A SIMPLE AND RAPID ASSAY FOR TYROSINE AMINOTRANSFERASE

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1. Introduction

The activity of hepatic tyrosine aminotransferase (TAT; L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) is increased by several hormonal [1–7] and nutritional [6–9] agents. This fact, together with the short half-life in vivo, has contributed to TAT being chosen by many workers as a model for studies of hepatic enzyme turnover, both in vivo and in vitro. TAT activity, particularly in 'fed control' conditions, is quite low, so that appreciable amounts of sample are required for spectrophotometric procedures [10–14]. The elaboration of techniques for handling isolated hepatocytes and liver-derived tumour cell lines dictates a parallel development of more sensitive assay systems.

Spectrophotometric assays are prone to high blanks when used with crude extracts. Greater sensitivity may be achieved with radioisotopic methods, although these require physical separation of substrate and product; ion-exchange chromatography [15–17] and extraction in organic solvents [18,19] have been used effectively, although these are cumbersome procedures when numbers of samples are involved.

In this paper we present a method using L-[*side chain*-2,3-^{3}H]tyrosine, which is simple, rapid, convenient, cheap and applicable to measurements in large numbers of crude extracts.

2. Materials and methods

2.1. *Materials*

L-Tyrosine, diethyldithiocarbamate and dithiothreitol were from the Sigma (London) Chemical Co., Kingston, Surrey. Pyridoxal phosphate and 2-oxoglutaric acid were from the Boehringer Corp (London), Lewes, Sussex. Bovine serum albumin was obtained from Armour Pharmaceutical Co., Eastborne, Sussex. PCS scintillation cocktail and Norit-GSX were from Hopkin and Williams, Chadwell Heath, Essex and Norit Clydesdale Co., Glasgow, respectively. L-[*side chain* 2,3-^{3}H]Tyrosine was obtained from the Radiochemical Centre, Amersham, Bucks. Ketoenol tautomerase was prepared according to [20].

2.2. *Preparation of extracts*

Rat livers were dispersed, in a Potter-Elvehjem homogenizer, in 4 vol. (w/v) 0.1 M-phosphate (Na^+) pH 6.9, containing pyridoxal phosphate (0.2 mM) and dithiothreitol (1 mM). The supernatant after centrifugation (105 000 × *g*, 30 rev/min, 4°C) was kept on ice used for subsequent assays.

In some experiments, TAT activity was increased by i.p. injection of tryptophan (1 g/kg body wt in 0.9% (w/v) NaCl, 0.1% (w/v) Tween-80). Livers were removed 3 h later and supernatant fractions were prepared as described above.

2.3. *Assay of TAT activity*

For comparative purposes, TAT was assayed by a previously published spectrophotometric method [11].

Our standard procedure was as follows. Each tube (1.5 ml conical plastic 'Eppendorf' tubes) contained 0.1 M-phosphate (K^+), 0.1 M 2-oxo-glutarate, 0.8 mM pyridoxal phosphate, 0.05% (w/v) bovine serum albumin, 0.425 mM L-tyrosine (containing 0.5 μCi L-[*side chain* 2,3-^{3}H]tyrosine) and extract in total vol. 50 μl; pH 7.6. Reaction was initiated by addition of tyrosine to tubes preincubated for 3–5 min at 30°C, and was stopped by further addition of 300 μl Norit GSX (20 mg/ml in 0.06% (w/v) bovine serum albumin). After thorough

mixing, all tubes were centrifuged (either immediately or after storage on ice) at 12 000 × *g* for 2 min at 4°C in an Eppendorf 3200 centrifuge. A portion of the supernatant (150 μl) was counted with 1 ml PCS in a minivial (Hopkin and Williams, Chadwell Heath, Essex) in a Packard 3375 liquid scintillation spectrometer. All assays were performed at least in duplicate with 'zero time' and 'zero enzyme' controls.

3. Results and discussion

This assay is based on the exchange of ^3H in the side chain with the solvent during catalysis [21]. Charcoal addition serves both to adsorb aromatic components, including tyrosine and *p*-hydroxyphenylpyruvate, and to stop further reaction. Use of acid to quench enzymic reaction was found to be unnecessary, and in fact increases background ^3H release from the substrate significantly.

The results of 'zero time' blanks with untreated [^3H]tyrosine reveal that approx. 0.6 total counts appear in the supernatant after charcoal addition. The background rate of ^3H release in similar 'zero enzyme' blanks is equivalent to approx. 8 pmol/min. These blank values are quite acceptable for routine assays.

If greater sensitivity is required, however, the [^3H]-tyrosine may be purified by adsorption onto Amberlite CG120(H^+), washing, elution with a small volume of 2 M $HCOONH_4$, and freezedrying; the solid residue is stored below −70°C. With material purified in this way, the 'zero time' blank is <0.25% and the blank rate <2.5 pmol/min. Results with crude extracts are identical with both standard and purified [^3H]tyrosine.

Figure 1 shows that the time course of reaction in this assay system is linear for a considerable time (see also fig.2B). The results in fig.2 indicate that reaction rate is a linear function of extract concentration. The lower useful limit of this new assay (fig.2B) is at approx. 20 μU (spectrophotometric assay) of TAT activity. This compares favourably with the sensitivity of the more complex radiometric assay [19], and is well below that necessary for activity measurements in, for example, tissue culture-derived cell extracts.

Measurements of reaction rate as a function of increasing substrate concentration (with constant radiochemical specific activity) yield a K_m for L-tyrosine of 1.92 mM (fig.3), close to values published [22,23]. The V_{max}, however, is lower than predicted from parallel spectrophotometric assays, the mean from six determinations being 0.198 ± 0.013 of the

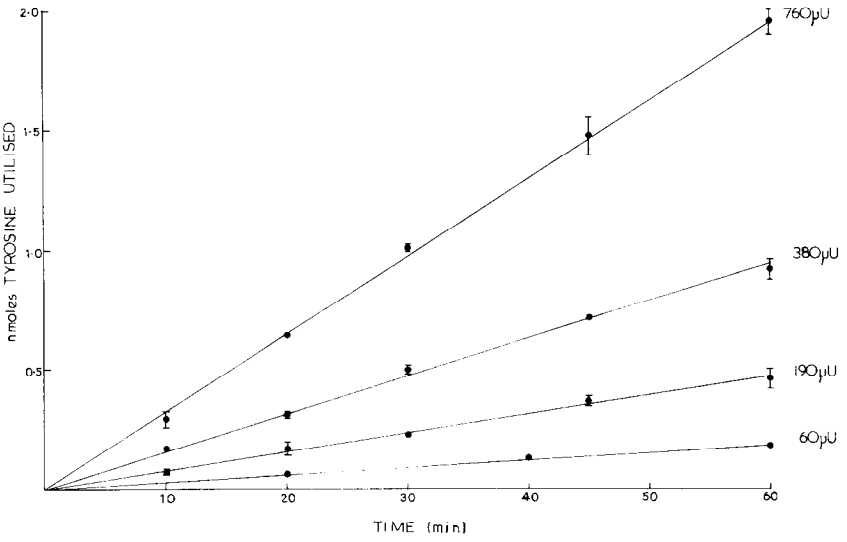

Fig.1. Time course of ^3H release from 0.425 mM L-[*side chain* 2,3-^3H]tyrosine with various amounts of crude extract. The enzyme activities indicated were measured spectrophotometrically [11]. Points represented the means ± SD of 5 determinations.

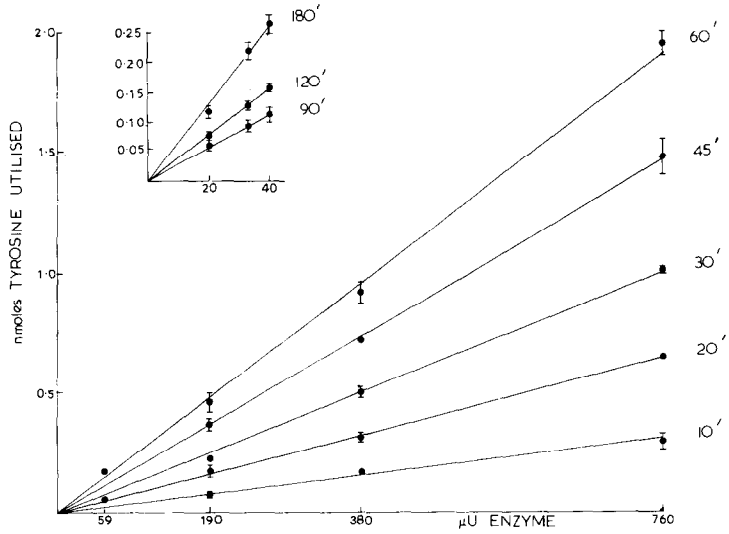

Fig.2(A) Effect of increasing enzyme concentration in crude extracts on ^3H release from 0.425 mM L-[*side chain* 2,3-^3H]-tyrosine. Enzyme activities indicated on the abscissa were determined spectrophotometrically. (B) As A, but with smaller amounts of extract. Points represent the means ± SD of 5 determinations.

calculated value. Nevertheless, assays with the new procedure (with 0.425 mM L-tyrosine) may be directly related to those using spectrophotometric measurement [11] at 4.25 mM L-tyrosine) by a standard conversion factor: rates of appearance of ^3H_2O should be multiplied throughout by 19.6 ± 1.14 (6 observations).

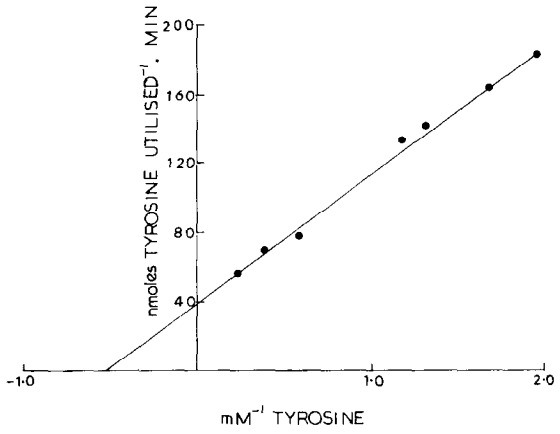

Fig.3. Lineweaver-Burk plot derived from measurements of ^3H release from varying concentrations of L-[*side chain* 2,3-^3H]tyrosine of constant specific activity.

If required, radiometric assays may be performed with 4.25 mM L-tyrosine, provided that the amount of charcoal is increased to 600 μl 100 mg/ml.

Results have been calculated throughout on the assumption that ^3H is exchanged from both side chain C-2 and C-3 positions. This has been demonstrated to hold for other transaminases [24–26], and a mechanism for such exchange has been proposed [25]. Additionally, in the case of TAT, crude extracts contain a keto-enol tautomerase activity (EC 5.3.2.1. [11]), which presumably catalyses the exchange of side chain C-3 protons. A deuterium isotope effect has been shown for aspartate aminotransferase [27], suggesting that proton transfer may be a limiting step in the reaction mechanism. The low value for V_{max} seen with this present assay may thus be attributable to a tritium isotope effect of approx. 5 (cf. [26]).

Participation of keto-enol tautomerase in the tritium exchange at side chain C-3 might theoretically render the assay unsuitable for induction studies in tissues, such as liver, which contain relatively low tautomerase activity. That the exchange is more probably a property of the aminotransferase itself, however, is shown in experiments where TAT activity is induced and

Table 1
Measurement of increased TAT activity after L-tryptophan administration

Assay	Rate (μmol.min^{-1}.g.liver^{-1})		Ratio
	Control	+Tryptophan	(tryptophan:control)
Spectrophotometric	0.382	3.87	10.1 : 1
Radiometric	0.347 ± 0.026	3.63 ± 0.37	10.4 : 1

Activity in each extract was measured both spectrophotometrically [11] and by release of ^3H_2O from L-[*side chain* 2,3-^{3}H]tyrosine. Rates (means of 6 observations ± SD) with the radiometric assay were calculated using the factor discussed in the text

measured by two independent assays (table 1). The ratio of induced to basal activity is similar in both cases.

The present radiometric assay thus offers several advantages over established procedures. The principle involved suggests that similar systems might be readily evolved for the sensitive assay of any aromatic amino acid transaminase activity.

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