# ASSOCIATION BEHAVIOUR OF RAT LIVER GLUTAMATE DEHYDROGENASE*

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## 1. Introduction

Most of the glutamate dehydrogenase (GluDH, EC 1.4.1.3) from liver mitochondria exhibit in solution an association–dissociation equilibrium [2, 3]. Although the amino acid composition and the peptide maps of rat liver GluDH [4, 5] are quite similar to the beef liver enzyme this enzyme protein lacks the ability to undergo a concentration-dependent reversible association reaction even in the presence of nucleotides promoting the association of the beef liver enzyme [5]. From the similarity of the peptide maps it can be assumed that the conformations are similar, too, and that the relatively small differences in the primary structure in the case of the rat liver enzyme have caused a loss of only some of the structural characteristics of the rat liver enzyme which, in the case of the beef liver enzyme, are responsible for the association reaction. Therefore, it can be expected that: 1) conditions which support the association process and cause an association of the rat liver enzyme may exist, and 2) that even if self association does not occur it might be possible to hybridize rat and beef liver GluDH, although the remaining structural characteristics are not sufficient under the conditions investigated till now for an association process.

In the present paper hybridization between oligomers (mixed association) and sedimentation experiments are reported indicating that rat liver GluDH has a tendency to associate and to hybridize.

* Studies on glutamate dehydrogenase, part IX; for part VIII see [1].

## 2. Experimental

Beef liver GluDH was purchased from C.F. Boehringer and Soehne GmbH (Mannheim, Germany), rat liver GluDH was prepared with slight modifications according to Fahien et al. [6] and all the other chemicals were of reagent grade from commercially available sources. The GluDH concentration was determined using an absorbance factor $A_{280} = 0.97$ [$cm^2\ mg^{-1}$]. Sedimentation velocity and diffusion measurements were made with the Spinco model E analytical ultracentrifuge (Beckman Instruments, München, Germany) and the calculation of the hydrodynamic properties were performed as previously described [7]. For the experiments the enzyme solutions were dialyzed for 36 hr at 2° to 4° against 0.067 M sodium phosphate buffer, pH 7.6, or citrate buffer ($\mu = 0.04$), pH 6.0 or 6.9 and the ionic strength was increased as noted by adding sodium chloride. The hydridization was obtained by mixing the two enzyme solutions. For the experiments in the presence of benzene, 0.5 ml enzyme solution was equilibrated with 0.5 ml benzene which was layered over the enzyme solution at room temp.

## 3. Results and discussion

The investigation of the sedimentation coefficient of the rat liver enzyme at different protein concentrations shows a slight ascent in the sedimentation coefficient with increasing protein concentration. In the range investigated (1–8 mg/ml) this dependence can be described according to the equation

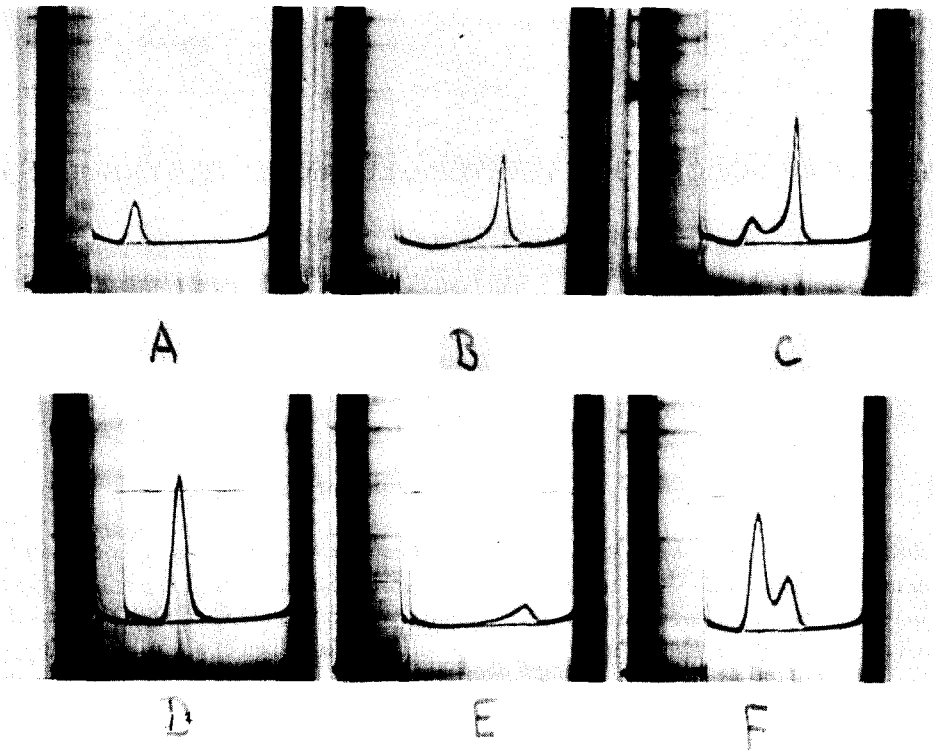


Fig. 1. Hybridization between GluDH from beef and rat liver. The sedimentation behaviour measured in 0.067 M sodium phosphate buffer, pH 7.6, at 60,000 rpm, 20°, and at an angle of the Schlieren diaphragm of 60° in 12 mm double sector cells. Rat liver GluDH: A (1.9 mg/ml) and D (8.3 mg/ml), beef liver GluDH: B (5.6 mg/ml) and E (2.3 mg/ml) and the mixture of both C (A plus B) and F (D plus E). The pictures were taken 15 min after the beginning of sedimentation (A–C) and 18 min (D–F), respectively.

$s_{20,w} = s^0_{20,w}(1 + k_s c)$ where $c$ is the protein concentration in mg/ml and $k_s$ a constant (= 0.0086 ml/mg); $s^0_{20,w}$ was obtained to 12.75 S. This result indicates a slight association as protein concentration is increased. With $D^0_{20,w}$ 3.95 $F$ ($k_D$ = -0.026 ml/mg) we calculate the molecular weight of rat liver GluDH at an infinite dilution to be 313,000.

The sedimentation behaviour of a mixture of rat and beef liver GluDH clearly shows that the area under the concentration gradient of the fast component (beef enzyme, $s_{20,w}$ = 26.7 S) increases whereas the area corresponding to the slow component (rat enzyme, $s_{20,w}$ = 12.8 S) decreases compared to the controls (fig. 1). Being dependent on the protein concentration of the beef liver enzyme (at a constant concentration of 1.9 mg/ml of the rat liver enzyme) and the area, the concentration of the rat liver enzyme in the slow component therefore decreases up to 40% if the beef liver enzyme is present at a concentration of 10 mg/ml. If the sedimentation behaviour is investigated at low concentration of the beef enzyme (2.3 mg/ml) and high concentration of the rat enzyme (8.3 mg/ml, fig. 1F), an analogous result is obtained. The $s_{20}$ value of the faster component is 19.9 S as compared to 24.0 S of the control. These results clearly demonstrate at least partial association of the rat liver GluDH with the beef enzyme and, therefore, suggests hybridization between both enzymes.

Low pH and low ionic strength [8] as well as the addition of toluene, or even more so of benzene [9, 10], intensify the association of beef liver GluDH. The same is observed for the rat liver enzyme (fig. 2). Under these conditions the sedimentation coefficient of the rat liver increases up to 33.3 S in citrate buffer (pH 6.0, $\mu$ = 0.04) saturated with benzene (see table 1). In all the solvents investigated the rat liver enzyme sediments slower than the beef liver enzyme, indicating a lower degree of association.

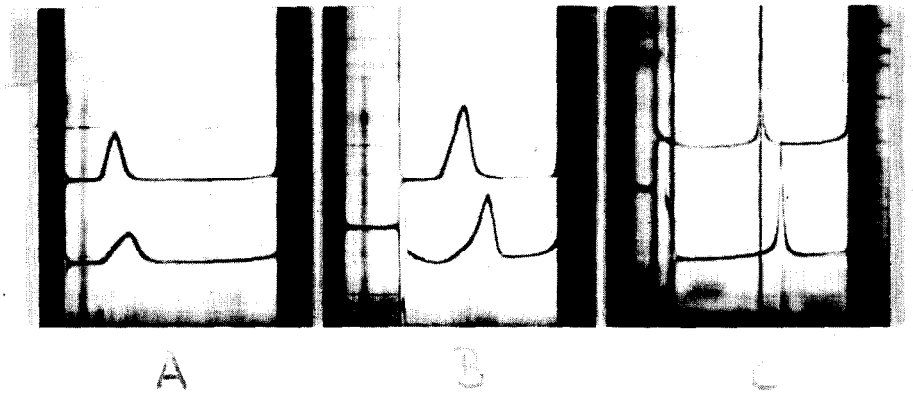


Fig. 2. Sedimentation pattern of beef and rat liver GluDH in different solvents. Experimental conditions as in fig. 1 with the exception that the experiments were done in normal and wedge window cells. Pictures were taken after 11 min (A), 15 min (B), and 13 min (C). A) Rat liver GluDH (2.5 mg/ml) in citrate buffer, $\mu$ = 0.115, pH 6.8 (upper curve) and $\mu$ = 0.04, pH 6.0 (lower curve). B) Rat liver GluDH in citrate buffer, pH 6.0 and $\mu$ = 0.04 in the presence of 4.4 mM $NAD^+$ (lower curve, enzyme 4.6 mg/ml, upper curve in the absence of $NAD^+$, enzyme 4.9 mg/ml). C) Beef (3.9 mg/ml, upper curve) in 0.067 M sodium phosphate buffer, pH 7.6, and rat liver GluDH (2.5 mg/ml, lower curve) in citrate buffer, pH 6.0, $\mu$ = 0.04, in the presence of benzene.

Our results clearly demonstrate that rat liver GluDH, like the other glutamate dehydrogenases, also possesses the ability to associate. At high ionic strength and at neutral pH, the association is very weak. An increase of the association is observed with decreasing ionic strength and pH, the addition of $NAD^+$ and particularly the addition of aromatic compounds. The association observed with the beef liver enzyme (table 1) proceeds in the same direction, but is much stronger, indicating that the same type of non-covalent bonds are involved in the association of the oligomers of both enzymes.

The greatest differences in the amino acid composition between beef and rat liver GluDH are found for valine (about three more in the rat enzyme) and phenylalanine (about three less in the rat enzyme *) [5]. The extension of the organization of the enzyme protein into a more highly ordered structure upon addition of benzene may indicate: 1) a lack of phenylalanine residues at important positions in the rat enzyme, which in the beef enzyme are involved in the hydrophobic interactions between the oligomers and 2) that benzene can partly substitute for the aromatic part of these phenylalanine residues. On the other hand it is also possible that benzene interacts with both enzymes in the same manner, but the lack of groups e.g. phenylalanine which may be important

Table 1
Sedimentation coefficients of rat and beef liver GluDH under various conditions.

| Solvent | $s_{20}$ (S) | |
|---|---|---|
| | Rat liver GluDH | Beef liver GluDH* |
| 0.067 M sodium phosphate buffer, pH 7.6 (control) | 12.4 | 25.3 |
| Saturated with benzene | 16.5 | 33.3 |
| Citrate buffer, pH 6.9, $\mu$ = 0.04 | 13.0 | 26.8 |
| Citrate buffer, pH 6.0, $\mu$ = 0.04 | 15.9 | 30.9 |
| Plus 4.4 mM $NAD^+$ | 20.2 | 33.2 |
| Saturated with benzene | 33.3 | 38.3 |

* cf. also [8].
Measurements at 20° and at protein concentrations between 1.9 and 4.0 mg/ml.

* The total amount of amino acid residues for rat and beef liver GluDH in the published analysis are 432 and 427, respectively [5]. On the other hand the analysis of the primary structure yielded 506 amino acid residues for the beef enzyme [11] and, therefore, the data given for valine and phenylalanine should be corrected on this basis.

for the association, may be responsible for a decrease in the equilibrium constant for the association–dissociation equilibrium of rat liver GluDH.

It is unknown if the ability of GluDH to associate was acquired during an early state of evolution. From the fact that GluDH from animals which are lower on the phylogenetic scale than rats associate more easily, i.e. have a stronger association than the rat enzyme, it can be concluded that the ability to associate was developed during an early state of evolution and that the rat enzyme lost this ability during a later state.

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