Electrophoretic analysis of soluble chromatin DNA fractions showed that DNA of soluble intranuclear DNP, treated with distamycin, was hydrolyzed by Ca, Mg-dependent endonuclease to a much lesser degree than DNA from the control nuclei (Fig. 2). This is clear from the proportion of fractions possessing the highest electrophoretic mobility: It is smaller in the first case.

It can thus be concluded from the results of this investigation that distamycin is the most universal inhibitor of hydrolysis of both free and intranuclear DNA by DNase I and by intranuclear endonucleases. Its advantage over intercalating agents is particularly clearly marked in the case of inhibition of the action of intranuclear DNase I.

LITERATURE CITED

1. G. Ts. Georgiev, Biokhimiya, 24, 472 (1969).
2. I. Yu. Karyagina, A. A. Myul'berg, L. I. Tishchenko, et al., Biokhimiya, 41, 1136 (1976).
3. A. M. Kolchinskii, A. D. Mirzabekov, A. S. Zasedatelev, et al., Mol. Biol., 9, No. 1, 19 (1975).
4. A. S. Spirin, Biokhimiya, 23, 656 (1958).
5. R. Axel, Biochemistry (Washington), 74, 2921 (1975).
6. D. R. Hawish and L. A. Burgoyne, Biochem. Biophys. Res. Commun., 52, 475 (1973).
7. M. Kunitz, J. Gen. Physiol., 33, 349 (1950).
8. H. Weintraub and M. Groudin, Science, 193, 848 (1976).

PARTIAL PURIFICATION AND SOME PROPERTIES OF γ-GLUTAMYL TRANSFERASE FROM RAT LIVER AND HEPATOMA

V. A. Loginov, N. N. Chernov, and T. T. Berezov

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γ-Glutamyl transferase (GGT) is a membrane-bound enzyme which catalyzes the transfer of the γ-glutamyl radical from glutathione and other γ-glutamyl compounds to amino acids and oligopeptides and also, to a much lesser degree, catalyzes the hydrolysis of these compounds. High GGT activity is found in the mammalian kidney where, it is considered, the enzyme is responsible for transport of certain amino acids through the cell membrane [4, 5]. The functional role of GGT in the liver has not been explained, but there are grounds for considering that it is evidently considerably wider in the liver than in the kidneys [1]. A sharp increase in specific activity of GGT has been demonstrated in hepatomas by comparison with the normal liver [1, 2, 6, 7], but no comparative studies of the enzyme from normal liver and hepatoma for the same species of mammals have hitherto been undertaken.

The object of this investigation was to study partially purified preparations of GGT from rat liver and from transplantable G-27 rat hepatoma.

EXPERIMENTAL METHODS

Preparations of washed membranes were obtained by the method described previously [1]. GGT activity was determined in the reaction between 5 mM L-γ-glutamyl-p-nitroanilide (from Calbiochem, U.S.A.) and 50 mM glycyl-glycine (from Reanal, Hungary) at pH 8.1; the reaction velocity was determined by measuring the increase in optical density of liberated p-nitroaniline at 405 nm.

Department of Biochemistry, Patrice Lumumba Peoples' Friendship University, Moscow.

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TABLE 1. Substrate Specificity of GGT from Rat Hepatoma and Liver

Acceptor	Concentration, mM	Activity, %		Acceptor	Concentration, mM	Activity, %	
		hepatoma	liver			hepatoma	liver
—	—	100	100	L-lysine	40	130	125
Glycine	50	122	143	L-arginine	50	150	142
L-serine	50	147	167	L-histidine	25	89	91
L-homocysteine	82	211	241	Glycyl-glycine	50	703	736
L-alanine	50	187	171	Glycyl-glycyl-glycine	50	175	156
L-valine	19	131	117				
L-leucine	25	125	121	Glycyl-D,L-norleucine	19	159	146
L-isoleucine	25	125	100				
D,L-phenylalanine	19	127	100				
L-methionine	50	213	200	L-histidyl-L-leucine	7	118	109
L-asparagine	25	140	129				
L-glutamine	50	197	188	Methylamine	50	103	102
L-aspartic acid	11	87	98	Spermine	50	115	108
				Spermidine	50	115	103
L-glutamic acid	2	111	105	Putrescine	50	123	126
				Cadaverine	50	110	118

EXPERIMENTAL RESULTS

Solubilization of GGT was carried out in aqueous solution at 37°C in the presence of papain (1 mg/15 mg membrane protein), 0.005 M EDTA, and 0.005 M EGTA. After incubation for 1 h, protamine sulfate was added (1 mg to 20 mg membrane protein) to precipitate the nucleic acids. Under these conditions almost 100% of the enzyme activity was solubilized from the hepatoma membrane, but only 20% from the liver membranes; in the latter case, 0.3% sodium deoxycholate had to be added to obtain complete solubilization. Membranes not containing GGT activity were removed by ultracentrifugation at 100,000g for 1.5 h.

For further purification of the solubilized GGT preparations gel-filtration on Ultragel AcA-44 (from LKB, Sweden) in Tris-HCl, pH 8.6, and ion-exchange chromatography on DEAE-Molselect A-50 (from Reanal, Hungary) in the same buffer, with subsequent gradient elution with KCl, were used. All types of chromatography were carried out on glass columns (from East Germany) at 4°C, using automatic equipment from Gilson (France). The enzyme preparations were concentrated by means of Dyflo PM-10 Ultramembranes (from Amicon, Holland). To purify GGT from the hepatoma, fractionation with ammonium sulfate (50-75% saturation) was used. However, this procedure was accompanied by considerable loss of total enzyme activity, and it was therefore not used in the purification of GGT from rat liver, in which the initial level of activity was much lower. At all stages of purification tests were carried out to determine whether nonspecific hydrolase activity could be exhibited, by omitting glycyl-glycine from the incubation sample. GGT preparations from liver and hepatoma were purified by 160 and 220 times respectively.

Both GGT preparations from liver and hepatoma had a pH-optimum at 8.0-8.2 in Tris-HCl and K-phosphate buffers. At pH 6.9, activity of GGT was reduced by half. The pH-optimum of the autotransferase reaction (transfer of a γ-glutamyl residue from one molecule of L-γ-glutamyl-p-nitroanilide to another) lay between 9.3 and 9.5. The character of the pH-optimum curve for the two GGT preparations was independent of the nature of the buffer used.

Apparent values of the Michaelis constant for L-γ-glutamyl-p-nitroanilide (LGGN) in the liver and hepatoma preparations were 1.9 and 2.1 mM respectively, and for glycyl-glycine 10.5 and 5.6 mM. These values are close to those given in the literature for GGT from kidneys and other sources [3, 5, 7] when GGT activity was determined by the same method.

After disc electrophoresis in 5% polyacrylamide gel in Tris-potassium-phosphate buffer, pH 7.8, GGT activity was detected in the reaction with LGGN or with L-γ-glutamylnaphthylamide (0.5 mM). In both cases one diffuse band of enzyme activity was found; the mobility of GGT from the liver was considerably higher than the mobility of the enzyme from the hepatoma.

Isoelectric focusing, on the instrument from LKB (Sweden), within the pH range 3.0-10.0 on a column with a capacity of 110 ml, demonstrated the heterogeneity of the GGT preparations. In the case of enzyme from hepatoma two main peaks of activity were detected with isoelectric points (IEP) of 6.5 and 7.0, and two minor peaks with an IEP of 5.7 and 6.0, whereas for GGT from liver three peaks were obtained with IEP of 3.9, 4.2, and 4.4 respectively. Heterogeneity of GGT during isoelectric focusing has been demonstrated for the enzyme from rat kidney [5], and the authors cited explained this phenomenon by the presence of different

quantities of sialic acids bound with the enzyme. A similar situation evidently is observed also in the case of GGT from rat liver and hepatoma: Enzyme from the liver contained more sialic acids, and this may perhaps have accounted for the difficulty of its solubilization from the membranes.

The reaction with LGGN (as donor of the γ-glutamyl radical) was used to test 25 different compounds capable of serving as acceptors of this radical. The results, expressed in percentages of the autotransferase reaction, in which LGGN itself was used as the direct acceptor (5 mM), are given in Table 1.

The best substrate, just as for GGT from rat kidney [5], was glycyl-glycine; the same tendency as with the enzyme from different sources was observed for preferential utilization of neutral amino acids as acceptors. Polyamines were the first to be tested in this system, although of these only putrescine exhibits measurable acceptor activity. The possibility that GGT may participate in the formation of γ-glutamyl derivatives of the polyamines has been demonstrated in the brain [8].

Similarity of behavior of GGT preparations from rat liver and hepatoma toward acceptors will be noted. The activity of the two GGT preparations also was determined at pH 7.0 with glycyl-glycine, L-serine, and L-methionine: The percentage ratio between their activities remained the same under these circumstances as at the pH-optimum of 8.1.

Preparations of folic acid (USSR) and methotrexate (from Lederle, U.S.A.) inhibited GGT activity from rat liver and hepatoma by 50% both at pH 8.1 and at pH 7.0 in concentrations of 3.5 and 0.9 mM respectively. For comparison it may be noted that glycine has the same inhibitory action in a concentration of 25 mM; L-methionine in a concentration of 45 mM, whereas glycyl-glycyl-glycine (13 mM) had practically no effect on GGT activity from rat hepatoma and liver.

The effects of folic acid and its analogs on GGT calls for further investigation. The study of this problem could be extremely useful in elucidating the functional role of the enzyme, more especially because administration of methotrexate to rats (2.7 mg/kg body weight intramuscularly over a period of 14 days, followed by 6.0 mg/kg intraperitoneally during the next 14 days) leads to an increase of 50-100% in the specific activity of the enzyme in the liver.

LITERATURE CITED

1. V. A. Loginov, in: Enzymology of Tumors [in Russian], Moscow (1979), pp. 73-82.
2. S. Fiala, A. E. Fiala, and B. Dixon, J. Natl. Cancer Inst., 48, 1393 (1972).
3. T. Hada, K. Higashino, H. Yamamoto, et al., Clin. Chim. Acta, 85, 267 (1978).
4. A. Meister, Science, 180, 33 (1973).
5. A. Meister and S. S. Tate, Annu. Rev. Biochem., 45, 559 (1976).
6. A. Szewczuk, H. Milnerowicz, and K. A. Sobiech, Neoplasma, 25, 297 (1978).
7. N. Taniguchi, J. Biochem. (Tokyo), 75, 473 (1974).
8. M. Tsuji, Y. Matsuoka, and T. Nakajima, J. Neurochem., 29, 633 (1977).