A POSSIBLE LINK BETWEEN THE SUBFORMS OF TYROSINE AMINOTRANSFERASE AND ITS INDUCIBILITY BY DIBUTYRYL CYCLIC AMP

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1. Introduction

Reports that rat L-tyrosine:2-oxoglutarate aminotransferase (tyrosine aminotransferase; EC 2.6.1.5) occurs in more than one form have led to much speculation of the nature of the enzyme. The subforms are not yet recognised as isoenzymes because they appear to possess identical physico-chemical properties including immunological identity [1]. There is disagreement as to whether these subforms have an in vivo significance [1–10] or arise as in vitro artifacts [11–13].

Rats are omnivorous animals whilst rabbits are herbivores. Tyrosine aminotransferase is a glucogenic enzyme and thus rabbit tyrosine aminotransferase might behave differently from its counterpart in the rat. Here, rat and rabbit tyrosine amino transferase were compared at various stages of purification. In addition, the induction of the enzyme has been investigated in primary rat and rabbit hepatocytes.

The results support the contention that the control of rat tyrosine aminotransferase is different from that of rabbit tyrosine aminotransferase and suggest that the subform phenomenon of rat tyrosine aminotransferase is linked to either the induction or degradation cycle of the enzyme.

2. Experimental

2.1. *Enzyme purification*

Rat and rabbit tyrosine aminotransferase were purified by an unpublished procedure [14]. The enzyme from both sources had similar physico-chemical properties. A minor difference observed was that the rabbit enzyme was salted out with ammonium sulphate between 30–60% saturation whilst the rat enzyme was precipitated from 35–70% ammonium sulphate. The rat and rabbit tyrosine aminotransferase, however, behaved rather differently when subjected to hydroxylapatite chromatography (spheroidal hydroxylapatite supplied by BDH) irrespective of the stage in the purification at which this step was performed. It was carried out by dialysing the various preparations against 5 mM potassium phosphate buffer (pH 6.8) containing 1 mM mercaptoethanol and 0.2 mM pyridoxal 5′-phosphate. The dialysate was centrifuged at 105 000 × *g* for 1 h. The supernatant was applied to an hydroxylapatite column (2.6 × 20 cm) pre-equilibrated with 5 mM potassium phosphate (pH 6.8) containing 1 mM mercaptoethanol. The column was washed with starting buffer at a flow rate of 30 ml/min. When no more protein appeared in the eluate chromatography was done using a linear gradient of increasing phosphate concentration generated by mixing 500 ml starting buffer with 500 ml of this buffer containing 500 mM potassium phosphate (pH 6.8). The eluate was collected in 6 ml fractions by means of a Golden Retriever Isco MSE fraction collector. The protein in the eluate was detected by a UA-5 absorbance monitor reading at 280 nm in conjunction with a multiplexer expander.

2.2. *Disc gel electrophoresis*

Non-denaturing gels were prepared in glass tubes (0.5 × 14 cm) by a modification of the method in [15]. The running gel (12 cm long) was 7% acrylamide and the stacking gel consisted of 4% acrylamide. The electrophoretic buffers were as in [16], except that dithiothreitol was replaced by 2-mercaptoethanol and 2-oxoglutarate was omitted from all the buffers. The latter was omitted because its presence

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in the cathode buffer greatly reduced the rate of electrophoretic separation and also complicated the interpretation of the staining patterns.

Electrophoresis was done for 3–4 h at 8°C at a constant 2.5 mA/tube. At the end of the electrophoresis the gels were removed from the siliconized tubes by rimming and rinsed with distilled water. The gels were stained for protein by Coomassie brilliant blue or analysed for tyrosine aminotransferase activity. The latter process entailed incubating the gels at 37°C for 30 min in a tyrosine aminotransferase-locating medium [16]. The gels were subsequently fixed in 7.5% acetic acid and scanned at 510 nm in a Gilford 240 Spectrophotometer fitted with a model 2410S linear transport.

2.3. *Preparation and culture of hepatocytes*

Rat hepatocytes were prepared as in [17]. Rabbit hepatocytes were also obtained by complete liver perfusion since the metabolic capacity of parenchymal cells is reported to be partially dependent on their position within the liver [18]. In addition, the level of tyrosine aminotransferase activity produced by induction with hydrocortisone appears to be dependent on the position of the cells relative to the portal vein [19]. Rabbits were killed by a blow on the head and then bled. The cannulation procedure adopted was as that used for the rat [17]. Heparin (1500 units) dissolved in the perfusion mixture were passed into the liver via the cannula. In early experiments the bile duct was cannulated and the gall bladder isolated. However, this did not appear to have any effect on the cell preparation and it was decided that it was more prudent to leave these structures untouched. The perfusate was recycled and the flow rate was adjusted to 100 ml/min. This rate caused little swelling and gave the optimum number of intact cells. The liver was removed from the apparatus after collagenase digestion, and gently placed on a piece of clean polythene. The gall bladder and its underlying hepatic tissue, together with any extrahepatic material were now carefully excised. Sections of each liver lobe were then taken and the hepatocytes treated as previously described. Hepatocytes were cultured and samples taken and analysed as in [17].

3. Results

Hydroxylapatite column chromatography resolved the rat tyrosine aminotransferase preparation into 3 peaks (fig.1). However, identical treatment of the rabbit enzyme gave a single peak of tyrosine aminotransferase activity (fig.2). The traces shown were of preparations which had been partially purified by differential centrifugation, ammonium sulphate precipitation, heat treatment and DEAE-cellulose chromatography [14]; however, identical results were obtained when crude cytosol preparations were subjected to hydroxylapatite fractionation. The peaks of activity were recognised by:

(i) Dependence on the substrate tyrosine;
(ii) Insensitivity to inhibition by aspartate [20]:
(iii) The inability of oxaloacetate to replace 2-oxoglutarate in the assay system [20];
(iv) The dependence of the reaction of pyridoxal 5′-phosphate.

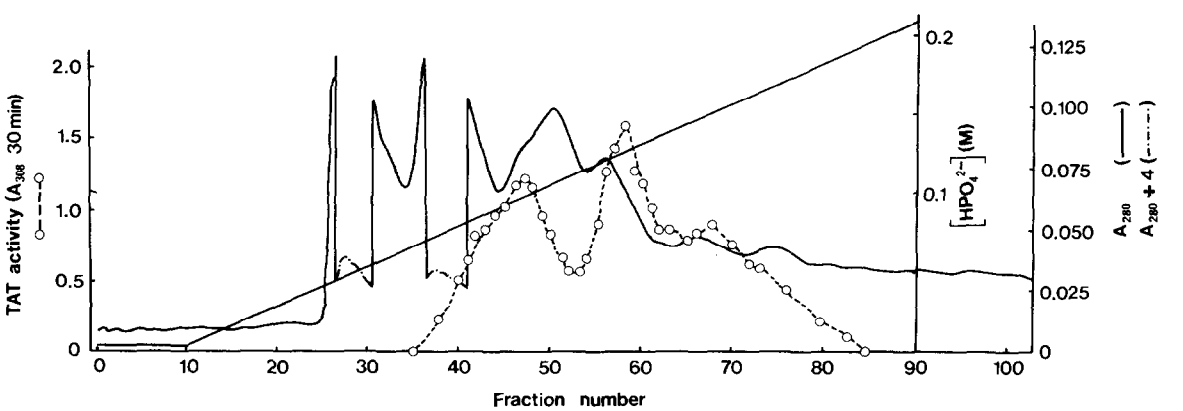

Fig.1. Separation of cytosolic rat tyrosine aminotransferase into subforms by hydroxylapatite chromatography.

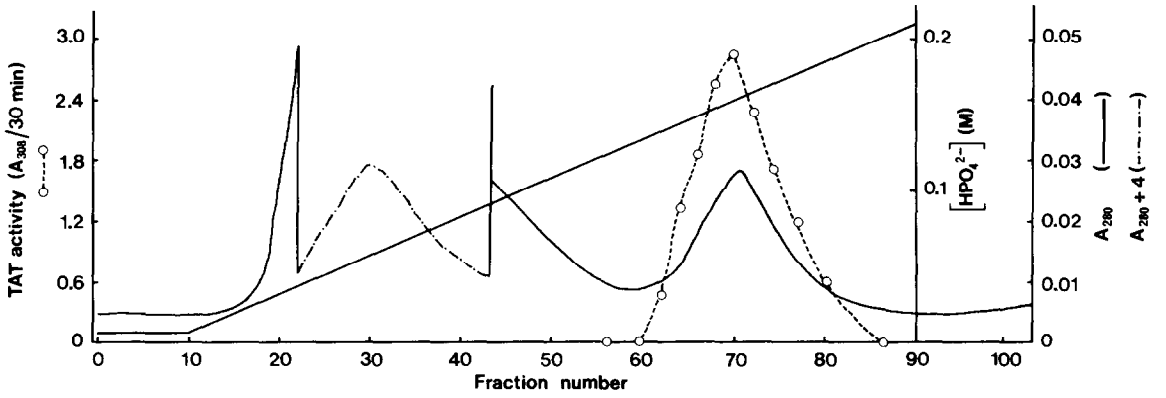

Fig.2. Hydroxylapatite chromatography of rabbit cytosolic tyrosine aminotransferase.

Preparations analysed following DEAE-cellulose chromatography produced 2 distinct forms of rat tyrosine aminotransferase (fig.3). By this procedure the rabbit preparation initially appeared to give as many as 5 bands of enzymic activity. However, detailed investigation showed clearly that only one band (band 2, fig.4), was due to cytosolic rabbit tyrosine aminotransferase [14].

The effect of dibutyryl adenosine 3′:5′-cyclic monophosphate (db-cAMP 5×10^{-6} M) on the tyrosine aminotransferase activity in rat and rabbit hepatocytes is shown in fig.5. No combination of insulin, glucagon, db-cAMP nor dexamethasone produced an increase of rabbit tyrosine aminotransferase over the culture period.

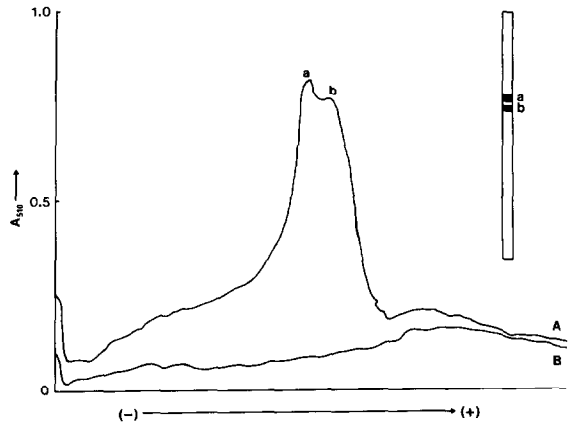

Fig.3. Disc gel analysis of rat tyrosine aminotransferase. See text for the electrophoretic and enzyme location procedures. Gels were loaded with 93 μg protein: (a,b) bands of tyrosine aminotransferase activity; (A) scan of gel incubated in the complete locating mixture; (B) scan of gel incubated in the locating mixture minus monoiodotyrosine.

4. Discussion

Hydroxylapatite chromatography, using a linear phosphate gradient, separated rat tyrosine aminotransferase into 3 distinct peaks (fig.1) while rabbit tyrosine aminotransferase remained as a single symmetrical peak (fig.2). The presence of tyrosine aminotransferase subforms in the rat and their absence in the rabbit was confirmed by disc-gel electrophoresis (fig.3,4). The rabbit results emphasize the caution which must be exercised before a coloured band can be equated with tyrosine aminotransferase activity [14].

Induction studies on hepatocytes from rat and rab-

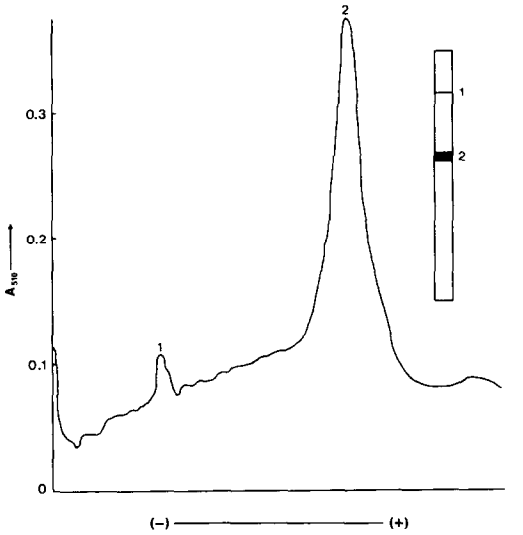

Fig.4. Disc gel analysis of rabbit tyrosine aminotransferase. See text for the electrophoretic and enzyme location procedures. Gels were loaded with 25 μg protein: (1) apparent band of tyrosine aminotransferase activity; (2) band of tyrosine aminotransferase activity.

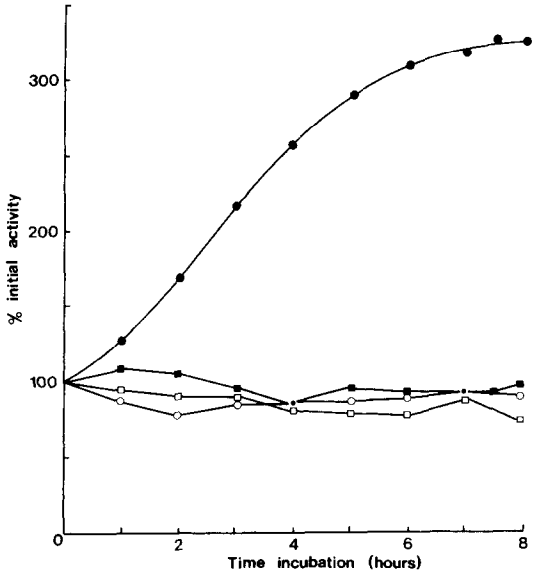

Fig.5. The effect of db-cAMP on the activity of tyrosine aminotransferase in primary rat and rabbit hepatocytes. Basic incubation medium supplemented with Hepes (25 mM), $CaCl_2$ (1.8 mM) and db-cAMP (5×10^{-6} M). The initial tyrosine aminotransferase activity in rat hepatocytes was 12.9 ± 1.74 mU/mg protein (n = 16 separate cell preps) and that in the rabbit was 14.3 ± 1.54 mU/mg protein (n = 4 separate cell preps): (●) rat tyrosine aminotransferase; (■) rat lactate dehydrogenase; (○) rabbit tyrosine aminotransferase; (□) rabbit lactate dehydrogenase.

bit showed that, although the cells of both animals could be isolated and cultured by similar techniques, only the rat tyrosine aminotransferase was inducible by db-cAMP. The rat hepatocytes were uninduced prior to culture [17] and had a basal level of 12.9 ± 1.74 mU/mg protein (n = 16 separate cell preps). The rabbit hepatocytes had a basal tyrosine aminotransferase level of 14.3 ± 1.54 mU/mg protein (n = 4 separate cell preps) which agrees favourably with the value of 15.94 ± 1.40 (n = 11 separate preps) obtained for rabbit homogenates. The observed low tyrosine aminotransferase level is evidence that the rabbit hepatocytes were not primed prior to the addition of db-cAMP. In vivo, rabbit tyrosine aminotransferase was not induced by dexamethasone [21]. Here, neither insulin (10 mU/ml), glucagon (10^{-7} M), db-cAMP (5×10^{-6} M), dexamethasone (10^{-6} M) nor any combination of two or more were found to increase tyrosine aminotransferase levels in primary rabbit hepatocytes.

It was observed [22] that frog tyrosine aminotransferase cannot be induced by glucocorticoids but that the enzyme level in this species is raised by db-cAMP. Frog tyrosine aminotransferase occurs in multiple forms [23]. Based on [22,23] and these findings there appears to be a link between the ability of tyrosine aminotransferase to be induced by db-cAMP and the occurrence of subforms of this enzyme.

Acknowledgement

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