CHROM. 4892

A simple technique for the estimation of mitochondrial glutamate–oxaloacetate transaminase in serum and tissues

It is thought that the determination of mitochondrial glutamate–oxaloacetate transaminase* (GOT II) activity in serum, during liver diseases, might give information concerning the degree of disturbed permeability of the mitochondria, and reveal the nature and severity of the underlying cellular damage[1–7].

Several methods[8–33] have been described for the separation of GOT into its mitochondrial and cytoplasmic isoenzymes. However, since the serum activity of GOT II is very low in most cases, and very labile[32,33], the characterisation of this isoenzyme is technically difficult, and therefore it is rarely used in clinical practice.

A simple and sensitive chromatographic method for the characterisation of GOT II in serum and tissue homogenates is described in this paper.

Methods

Columns (diameter 0.9 cm and length 15 cm) with a top feeding funnel, supplied by the Pharmacia Co. (Uppsala, Sweden), are used for chromatography.

Absorbent. DEAE Sephadex A 50 Medium. The ion exchanger (activated according to the directions of the Pharmacia Co.) is poured into the column, at room temperature, to give a final height of the settled suspension of 7–8 cm, and then repeatedly washed with 0.008 *M* Na phosphate buffer, pH 7.

The samples (sera or homogenates) are dialysed for 4 h in a continuous-flow apparatus, against 25 l of 0.008 *M* Na phosphate buffer, pH 7, at 4°. After dialysis, the total GOT activity is assayed. 1 ml of the dialysed sample is applied to the column at room temperature. After the sample has soaked into the column, elution is performed with 15 ml of 0.008 *M* Na phosphate buffer, pH 7. The entire chromatographic procedure is performed, at room temperature, within 15 to 30 min.

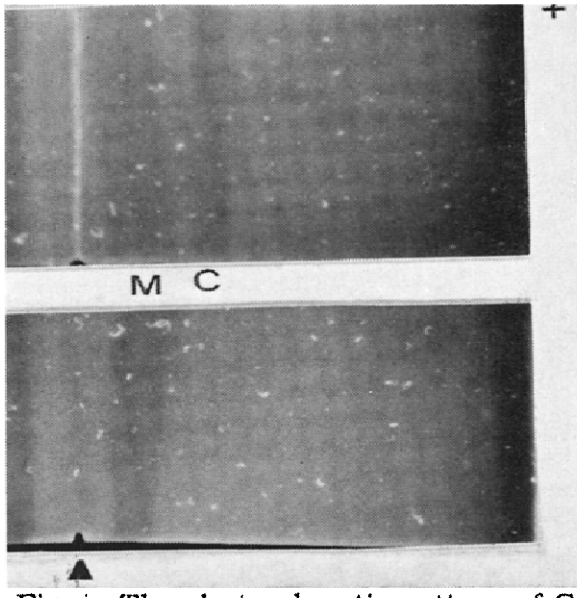
GOT activity is assayed in the eluate by the UV test (KARMEN's method modified by BERGMEYER[34]). If the enzyme activity in the sample is very low, it can be assayed by the MonoTest (Biochemica-Boehringer Co.), by dissolving the reagents in the eluate.

Results

The cytoplasmic isoenzyme of GOT (GOT I) is adsorbed by anion exchangers such as DEAE-Sephadex, while the mitochondrial isoenzyme (GOT II) remains unadsorbed. Therefore, only GOT II occurs in the eluate. GOT I activity can be calculated, if necessary, by subtraction of the mitochondrial activity from total GOT activity of the dialysed sample.

Sensitivity. Rat liver GOT II, obtained from washed mitochondria[36], and tested for purity by electrophoresis (Fig. 1), was diluted to give samples containing different amounts of GOT II activity. These samples were then chromatographed by the method described above. The results are reported in Table I. As little as 2.6 mU of GOT activity were completely recovered after chromatography.

* Glutamate–oxaloacetate transaminase (E.C.2.6.1.1.) = aspartate–aminotransferase = GOT. GOT I = cytoplasmic GOT; GOT II = mitochondrial GOT.



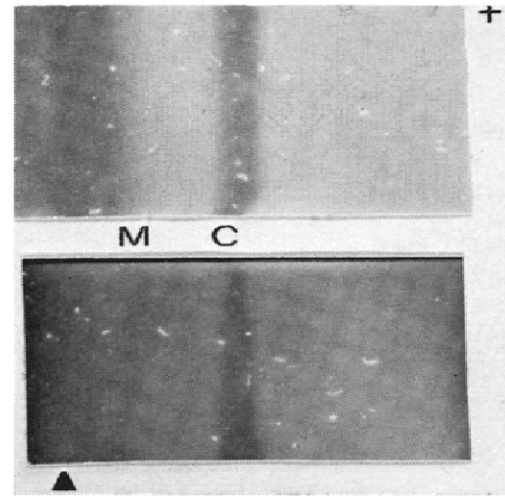


Fig. 1. The electrophoretic pattern of GOT from washed rat liver mitochondria shows no contamination by the cytoplasmic iso-enzyme (left, below). The electrophoretic technique used has been described by FIORELLI *et al.*[31]. The electrophoretic pattern of GOT in whole rat liver homogenate is shown for comparison (left, above).

Fig. 2. Electrophoretic pattern of GOT activity in rat liver cytoplasm, purified by cation-exchange column chromatography (right, below). Columns: diameter 0.9 cm, length 15 cm. Absorbent: CM cellulose C II (Whatman). Final height of the settled suspension: 8 cm. 1 ml of dialysed cytoplasm was applied to the column, and eluted with 15 ml of 0.008 *M* Na phosphate buffer, pH 7. The cytoplasmic isoenzyme is not adsorbed by the cation exchanger, and is therefore recovered in the eluate. The electrophoretic pattern of GOT in whole rat liver homogenate is shown for comparison.

Selectivity. Cytoplasmic GOT (which is usually contaminated by mitochondrial isoenzyme) was purified by chromatography on CM-cellulose. The degree of purification was tested by electrophoresis (Fig. 2). The eluate was then diluted to give samples containing different amounts of GOT I activity. These samples were then rechromatographed by the technique described above. The results are reported in Table II. No GOT activity was found in the eluates, even when as much as 450 mU of GOT were applied to the column.

Reproducibility. Five samples of the same serum* were chromatographed by

TABLE I

CHROMATOGRAMS OF DIFFERENT AMOUNTS OF GOT II

GOT activity applied to the column (mU)	*GOT activity recovered in the eluate (mU)*	*Recovery (%)*
95	93.6	98.5
47	46	97.8
27.6	26.13	94.6
4.2	4.28	101.9
2.6	2.68	103.4

* Serum from a patient with infectious hepatitis.

TABLE II

CHROMATOGRAMS OF DIFFERENT AMOUNTS OF GOT I

GOT activity applied to the column (mU)	*GOT activity recovered in the eluate (mU)*	*Recovery (%)*
18.6	0	0
51.75	0	0
103.5	0	0
225	0	0
450	0	0

TABLE III

VARIATIONS OF GOT ACTIVITY ON REPEATED CHROMATOGRAMS

Chromatogram	*GOT II activity in the eluate (mU)*
1	12.6
2	13.1
3	12.3
4	13.3
5	12.8

the technique described above. The reproducibility of GOT II activity in the samples proved to be satisfactory, as shown in Table III.

Discussion

The chromatographic technique described in this paper is sensitive enough to detect as little as 2.6 mU/ml of mitochondrial GOT activity. No contamination by cytoplasmic GOT has been found in the eluates by selectivity tests. The amount of mitochondrial GOT in samples is reproducible within narrow limits. This quantitative technique is more sensitive than the semiquantitative or qualitative methods reported by other authors. It is also much less time consuming than the method described by SCHMIDT *et al.*[30]. In fact, the continuous-flow apparatus permits good results with dialysis periods as short as 4 h. This is very important, since previous studies have shown that longer dialysis periods cause a striking loss of GOT II activity[36]. This might account for the data reported by SCHMIDT *et al.*[7], who were unable to detect GOT II activity in normal human serum, in contrast to BOYDE[28] and the data obtained in our laboratory by means of the technique described in this paper[35].

Moreover, the entire chromatographic procedure requires one elution only, and can be carried out, at room temperature, within 15–30 min. The simplicity of this method makes it suitable for clinical practice.

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Received June 22nd, 1970

J. Chromatog., 52 (1970) 162–165

CHROM. 4895

Polyacrylamide gel electrophoresis and gel filtration of dyed polysaccharides

At present, polyacrylamide gel electrophoresis is a widely used method in the field of biopolymer chemistry. However, application of this useful and selective method for resolving mixtures of polysaccharides has hitherto been limited to glycoproteins[1] and mucopolysaccharides[2]. Recently, we have shown that the method can be applied to the analysis of neutral and uronic acid-containing polysaccharides[3]. The method possessed a satisfactory resolving power and reproducibility. Nevertheless, this technique had one substantial disadvantage; detection of polysaccharides