c] 1	2	3	4	5	6	7	8	9
Trp[d]	2									
Lys[d]	1									
Thr	1	1.0	0.98	0.95	0.97	0.95	0.95	1.0	0.95	0.93
Glu	1	1.0	0.99	1.1	1.1	1.0	0.57	0.55	0.56	0.55
Pro	1	1.1	1.1	1.1	1.1	1.0	1.1	0.65	0.65	0.63
Gly	2	2.2	2.2	2.1	2.1	2.1	1.9	2.1	2.2	2.2
Ala	2	2.1	2.1	2.1	1.4	1.4	1.4	1.4	1.4	1.1
Val	2	2.0	1.2	1.1	1.1	1.2	1.1	1.1	0.7	0.7
Ile	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Leu	1	0.99	0.95	0.33	0.30	0.31	0.28	0.28	0.26	0.27
Tyr	1	0.90	0.88	0.87	0.82	0.42	0.35	0.31	0.30	0.28

[a]The CAP-labeled active-site peptide was treated initially with 0.01 N NaOH to convert the esterified glutamyl residue to the free acid. The resulting peptide (0.8 μmoles) was subjected to degradation. After each cycle, a sample representing about 0.02 μmoles of peptide was removed, hydrolyzed with 6 N HCl at 110° for 21 hr, and analyzed on a Beckman Model 120C amino acid analyzer.

[b]Isoleucine was arbitrarily set at 1.0.

[c]Underlining indicates the amino acid disappearing at each cycle.

[d]Tryptophan and lysine were assayed only in the initial peptide.

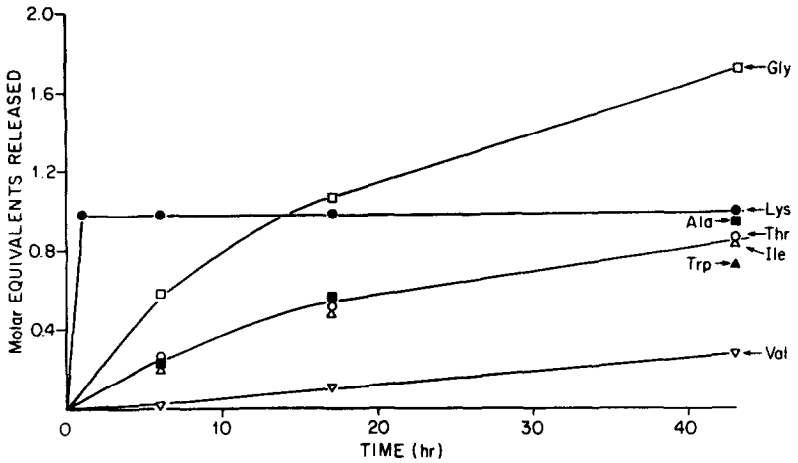

Fig. 1. Time-course of amino acids released from the active-site peptide upon digestion with a mixture of carboxypeptidases A and B. The peptide (0.5 μmoles) was dissolved in 2.5 ml of N-ethylmorpholine hydrochloride (0.1 M, pH 8.0) containing 0.15 mg of carboxypeptidase A and 0.02 mg of carboxypeptidase B. Periodically, samples containing 0.05 μmoles of original peptide were withdrawn, neutralized to pH 2.0, and subjected to amino acid analysis on a Beckman Model 120 C amino acid analyzer.

The positions in the active-site peptide of the alanyl and valyl residues released by carboxypeptidase were already established by the Edman degradations. A peptide, with the moiety derived from modification by CAP still intact, composed of equivalent molar amounts of Trp, Glu, Pro, Ala, Val, Tyr was isolated from a chymotryptic digest of the original peptide by chromatography on Aminex AG50W-X2. Thus, tryptophan occupies position 10. The order of threonine and isoleucine remain to be established in the active-site peptide:

Trp-Val-Leu-Ala-Tyr-Glu-Pro-Val-Ala-Trp-(Thr, Ile)-Gly-Gly-Lys

Perhaps the most striking feature of the active-site peptide is that ten of the fifteen residues are hydrophobic, a characteristic possessed by many enzymic active sites which have been studied in detail (8-10). The mechanism of the TPI-catalyzed interconversion of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate is by intramolecular proton transfer (11, 12). From the dependence of V_{max} on pH, Rose (11) has calculated that groups with pK's of 6.5 and 9.5 are

involved in catalysis. Studies on the inactivation of TPI by iodoacetic acid and photooxidation have led Burton and Waley (13) to suggest that histidine is the active-site residue with pK 6.5 and is responsible for proton transfer. In view of the hydrophobic residues in the vicinity of the essential glutamyl residue in TPI, one cannot exclude the possibility of its pK being abnormally high. For example, a catalytically functional glutamyl residue of lysozyme is in a hydrophobic environment and is thought to have a pK of 6.3 (14, 15).

Conceivably, the prolyl residue, which is adjacent to the essential glutamyl residue, plays an important role in establishing the proper geometric orientation between certain groups of the active site.

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