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Note

The separation of glyoxalase I and glyoxalase II by paper electrophoresis

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The glyoxalase enzyme system, consisting of the two enzymes glyoxalase I (S-lactoylglutathione methylglyoxal lyase; Gl. I) (E.C. 4.4.1.5) and glyoxalase II (S-2-hydroxyacylglutathione hydrolase; Gl. II) (E.C. 3.1.2.6) catalyses the following reactions[1]:

$$\text{methylglyoxal} + \text{glutathione} \xrightarrow[\text{Gl. I}]{} \text{S-lactoylglutathione} \xrightarrow[\text{Gl. II}]{} \text{D-lactate} + \text{glutathione}$$

Investigations on the physico-chemical properties of the glyoxalases have been reported[2–13], but no data on the electrophoretic properties of these enzymes have been published.

This paper reports the results of investigations on the feasibility of separating the enzymes of this system by electrophoresis, and on the factors that determine the electrophoretic behaviour of the glyoxalases, particularly Gl. I.

MATERIALS AND METHODS

Yeast Gl. I and glutathione (GSH) were purchased from Boehringer (Mannheim, G.F.R.) and crystalline albumin from BDH (Poole, Great Britain). Methylglyoxal was obtained by distillation of glyceraldehyde (Reanal, Budapest, Hungary)[14] and S-lactoyl-GSH was prepared enzymatically[15]. All other reagents were of analytical purity.

Ox liver homogenates (1:6 in 0.15 *N* potassium chloride solution) were prepared by using a Potter–Elvehjem homogenizer and centrifuged in a refrigerated centrifuge at 20,000 *g* for 30 min. The supernatant solutions were used for electrophoretic studies.

Purified Gl. I and Gl. II were obtained by the molecular filtration method as described by Jerzykowski *et al.*[13]. The fractions with maximum activity were used for electrophoretic studies (both before and after precipitation with ammonium sulphate as described in the text).

Electrophoresis was conducted with a standard apparatus for serum paper electrophoresis, using Whatman No. 1 paper, at 220 V for 5.5 hours with an ionic strength of 0.1. Paper strips 3 mm wide were eluted in 2 ml of phosphate buffer (1/15 *M*, pH 6.8) by shaking them in the solution for 1 h.

Gl. I and Gl. II in the eluates from the paper strips and in the glyoxalase preparations (column fractions) were determined by the method of Racker as described by Jerzykowski *et al.*[12,13]. Units of enzyme activity are expressed as μmoles of lactoyl-GSH produced (Gl. I) or decomposed (Gl. II) in 1 min (initial velocity) at room temperature under the experimental conditions used.

Protein concentrations were determined by the method of Warburg and Christian[16].

RESULTS AND DISCUSSION

Liver glyoxalases

Most of the experiments were carried out with the supernatant solutions from ox liver homogenates, which exhibited considerable activity of both glyoxalases. Several preliminary tests on the influence of pH on glyoxalase mobility were conducted at pH 5–10 and with veronal, Tris–hydrochloric acid and phosphate buffers. The electrophoresis time (5.5 h), voltage (220 V) and ionic strength (0.1) were constant. The purpose of these tests was to determine as far as possible the optimal conditions for the separation of Gl. I and Gl. II in supernatant solutions from ox liver homogenate. These conditions were found to be the use of veronal buffer at pH 8–9.

The relative electrophoretic mobility of both glyoxalases in veronal buffer (pH 8.6) in comparison with human serum (Fig. 1a) is shown in Fig. 1d. In this instance, Gl. I migrates to the anode, exhibiting a mobility similar to that of the albumin fraction in the electrophoresis of blood serum, while Gl. II apparently remains in the initial position. Fig. 2 shows the distribution of enzyme activity after electrophoresis at pH 8.0. At room temperature, about 50% of the initial activity of Gl. I and about 50% of the activity of Gl. II can be obtained from electropherogram analysis, and the enzyme separation is complete. This suggests the feasibility of using this buffer and pH to achieve enzyme separations by continuous curtain electrophoresis. Further investigations were conducted in the selected range of pH.

Red cell glyoxalases

Both Gl. I and Gl. II are present in blood red corpuscles. Electrophoretic testing of the supernatant solution from haemolized human red corpuscles (after prior centrifugation and repeated washing with 0.9% sodium chloride solution) showed a glyoxalase activity distribution similar to that found for glyoxalases from ox liver homogenate.

Purified yeast glyoxalase I

Several experiments were performed with yeast Gl. I obtained from Boehringer. It can be seen from Fig. 1b that yeast Gl. I moves toward the cathode. This migration is only apparent, as actually the dextran determined line of zero flow[17] is in the position marked by the arrow (Fig. 1a), which provides evidence that under these conditions Gl. I does not migrate owing to the presence of a charge.

Purified liver glyoxalase I

A behaviour similar to that described above was found for Gl. I purified by a molecular filtration method and then precipitated with ammonium sulphate at a

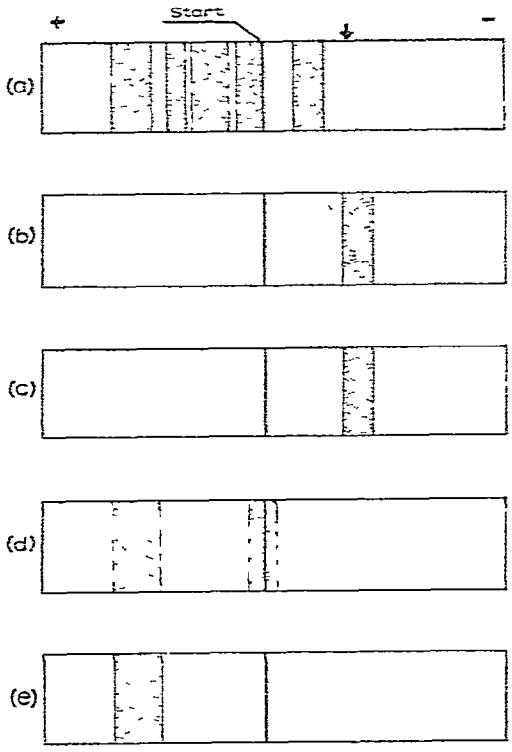

Fig. 1. Low-voltage electropherograms of glyoxalases (veronal buffer, pH 8.6, ionic strength 0 1, 220 V, time 5 5 h, paper electrophoresis). (a) human serum; (b) purified yeast GI. I; (c) GI. I precipitated with ammonium sulphate, (d) GI. I and GI. II from liver homogenate supernatant, (e) GI I purified by molecular filtration, after the addition of ox albumin.

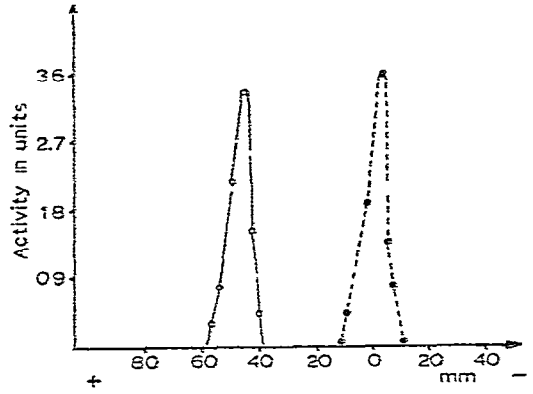

Fig. 2. Distribution of GI. I (○—○) and GI. II (●—●) activity after paper electrophoresis (veronal buffer, pH 8.0, ionic strength 0.1, 220 V, time 5.5 h).

degree of saturation >0.7 (Fig. 1c). This glyoxalase preparation exhibits a specific activity five times greater than that of Gl. I from the supernatant solution. However, if this preparation is diluted with water, or if the degree of saturation with ammonium sulphate is less than *ca.* 0.7, the Gl. I moves to the anode, in the same manner as Gl. I from homogenate. The mobility is the same when the dilution is made with 8 *M* urea solution, or Mg^{2-} ions (magnesium sulphate, 10^{-3} *M* solution). However, a lack of mobility, or more accurately an apparent mobility towards the cathode, is found after adding EDTA (10^{-3} *M* solution).

An exceptional behaviour is shown by Gl. I prepared by molecular filtration and not precipitated with ammonium sulphate. This preparation is probably completely deactivated by electrophoresis, as no enzyme activity can be detected over the whole electropherogram, despite the high activity of the initial preparation. It should be noted that GSH ($5 \cdot 10^{-2}$ *M*), which is a substrate for Gl. I, shows no protective effect on the behaviour of this preparation of Gl. I. However, this preparation maintains its activity when ox serum albumin is added to it. In this instance, the enzyme moves towards the anode and can be detected in the same position in which it is found after direct electrophoresis of ox liver preparation (Fig. 1e). Hence, it was reasonable to assume that the enzyme migrated in association with the added protein. In order to test this assumption, a detailed comparative analysis was made of the molecular weights of Gl. I and mixtures of Gl. I and albumin (by the method described by Jerzykowski *et al.*[13]). These experiments provided no evidence that albumin combined with Gl. I. However, it should be stressed that the conditions under which the molecules migrate during electrophoresis do not correspond precisely to the conditions that exist during the determination of molecular weights. This aspect appears to require further elucidation.

CONCLUSIONS

Electrophoretic tests on glyoxalase I preparations have been described. The electrophoretic mobility shown by this enzyme depends on the method of preparation. It was demonstrated that under the electrophoretic conditions determined (paper electrophoresis, veronal buffer, ionic strength 0.1, pH 8.0), Gl. I and Gl. II can be completely separated. Studies were conducted using glyoxalases from ox liver, human red corpuscles and also yeast Gl. I.

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