FRACTIONATION OF RAT LIVER TYROSINE AMINOTRANSFERASE DURING THE COURSE OF PURIFICATION. FURTHER EVIDENCE FOR MULTIPLE FORMS OF THE ENZYME

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Tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) (TAT) of rat liver has been purified by a number of workers and evidence for homogeneity has been obtained in the ultracentrifuge and by immunological criteria using Ochterlony double diffusion [1–3]. However, the electrophoresis of the purified enzyme in polyacrylamide gel has often revealed the presence of two or more bands containing enzyme activity [2–5]. It has been suggested that heterogeneity detected by this method is due either to aggregation or to degradation of the protein under the conditions of electrophoresis. Miller and Litwack [6] have demonstrated that during the course of enzyme purification following with cortisol, the relative initial velocity of the reaction measured with monoiodotyrosine and tyrosine as substrates, shows a steady decrease. They have suggested that this phenomenon is due to formation of physical conformers of the enzyme during induction.

Holt and Oliver [7] have suggested that TAT may consist of three active forms *in vivo* and have subsequently shown that the gel electrophoretic pattern of enzyme activity in crude liver extracts responds to different hormonal stimuli in the intact animal [8]. On the basis of these findings, the stepwise purification of the enzyme has been monitored with polyacrylamide gel electrophoresis in order

Table 1
Purification of TAT

Fraction and stage	Enzyme activity (Total units)	Total protein (mg)	Specific activity	% Recovery
(2) Crude extract	23200	16600	1.4	–
(3) After 1st ammonium sulphate	23600	10300	2.3	100
(4) After heat treatment	20800	2160	9.6	93
(5) After 2nd ammonium sulphate	13700	1000	13.7	59
(6) After dialysis	9100	955	9.5	39
(7) DEAE eluate (Lyophilized)	5800	26	225.0	25

The unit of enzyme activity is 1 μmole *p*-hydroxyphenylpyruvate produced per hour. Specific activity represents units/mg protein.

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to assess the possibility of artefacts arising during manipulation of tissue extracts.

1. Enzyme purification

The method used for purification [3] consisted of: (1) Pre-induction of tyrosine aminotransferase with hydrocortisone in the adult rat; (2) homogenization of the liver in KCl-EDTA; (3) fractionation with ammonium sulphate; (4) heat treatment: (5) fractionation with ammonium sulphate; (6) dialysis; (7) DEAE-cellulose chromatography and lyophylization.

2. Polyacrylamide gel electrophoresis

Samples taken at stages 2–6 above were electrophoresed in polyacrylamide gel and the gel sectioned and assayed for enzyme activity as previously described [8].

Table 1 shows the recoveries and specific activities obtained at each stage of the purification. The final product had a specific activity of 225, which represented a 450-fold increase over the specific activity of the basal uninduced enzyme.

In fig. 1 are shown the electrophoretic patterns of the enzyme demonstrable at different stages of the purification. Samples from each stage were subjected to polyacrylamide gel electrophoresis a number of times to gain a reproducible picture of the distribution of enzyme activity in the gel.

The pattern of enzyme activity in fig. 1(a) is from stage 2 of the purification. It can be seen that three peaks of enzyme activity are present. On the basis of electrophoretic mobility, these apparently correspond to forms A, B and C described previously by Holt and Oliver [8]. These authors have shown that approximately equal amounts of the three forms are present in normal adult rat liver and that the synthesis of forms B and C in perinatal rat liver is stimulated by the administration of hydrocortisone. The data presented in fig. 1(a) suggest that following hydrocortisone induction in the adult, higher levels of forms B and C are present than of form A, with C predominating slightly. Samples taken at stages 3 and 4 of the preparation, where a small overall purification with little

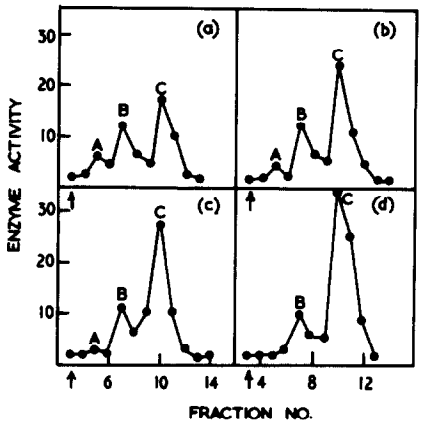

Fig. 1. Polyacrylamide gel electrophoresis of tyrosine aminotransferase. The patterns of enzyme activity obtained at stages 2,5,6 and 7 of the purification are shown in figs. a, b, c and d respectively. Enzyme activity is expressed as μg *p*-hydroxyphenylpyruvate produced per hour.

loss of total activity was obtained, produced patterns of activity qualitatively similar to that seen in fig. 1(a).

The second ammonium sulphate fractionation (step 5), during which 34% of total activity was lost, produced enzyme with a pattern of activity seen in fig. 1(b). The relative ratios of the three forms have altered showing enrichement of form C and diminution of the relative content of A and B.

The final two stages of the purification yielded the patterns of enzyme activity shown in fig. 1(c) and 1(d). During dialysis, a loss of specific activity occurred (table 1) with little change in the qualitative pattern previously shown. Electrophoresis of the final product obtained after DEAE cellulose column chromatography and lyophylization produced the distribution of TAT activity in the gel seen fig. 1(d). It can be seen that form A, which had previously been undergoing impoverishment in the preceding steps of the purification, could no longer be detected. A further increase in the ratio of form C to that of form B is also apparent.

The alteration in the electrophoretic profile of liver TAT during the course of purification could arise by fractionation of the 3 initial components or by preferential denaturation of forms A and B. Since the fractionation procedure progressively enriches the preparation in form C it is highly likely that further purification would result in electro-

phoretic homogeneity of this single form. Valeroite et al. [5] have recently reported a more extensive purification that yields an enzyme with higher specific activity than any previously reported. The enzyme was 95% homogeneous in the ultracentrifuge but still contained traces of enzymic activity with lower electrophoretic mobility (in gel) than the major band. These contaminants were attributed by the authors to polymerized forms and although zone centrifugation appeared to confirm this suggestion, the major contaminants apparent in the analytical ultracentrifuge were of lower sedimentation coefficients than the major component.

The data reported in this paper, showing the progressive elimentation of electrophoretic components found initially in crude liver extracts suggests that the heterogeneity of soluble TAT in rat liver is not an artefact of gel electrophoresis, of polymerization [5,6] or of degradation since artefacts are more likely to arise during purification. Preincubation of extracts with hormones *in vitro* does not alter the electrophoretic profile of enzyme activity and it has already been shown [8] by analysis of crude liver extracts from neonatal animals that specific induction of the different forms can be achieved by treatment of the intact animal with different hormones.

The data further suggest that the immunological characterization of rat liver TAT requires further close investigation since purification appears to select largely one form and it is likely that antibody to this form will cross-react with other forms of the enzyme.

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