The Effect of Guanidinium Chloride on the Self-Association of Bovine Liver Glutamate Dehydrogenase: A Gel Filtration Study

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The associative behaviour of bovine liver glutamate dehydrogenase has been studied by gel chromatography at neutral pH in 1 M guanidinium chloride and 1 M sodium chloride. In guanidinium chloride both the elution volume and the elution profile of the enzyme are independent of protein concentration, whereas in sodium chloride they are strongly dependent on it. In NaCl the enzyme behaves as expected according to the well established random association model, whereas in guanidinium chloride it appears to have completely lost the self-associative property. Furthermore, since the elution volume of the enzyme in guanidinium chloride corresponds to that of an hexamer, trimer formation reported to occur in these conditions is not confirmed by this technique.

Introduction

The mitochondrial enzyme glutamate dehydrogenase from bovine liver (EC 1.4.1.3.) presents a variety of interesting properties, among which a reversible association-dissociation equilibrium between hexamers and higher oligomers [1]. This process, well described by a model of open linear polymerization, *i.e.* with no limit imposed on the chain length, is affected by many parameters among which the enzyme concentration and solvent composition. In contrast with this behaviour, further dissociation of hexamers into subunits, caused by denaturating agents such as urea or GuCl, has always been found to be accompanied by irreversible denaturation in all cases investigated [3]. More recently it has been proposed that GuCl at moderate concentration (< 2 M) promotes a reversible dissociation of hexamers to trimers [4, 5]. By comparison of the residual enzymatic activity in guanidinium chloride with that in sodium chloride, trimers were deduced to be inactive species [6, 7]. It was in fact assumed that inactive trimers form in GuCl and not in NaCl, the less pronounced loss of activity observed in NaCl being completely attributed to ionic strength effects [6]. However, as several other studies on the reversible inactivation and self-association of glutamate dehydrogenase by ionic compounds have pointed out, many ions exhibit rather specific effects in addition to those of ionic strength, *e.g.* [8–11], which generally parallel their position in the empirical Hofmeister series [12]. The specific effects induced by moderate concentrations of guanidinium ions and other electrolytes on structural and functional properties of proteins have been thouroughly investigated in a variety of systems, *e.g.* [13, 14]. In this report we have analyzed the chromatographic behaviour of glutamate dehydrogenase eluted either in 1 M GuCl or 1 M NaCl, by gel filtration at pH 7 in 50 mM phosphate buffer. The results obtained indicate that the self-association between hexamers is abolished in the presence of 1 M GuCl whereas it is maintained in 1 M NaCl. Furthermore, in contrast with previous papers [4, 5] the formation of trimers in 1 M GuCl has not been confirmed by this technique.

Materials and Methods

Bovine liver glutamate dehydrogenase from Boehringer has been dialyzed and assayed as previously described [15]. Enzyme stock solutions have then been diluted in the appropriate solution and filtered through 0.8 μm Millipore membrane filters before each chromatographic run. GuCl, NaCl and other salts were from Merck. Gel chromatography runs have been carried out at 22 °C through a (100 × 2.5 cm) column filled with Sepharose CL-6B (Pharmacia) and previously equilibrated in either solvent. The eluted fractions have been monitored using Jasco instrumentation (Uvidec-510 spectrophotometer or FP-770 spectrofluorometer) and col-

Abbreviation: GuCl, guanidinium chloride.

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lected with an LKB Superrac fraction collector at a flow rate of 24 ml/h. Enzyme reactivation after each chromatographic run in either solvent has been checked by appropriate (100fold or more) dilution into assay solutions containing 0.25 mM NAD and 50 mM glutamate in 0.05 M pH 7 sodium phosphate buffer at 20 °C. Water purified through a Milli-RO plus Milli-Q coupled system (Millipore Corp., USA) was used throughout.

Results and Discussion

An identical molecular weight calibration curve for several proteins has been obtained in either solvent (1 M GuCl or 1 M NaCl, both at pH 7 in 50 mM phosphate buffer) using a column filled with Sepharose CL-6B and previously equilibrated (Fig. 1). The elution volume of glutamate dehydrogenase at 0.1 mg/ml or less from both solvents fits well into the calibration curve, assuming a molecular weight of 336,000.

Typical elution profiles of glutamate dehydrogenase in 1 M NaCl and 1 M GuCl are shown in Fig. 2 at two different enzyme concentrations. The values of the elution volume V_e and of the parameter α, a measure of the band asymmetry defined as $\alpha = (V_R - V_e)/(V_e - V_L)$, V_R and V_L being the elution volumes at the right and left half-height of each band, are plotted *versus* enzyme concentration in Fig. 3.

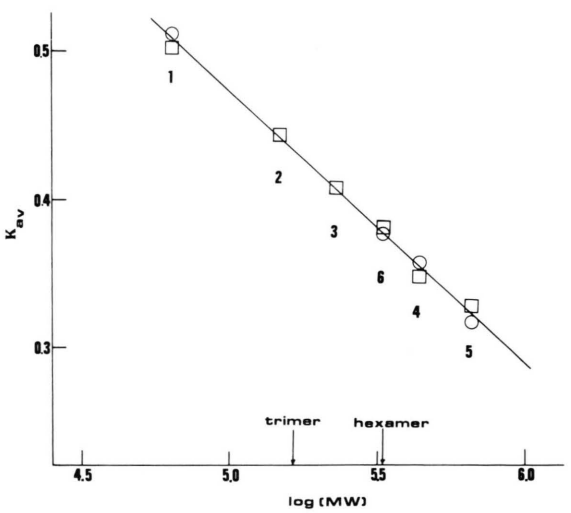

Fig. 1. Molecular weight calibration curve obtained using a 100 × 2.5 cm column filled with Sepharose CL-6B and eluting either with 1 M NaCl (□) or 1 M GuCl (○), at pH 7 in 50 mM phosphate buffer. Used standard proteins are: 1, bovine serum albumin; 2, yeast alcohol dehydrogenase; 3, catalase; 4, ferritin; 5, tyroglobulin. 6 is glutamate dehydrogenase (0.08 mg/ml). Proteins 2 and 3 are not shown in 1 M GuCl because denatured.

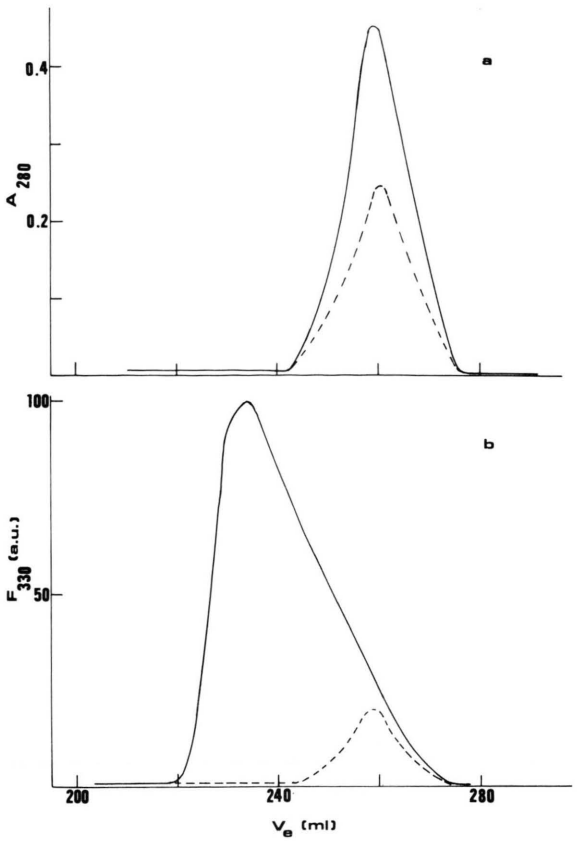

Fig. 2. Elution profiles of glutamate dehydrogenase at different concentrations (mg/ml). (a) 1 M GuCl: 2.22 (———) and 1 (----); (b) 1 M NaCl: 0.5 (———) and 0.08 (----). All runs have been done at pH 7 in 50 mM phosphate buffer. The eluted enzyme has been detected by its absorbance at 280 nm in GuCl and its fluorescence at 330 nm (excitation at 280 nm) in NaCl, respectively.

Though the observed marked variation of V_e in 1 M NaCl contrasts with the essentially constant value found in 1 M GuCl, the same value is obtained at very low enzyme concentration. Moreover, as the enzyme concentration is decreased the elution profile observed in 1 M NaCl becomes less asymmetric, whereas the peak symmetry in GuCl is always retained in the whole range investigated (0.08–2.2 mg/ml).

The chromatographic results described above clearly indicate that the associative behaviour of the

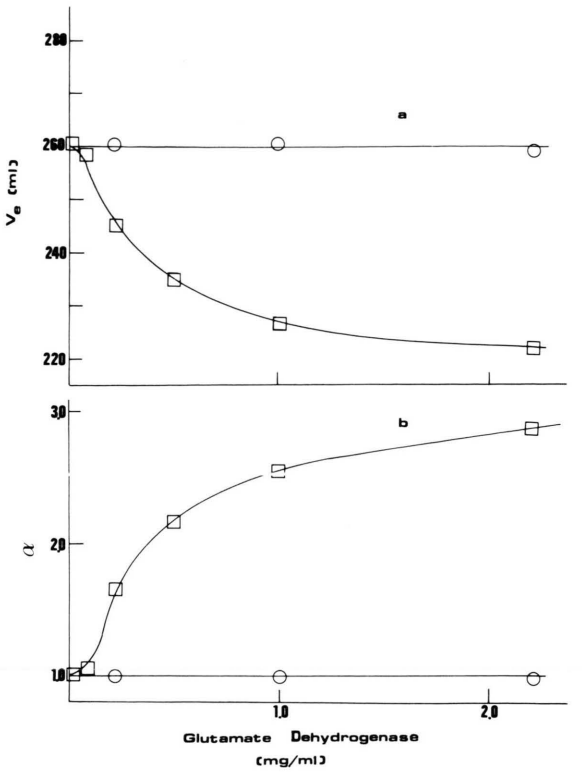

Fig. 3. (a) Elution volumes V_e and (b) asymmetry parameter α (defined in the text) relative to several concentrations of glutamate dehydrogenase in 1 M NaCl (□) and 1 M GuCl (○), at pH 7 in 50 mM phosphate buffer.

enzyme is different in the two saline systems examined.

In 1 M NaCl the average degree of association of glutamate dehydrogenase hexamers N_{av} depends on the enzyme concentration, its values ranging from that of a pentamer of associated hexamers at 2.2 mg/ml to that of a non-associated hexamer below 0.1 mg/ml, as deduced from the observed values of V_e. This behaviour, qualitatively consistent with the random association model peculiar of this enzyme [16], is quantified in Table I.

On the contrary, in 1 M GuCl the elution volume is constant and the band shape is symmetric throughout, independently of enzyme concentration. Both results are consistent with the existence of only one species having the molecular weight of an hexamer, as deduced by interpolation of V_e in Fig. 1.

Table I. Apparent average association number of glutamate dehydrogenase hexamers (N_{av}) as a function of loaded enzyme concentration (mg/ml), as deduced from our gel filtration data.

Enzyme [mg/ml]	N_{av} 1 M NaCl*	1 M GuCl*
0.016	1.0	–
0.08	1.1	–
0.22	1.9	1.0
0.5	2.8	–
1	4.2	1.0
2.21	4.6	1.0

* pH 7, 0.05 M phosphate buffer and 22 °C.

The polymer dissociating effect brought about by GuCl can be explained as mainly due to its amphoteric properties: many organic substances with both hydrophobic and hydrophylic moieties, such as dimethylsulfoxide, dioxane and polyalcohols are in fact known to promote dissociation of glutamate dehydrogenase into hexamers [1].

Concerning the stronger inactivating effect exhibited by GuCl than by NaCl, recently described by different authors [5–7], it has been explained as caused by the preferential formation of inactive trimers in GuCl, previously deduced from the light scattering measurements [4]. However, in contrast with this finding, our gel filtration results do not support the formation of trimers in 1 M GuCl. Whether this is due to different experimental conditions or techniques remains to be clarified. A potential source of error, which may become important when evaluating the molecular weight of proteins from light scattering and other techniques, arises from the assumption that the specific volume of the protein does not vary with the ionic strength. This problem has been clearly pointed out in the case of lactate dehydrogenase [17] and other proteins [18].

According to our data, we therefore suggest that inactive hexamers, rather than inactive trimers, are formed in 1 M GuCl.

Acknowledgement

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