THE CONCENTRATION DEPENDENCE OF THE ACTIVITY OF BEEF LIVER GLUTAMATE DEHYDROGENASE AS MEASURED BY RAPID MIXING AND ULTRACENTRIFUGATION TECHNIQUES

M. KEMPFLE, K.-O. MOSEBACH
Physikalisch-chemische Abteilung des physiologisch-chemischen Instituts der Universität Bonn, W. Germany

and

H. WINKLER
Max-Planck-Institut für biophysikalische Chemie, Göttingen, W. Germany

Received 24 August 1971
Revised version received 21 October 1971

1. Introduction

Beef liver glutamate dehydrogenase (EC 1.4.1.3) shows an interesting correlation between enzymatic activity and molecular weight. At concentrations under 0.01 mg/ml the molecular weight is 310,000 and increases to approximately 8 million for protein concentrations of about 50 mg/ml [1–3].

The species of molecular weight 310,000, which will be referred to as "monomer", is the smallest active unit and is itself composed of 6 subunits each of a molecular weight ca. 52,000 [2]. Depending on the concentrations of enzyme the monomers aggregate to dimers, trimers and up to large polymers of molecular weight 8 million.

We will present evidence in this communication to show that the enzymatic activity per monomer decreases with increasing protein concentration, but that the activity per *molecular species* remains constant, (i.e. monomers, dimers, trimers etc. show the same enzymatic activity per particle).

In contrast to our earlier conclusions we now find that the enzymatic activity no longer reaches a maximum with increasing enzyme concentrations [4]. The apparent maximum is the result of the use of high concentrations of substrate, which are inhibitory to the enzyme.

2. Materials and methods

A Durrum-Gibson stopped flow apparatus, modified by the addition of a logarithmic converter, was used to measure enzyme activity at high concentrations. Slower reaction rates were followed in a Zeiss PMQ II spectrophotometer and the rates were recorded on a Hewlett-Packard 4007-B X-Y-recorder. The reaction rates were monitored by following the absorbancy of NADH at 340 nm.

Beef liver glutamate dehydrogenase in 2 M ammonium suspension, NAD, NADH, α-ketoglutaric acid and glutamic acid were purchased from Boehringer and Söhne GmbH, Mannheim. The enzyme was dialysed for 24 hr against 3 changes of a solution containing 0.1 M potassium-sodium-phosphate buffer, and 0.1 mM EDTA at pH = 7.6 and finally centrifuged at 3000 *g* for 20 min.

Enzyme concentrations were determined spectrophotometrically, using an absorbance coefficient $A_{279}^{mg/ml} = 0.97$ [5]. Molecular weights were determined in a Phywe U 60 L analytical ultracentrifuge. All measurements were performed at 25°.

3. Results and discussion

In fig. 1 the activity of the enzyme, measured as

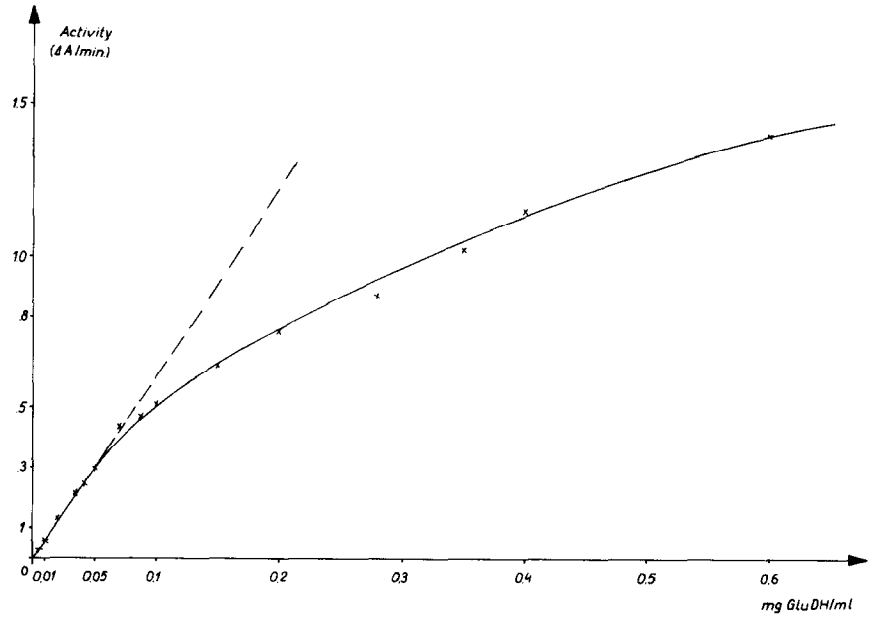

Fig. 1. Enzyme activity in Δ A/min versus glutamate dehydrogenase concentration as measured with stopped-flow techniques. Each point represents the means of 5 measurements. 0.1 M Potassium-sodium-phosphate buffer pH 7.6, 0.1 mM EDTA. Syringe 1: twice enzyme concentration given. Syringe 2: 2×10^{-2} M Glu, 2×10^{-3} M NAD^+.

Δ A/min is plotted against enzyme concentration. At low concentrations of enzyme the activity parallels enzyme concentration, but levels off at higher concentration, indicating that the polymerized enzyme is less active than the monomer, based on total protein concentration.

This is shown clearly, when turnover numbers are plotted against:

1) the moles of glutamate dehydrogenase monomers (310,000) per ml (fig. 2a) or
2) the moles of glutamate dehydrogenase per ml using a mean molecular weight as measured by ultracentrifugation [2] and light scattering [1] (fig. 2b) or
3) the total moles of molecular species per ml as present in solution (fig. 2c). Corresponding values were taken from Eisenkraft [10] having been obtained by the method of Steiner [11] or were yielded by our own calculations using the same source. Following this way, the concentrations of the monomers, dimers etc., c_i, in the formula expressing the weight-average molecular weight

$$M_w = \sum_i c_i M_i / \sum_i c_i$$

are substituted by c_1 and the equilibrium constants K_2, K_3 etc., obtaining

$$M_w c / M_1 c_1 = 1 + 2K_2 c_1 + 3K_2 K_3 c_1^2 + + 4K_2 K_3 K_4 c_1^3 + \ldots$$

This equation, in connection with a formula derived by Steiner [11] for the weight fraction of the monomers

$$\ln c_1/c = \int_0^c (M_1/M_w - 1)/c \, dc$$

makes is possible to calculate the equilibrium constants. The knowledge of these constants enabled us to compute the molecular species composition at various enzyme concentrations.

The actual calculations are shown in table 1.

The fact that the activity correlates with the number of particles suggests that each molecular species shows the same enzymatic activity. Thus the dimer and trimer have the same number of catalytic sites as the monomer, which in turn suggests that the contact

Table 1

Concentration of glutamate dehydrogenase in moles/ml and the turnover number in ($\frac{\text{moles NAD}^+}{\text{moles enzyme}}$/min).

a) Concentration of monomers of molecular weight 310,000 daltons

Conc. (mg/ml)	Molecular weight	Conc. (moles/ml) $\times 10^{-10}$	Turnover number	
0.005	310,000	0.16	300	(see fig. 2a)
0.01	310,000	0.32	300	
0.02	310,000	0.64	300	
0.05	310,000	1.6	300	
0.1	310,000	3.2	250	
0.15	310,000	4.8	210	
0.2	310,000	6.4	190	
0.25	310,000	8.0	175	
0.5	310,000	16.0	135	

b) Concentration of aggregates with the weight-average molecular weight determined by ultracentrifugation [2].

Conc. (mg/ml)	Molecular weight	Conc. (moles/ml) $\times 10^{-10}$	Turnover number	
0.005	310,000	0.16	300	(see fig. 2b)
0.01	310,000	0.32	300	
0.05	410,000	1.2	400	
0.1	520,000	1.92	410	
0.15	600,000	2.4	420	
0.2	750,000	2.7	430	
0.5	1,300,000	3.85	500	

c) Total moles of enzyme molecules per ml irrespective of molecular weight [10, 11].

Conc. (mg/ml)	Molecular weight	Conc. (moles/ml) $\times 10^{-10}$	Turnover number	
0.005	310,000	0.16	300	(see fig. 2c)
0.025	340,000	0.7	315	
0.05	410,000	1.45	310	
0.1	520,000	2.74	295	
0.2	750,000	4.84	290	
0.3	1,000,000	8.5	290	

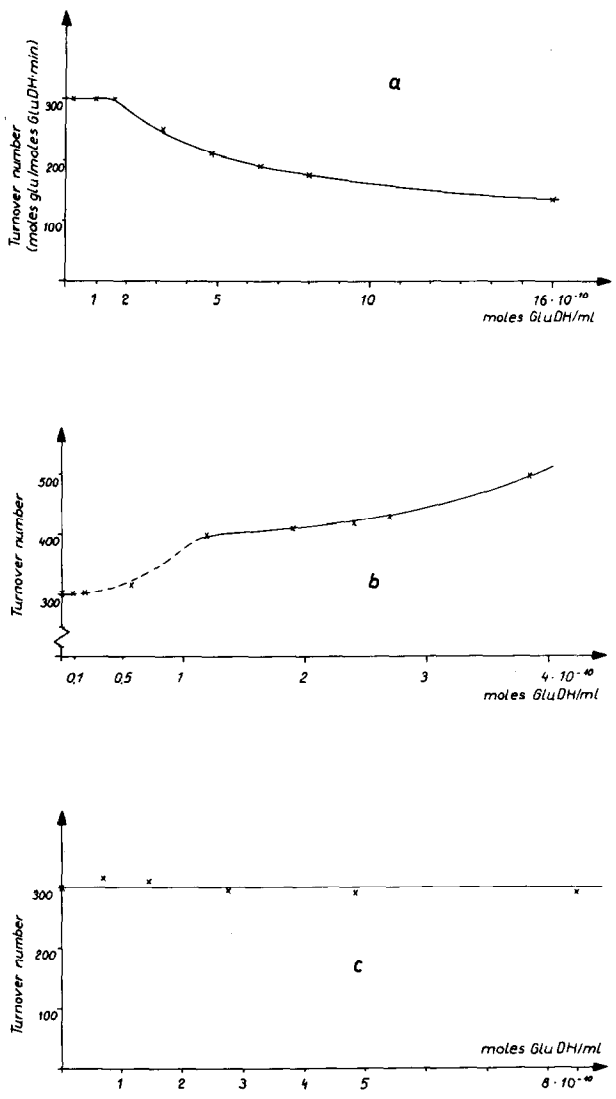

Fig. 2. Turnover number (in $\frac{\text{moles NAD}^+}{\text{moles enzyme}}$/min) versus enzyme concentration in moles/ml. a) Concentration of monomer of molecular weight: 310,000 daltons. b) Concentration of aggregates with the weight-average molecular weight determined by ultracentrifugation [2]. c) Total moles of glutamate dehydrogenase molecules per ml irrespective of molecular weight [10, 11].

regions in the aggregates overlap the catalytic sites. There exists independent evidence in support of this conclusion: diethylstilbestrole (DES) is a strong competitive inhibitor for α-ketoglutarate but not for NADH [6]. DES also promotes the disaggregation of the polymers to monomers. Binding [7] and kinetic studies [8] indicate that two molecules of DES per monomer are sufficient to effect the disaggregation.

Electron microscopy has shown that the aggregated species consist of long chains of monomers [9]. These studies and the DES-results suggest that the catalytic sites are at the aggregating ends of the monomers.

During the course of inhibition and the disaggregation of the polymers NADH has to be present, but the role of the NADH is not yet clear. Current efforts are in the direction.

Acknowledgements

We thank Frl. I. Klauditz for skillful technical assistance and EMBO for a short-term fellowship to MK.

References

[1] H. Eisenberg and G.M. Tomkins, J. Mol. Biol. 31 (1968) 37.
[2] K.-O. Mosebach and M. Kempfle, Z. Naturforsch. 24b (1969) 580.
[3] H. Sund and W. Burchard, European J. Biochem. 6 (1968) 202.
[4] M. Kempfle, K.-O. Mosebach and C. Blanke, Z. Naturforsch. 25b (1970) 273.
[5] J.A. Olson and C.B. Anfinsen, J. Biol. Chem. 197 (1952) 67.
[6] M. Kempfle and K.-O. Mosebach, Wintertagung der deutschen Gesellschaft für Biologische Chemie, Würzburg 1970.
[7] M. Kempfle, S. Kempfle and H. Winkler, to be published.
[8] M. Kempfle, K.-O. Mosebach, R. Müller and H. Winkler, to be published.
[9] H. Eisenberg: in: Pyridine Nucleotide Dependent Dehydrogenases, ed. H. Lund (Springer-Verlag, Berlin, Heidelberg, New York) p. 301.
[10] B. Eisenkraft, J.B. van Dort and C. Veeger, Biochim. Biophys. Acta 185 (1969) 9.
[11] R.F. Steiner, Arch. Biochem. Biophys. 39 (1952) 333.