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PARTIAL PURIFICATION AND SOME PROPERTIES OF γ-GLUTAMYL TRANSPEPTIDASE FROM HUMAN BILE

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Summary

1. γ-Glutamyl transpeptidase ((5-glutamyl)-peptide:amino acid 5-glutamyltransferase, EC 2.3.2.2) from human bile has been partially purified using protamine sulphate treatment, DEAE-cellulose chromatography and Sephadex G-200 filtration. The procedure resulted in 150-fold increase in specific activity with a 37% yield.

2. The partially purified enzyme showed a single zone of enzyme activity by polyacrylamide gel electrophoresis and eluted in the inner volume of Sephadex G-200.

3. The enzyme had a pH optimum of 8.1 and K_m of 1.52 mM using γ-glutamyl *p*-nitroanilide as substrate.

4. The effects of cations and different γ-glutamyl acceptors on the activity of the enzyme are reported.

5. As bile γ-glutamyl transpeptidase appears to be soluble in the absence of detergents, it is suggested that bile may prove to be a useful source for further studies of the kinetic properties and physiological role of human γ-glutamyl transpeptidase.

Introduction

γ-Glutamyl transpeptidase ((5-glutamyl)-peptide:amino acid 5-glutamyltransferase, EC 2.3.2.2) was first described by Hanes et al. [1] in sheep kidney. The enzyme catalyses the transfer of the γ-glutamyl moiety from γ-glutamyl peptides to other peptides or amino acids and a role for the enzyme in the transport of amino acids via the γ-glutamyl cycle has been proposed [2]. The presence of this enzyme has been shown in yeast [3], plant tissues [4] and in a number of mammalian species [5—11]. The enzyme has been purified to homogeneity from various sources [5—7,10] and the properties have been character-

ised. This paper deals with the purification of γ-glutamyl transpeptidase from human bile and describes some of its properties.

Materials and Methods

L-γ-glutamyl *p*-nitroanilide (anhydrous), L-glycylglycine, bovine serum albumin, Tris, Sephadex G-200 and G-100, and dipeptides were all obtained from Sigma, U.S.A.; DEAE-cellulose DE-52 was obtained from Whatman Limited, U.K. Protamine sulphate and all the amino acids used were from Nutritional Biochemical Corp., U.S.A. and Dextran Blue 2000 from Pharmacia, Sweden. Other chemicals used were of analytical reagent grade.

Enzyme assay. The enzyme was assayed according to the method of Szasz [12] with the following modification; because of the poor solubility of L-γ-glutamyl *p*-nitroanilide, the buffered substrate solution was prepared by dissolving 12.0 mg of substrate in 0.25 ml of 0.5 M HCl which was made up to 10 ml with 0.23 M Tris · HCl buffer, pH 8.5, and used within 3 h. The reaction mixture consisted of 0.2 M buffer, 4 mM substrate and 40 mM glycylglycine as acceptor in a final volume of 1.6 ml. The reaction was started by the addition of the enzyme. The release of *p*-nitroaniline was measured at 405 nm over 5 min using a Vitatron UC 200S colorimeter and a Vitatron UR 401 recorder, set to 0.25 *A* for full scale deflection

Enzyme unit. 1 unit/l is the amount of enzyme that converts 1 μmol of substrate per min at 37° C. The molar absorption coefficient of *p*-nitroaniline is $9.9 \cdot 10^3$ with 10 mm light path, using the equipment specified.

The specific activity is expressed in units/ml per mg of protein. Protein was determined by the method of Lowry et al. [13] using crystalline bovine serum albumin as the standard.

Purification of γ-glutamyl transpeptidase. Bile samples were collected from patients following cholecystectomy by 'T' tube drainage of the common bile duct. All procedures were carried out at room temperature.

Step 1. Protamine sulphate treatment: Protamine sulphate was used to remove pigment and lipid according to the method of Price et al. [14]. The sample was treated with an equal volume of protamine sulphate solution (400 mg/100 ml) and left overnight at 4° C. After centrifuging at 7000 rev./min for 30 min, the supernatant containing the enzyme activity was used for further steps of purification (Fig. 1).

Step 2. DEAE-cellulose DE-52 column chromatography: The sample from the previous step (370 ml; 418 mg protein) was adjusted to 0.02 M Tris · HCl, pH 8.3, by adding the appropriate volume of 1.0 M Tris · HCl buffer and then loaded on a DEAE-cellulose DE-52 column (2.4 cm × 24.0 cm, bed volume 105 ml) equilibrated with 0.02 M Tris · HCl, pH 8.3. After collecting the breakthrough, the column was washed with four bed volumes of the equilibrating buffer. The protein was eluted stepwise with equilibrating buffer containing increasing concentrations of NaCl (0.05—0.2 M), 15-ml fractions were collected. The enzyme was eluted at the end of the first bed volume and in the second bed volume of 0.1 M NaCl. The active fractions were pooled and concentrated for step 3.

Step 3. Gel filtration on Sephadex G-200: The concentrated sample (3.4 ml)

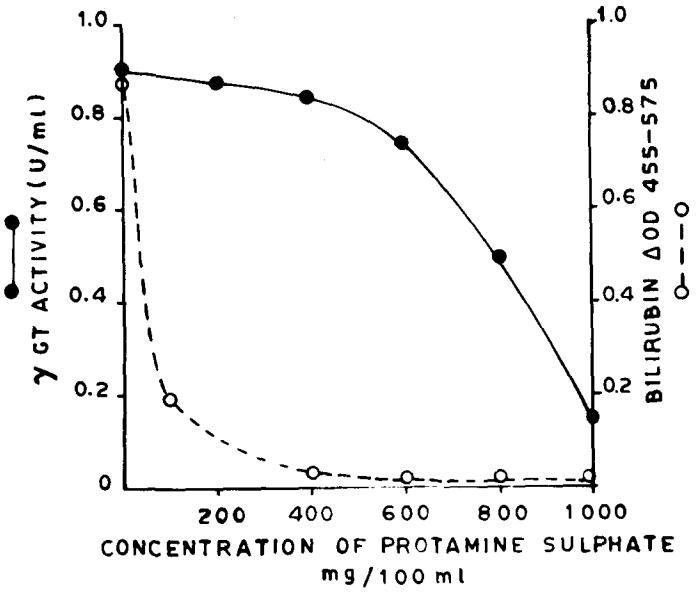

Fig. 1. γ-Glutamyl transpeptidase activity and bile pigment concentration after treatment of hepatic bile with protamine sulphate.

from the DE-52 column was applied to a Sephadex G-200 column (2.5 cm × 60 cm; bed volume 290 ml) previously equilibrated with 0.02 M Tris · HCl, pH 8.3. 3-ml fractions were collected at a flow rate of 20 ml/h. The enzyme appeared in the inner volume. The active fractions were pooled and used to study the properties of the enzyme.

Table I gives a summary of the steps in the purification procedure. The enzyme was purified about 150-fold with 37% overall recovery.

Results

Properties of purified γ-glutamyl transpeptidase. Within the limits of the assay procedure, the enzyme activity was linear with respect to the enzyme concentration.

TABLE I

PURIFICATION OF γ-GLUTAMYL TRANSPEPTIDASE FROM HUMAN BILE

Fraction	Total protein (mg)	Enzyme activity		Recovery (%)
		Total units	Specific activity	
1. Crude bile	992	528	0.53	100
2. Protamine sulphate treatment	418	492	1.17	93
3. DEAE-cellulose DE-52	20.37	293	14.4	55
4. Sephadex G-200	2.44	198	81.0	37.5

TABLE II

THE EFFECT OF AMINO ACIDS AS γ-GLUTAMYL ACCEPTORS

Acceptor	Enzyme activity	
	Activity relative to control (%)	Activity relative to glutamine (%)
None (control)	(100)	37
L-Alanine	120	45
L-Arginine	117	44
L-Asparagine	100	37
L-Aspartate	100	37
L-Cysteine	241	90
L-Glutamate	100	37
L-Glutamine	268	(100)
Glycine	87	32
L-Histidine	100	37
L-Hydroxyproline	115	43
L-Isoleucine	106	40
L-Leucine	112	42
L-Lysine	117	44
L-Methionine	161	60
L-Phenylalanine	93	35
L-Proline	115	43
L-Serine	126	47
L-Threonine	117	44
L-Tryptophan	112	42
L-Tyrosine	106	40
L-Valine	100	37

pH optimum. Tris · HCl buffer in the pH range of 7.0—9.0 was used in the assay of the enzyme. The optimum pH was found to be 8.1.

K_m. Using the synthetic L-γ-glutamyl *p*-nitroanilide the K_m value was found to be 1.52 mM.

Effect of cations. There was no activation or inhibition by the monovalent ions Na^+, Li^+ or K^+ at concentrations up to 300 mM. Similarly the divalent

TABLE III

THE EFFECT OF DIPEPTIDES AS γ-GLUTAMYL ACCEPTORS

Acceptor	Enzyme activity	
	Activity relative to control (%)	Activity relative to Gly-Gly (%)
None (control)	(100)	19
Glycine	87	16
Gly-Gly	535	(100)
Gly-Leu	106	20
Gly-His	120	23
Gly-Pro	153	29
L-Leucine	112	21
Leu-Ala	254	47

cations Ca^{2+}, Zn^{2+}, Mg^{2+} and Mn^{2+} at concentrations up to 2.5 mM did not have any effect on the activity of the enzyme. At a concentration of 2.5 mM, Co^{2+} caused a 35% inhibition of the enzyme activity.

Effect of L-amine acids and dipeptides as acceptors. The ability of various amino acids to act as acceptors of the γ-glutamyl moiety released from the substrate was tested by replacing the glycylglycine in the reaction mixture with different amino acids at a concentration of 20 mM. The results are shown in Table II expressed as a percentage of the activity with no added acceptor and as a percentage of the activity, with 20 mM glutamine as acceptor. The effect of dipeptides as acceptors is shown in Table III. Glycylglycine at 20 and 40 mM concentrations markedly increased the enzyme activity. Glycyl-L-proline and L-leucyl-L-alanine were also active as acceptors.

Discussion

This paper describes the partial purification of γ-glutamyl transpeptidase from human hepatic bile and reports some of its properties. Unlike serum which normally contains only small amounts of the enzyme (0.02 units/ml), hepatic bile contains the enzyme in high concentration (0.5—3.0 units/ml). Hepatic bile is therefore a useful source of the enzyme and the procedures described in this paper have enabled us to achieve an 150-fold increase in specific activity.

The enzyme from native bile appears in the void volume of Sephadex G-200, but after protamine sulphate treatment it elutes in the inner volumes of both Sephadex G-200 and G-100, although the mobility on polyacrylamide gel is unaltered. In native bile and after partial purification the enzyme migrates as a single band in the α_2-globulin region. The behaviour of the enzyme in native bile on Sephadex G-200 may therefore be due to an association with other macromolecular constituents, which is dissociated either by protamine sulphate or by electrophoresis. The pooled active fractions after Sephadex G-200 filtration, were used to study the properties of the enzyme as described earlier in the paper.

As shown in Table II, in the absence of an added acceptor, the rate of release of *p*-nitroaniline from the substrate was 37% of the activity observed with 20 mM glutamine as acceptor. The best acceptors were L-glutamine, L-cysteine and L-methionine. Similar results have been observed for the rat kidney enzyme [15]. For the human kidney enzyme, L-glutamine was reported to be the best of the amino acid acceptors, but L-cysteine and L-methionine were poor acceptors [10].

Unlike the human kidney enzyme [10], the partially purified bile γ-glutamyl transpeptidase is soluble in the absence of detergents. There is no evidence of aggregation of the partially purified enzyme from either the gel filtration studies or its behaviour on polyacrylamide gel electrophoresis. Aggregation of the purified enzyme from other sources has been reported [10,16]. Other apparent differences between the human kidney and bile enzymes are the K_m values and amino acid acceptor specificities.

In view of the problems associated with solubilisation of the tissue enzyme it is suggested that bile may prove to be a useful source for further studies of the kinetic properties and physiological role of human γ-glutamyl transpeptidase.

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