MOLECULAR PROPERTIES OF YEAST GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN THE PRESENCE OF ATP AND KCl

Peter Bartholmes and Rainer Jaenicke

Biochemie II, Fachbereich Biologie,
Universität Regensburg, Germany

Received March 25,1975

SUMMARY. The influence of ATP and KCl on the quaternary structure and the enzymatic activity of D-glyceraldehyde-3-phosphate dehydrogenase from yeast(Y-GAPDH) has been studied by ultracentrifugation, gel chromatography and standard optical tests. In 0.1 M imidazole buffer pH 7.0, at low temperature (0° C) both complete deactivation and dissociation to dimers occur in the presence of 2 mM ATP and 0.1 M 2-mercaptoethanol. In 0.067 M phosphate buffer pH 7.0, containing 2 mM ATP and 1 mM dithiothreitol, only slight deactivation paralleled by minor changes of the native quaternary structure is observed. In this same buffer, increasing temperature leads to stabilization of both the tetrameric state and the catalytic activity of the enzyme. Deactivation and dissociation in the presence of 0.15 M KCl (in 0.2 M glycine buffer $9.1 \geq pH \geq 8.0$) is a function of pH rather than electrolyte concentration; at neutral pH the enzyme is stabilized in its native state. Contrary to earlier assumptions in the literature, ATP and KCl under the above experimental conditions do not appear to play an important role in the in vivo regulation of Y-GAPDH.

INTRODUCTION. The effect of adenine nucleotides on the regulation of key enzymes of the carbohydrate metabolism, e.g. glycogenphosphorylase, phosphofructokinase and fructose-diphosphatase is well established (1-3). More recently similar effects including reversible dissociation and deactivation were reported for the interaction between D-glyceraldehyde-3-phosphate dehydrogenase and ATP (4-6). The allosteric effect of NAD^+ on Y-GAPDH (7-10) suggests that regulation of this enzyme by specific effectors is possible. On the other hand the experimental conditions leading to the observed alterations of the catalytic and structural properties are remote from physiological conditions (4, 5, 11). Therefore, the biological significance of the deactivation and/or dissociation by ATP and KCl was checked,

Abbreviations: Y-GAPDH, D-glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) from yeast; DTT, dithiothreitol; MEt, 2-mercaptoethanol

analyzing the influence of solvent parameters on the molecular properties of the enzyme. Subunit dissociation and deactivation are favoured by low temperature, alkaline pH and high concentrations of 2-mercaptoethanol as shown by ultracentrifugation, gel chromatography and enzymatic tests. At room temperature, pH 7.0 and low concentrations of dithiothreitol the native state is not affected by either ATP or KCl, thereby indicating that neither effector is important in the regulation of Y-GAPDH.

MATERIALS AND METHODS. Apo Y-GAPDH was purified according to Kirschner and Voigt (12). NADH, phosphoglycerate kinase, 3-phosphoglycerate and ATP were purchased from Boehringer, Mannheim; DTT from Calbiochem. All other substances were of A grade purity (Merck, Darmstadt). Quartz double distilled water was used throughout. Enzymatic activity was measured in a combined optical test using 3-phosphoglycerate as substrate and phosphoglycerate kinase as indicator enzyme (13). The decrease of NADH extinction at 340 nm was recorded in an Eppendorf spectrophotometer (25° C). The specific activity of the freshly prepared, electrophoretically pure enzyme was 130-140 IU/mg.
Ultracentrifugation analysis made use of sedimentation velocity and high speed sedimentation equilibrium experiments in an analytical ultracentrifuge (Beckman Model E) equipped with schlieren optics and a high sensitivity UV scanning system. 12 mm double sector cells were used in the wavelength range between 280 and 295 nm.

Ascending gel chromatography on Sephadex G-100 was performed at 4° C using Pharmacia columns 85 x 1.6 cm and Vario Perpex pumps (LKB, Uppsala) with flow rates of 5-7 ml/h. Incubation for deactivation experiments with the enzyme under various solvent conditions was performed for 0.5 - 2 hrs in the respective buffers. For the standard optical test the enzyme solution was pre-equilibrated in the test medium for 2 minutes at 25° C in thermostated quartz cuvettes, starting the reaction with phosphoglycerate kinase.

The temperature dependence of the enzymatic activity was measured after incubating the enzyme for 2 hrs at given temperatures. Immediately afterwards the optical test was performed at the same temperature, giving data used in Arrhenius plots.

RESULTS AND DISCUSSION. In order to investigate the effect of ATP or KCl on the structural and catalytic properties of Y-GAPDH, various parameters of the solvent which influence the activity of the enzyme were analyzed for their participation in the ligand induced effects.
As shown in Fig. 1 the enzymatic activity of Y-GAPDH is influenced by the solvent media which were used in earlier dissociation experiments (4, 5, 11) even in the absence of ATP and KCl. Complete deactivation of the enzyme is observed at 0° C in the presence of 0.1 M 2-mercaptoethanol, independent of the buffer used. However, deactivation in 0.1 M imidazole after applying

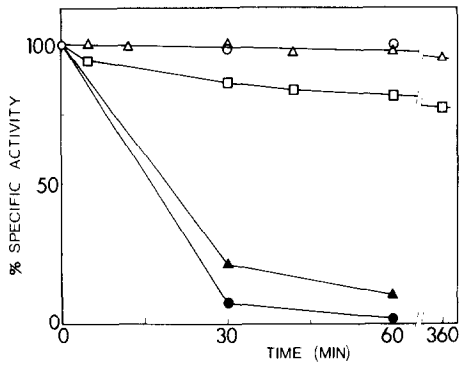

Fig. 1. Effect of different incubation media at 0°C on the enzymatic activity of Y-GAPDH in the absence of ATP (c = 0.01 mg/ml).
0.067 M phosphate buffer pH 7.0: △ + 1 mM DTT, ▲ + 0.1 M MEt
0.1 M imidazole buffer pH 7.0: □ + 1 mM DTT, ● + 0.1 M MEt, ○ + 0.1 M MEt + 0.15 M KCl. Incubation procedure as described in methods.

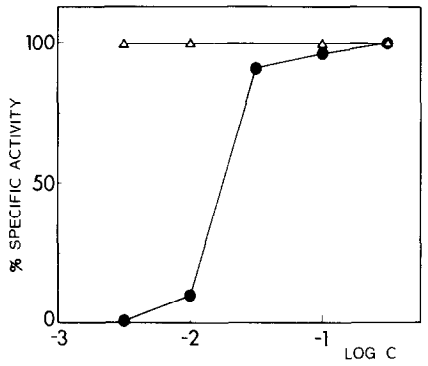

Fig. 2. Concentration dependence of the specific activity of Y-GAPDH in the absence of ATP(pH 7.0, incubation 1 hour). Enzyme concentration in mg/ml.
△ , 0.067 M phosphate buffer pH 7.0 + 1 mM DTT
● , 0.1 M imidazole buffer pH 7.0 + 0.1 M MEt

---

1 mM DTT as an SH-protecting agent does not exceed 25 % over a period of at least 6 hours. In phosphate buffer about 95 % of the activity is preserved within the given time range. A similar stabilizing effect of phosphate/DTT is observed for the concentration dependent deactivation of the enzyme (Fig.2). While the specific activity in 0.1 M imidazole/2-mercaptoethanol depends markedly on enzyme concentration, there is no change detectable in 0.067 M phosphate/1 mM DTT over the range of $0.002 \leq c \leq 0.5$ mg/ml. Further

evidence for the destabilizing influence of 2-mercaptoethanol is gained from a comparison of the effect of 2 mM ATP at 0° C in the presence of either 0.1 M 2-mercaptoethanol or 1 mM DTT in imidazole and phosphate buffer (Fig. 3): In the presence of 2-mercaptoethanol complete deactivation is observed while in the presence of 1 mM DTT only slight deactivation occurs. As reported by Constantinides and Deal (11) glycine buffer pH 9.1 at 0° C in the presence of 0.15 M KCl leads to deactivation which is reversed at 20° C. At neutral pH this effect disappears (Fig. 4) suggesting that deactivation is caused by high pH rather than increased electrolyte concentration. On the other hand data in Fig. 1 and Table 1 show 0.15 M KCl at neutral pH to have a stabilizing effect on both enzymatic activity and subunit structure.

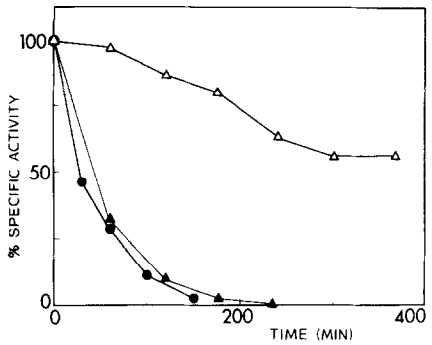

Fig. 3. Influence of 2mM ATP on the deactivation of Y-GAPDH(c=0.05 mg/ml). 0.067 M phosphate buffer pH 7.0 : △ + 1 mM DTT, ▲ + 0.1 M MEt 0.1 M imidazole buffer pH 7.0 : ● + 0.1 M MEt

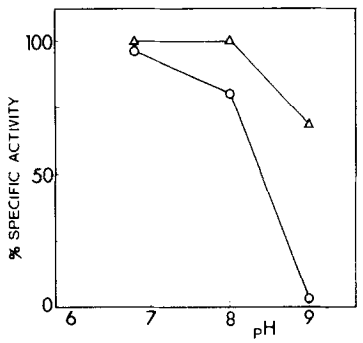

Fig. 4. pH dependence of the deactivation of Y-GAPDH(c=0.02 mg/ml) in 0.15 M KCl (0.2 M glycine buffer + 0.1 M MEt). △, 25°C ; ○ , 0°C

Table 1. Sedimentationcoefficients of Y-GAPDH in different buffer systems

Medium	pH	t°C	c	$s_{20,w}$ (S)
0.067 M phosphate + 1 mM DTT	7.0	33	0.10	7.45 ± 0.05
		20	0.10	7.49 ± 0.03
		4	0.10	7.50 ± 0.05
0.03 M phosphate + 1 mM DTT + 2 mM ATP	7.0	23	0.65	7.38 ± 0.08
		4	0.50	7.06 ± 0.08
0.1 M imidazole + 1 mM DTT	7.0	20	0.10	7.34 ± 0.05
		4	0.10	7.32 ± 0.06
0.2 M glycine + 0.15 M KCl + 0.1 M 2-mercaptoethanol	7.0	4	0.60	7.11 ± 0.05
0.2 M glycine + 0.15 M KCl + 1 mM DTT	7.0	4	0.70	7.51 ± 0.07

Enzyme was incubated at given concentrations (c : mg/ml) for 2 hours in the respective buffers. Centrifugation at various speeds in the range 40 000 - 56 000 rpm. Other conditions see Materials and methods.

Gel chromatography indicates that there is no appreciable dissociation of subunits under the conditions of inactivation unless 0.1 M imidazole + 0.1 M 2-mercaptoethanol + 2 mM ATP are applied (Fig. 5).

The deactivation under stabilizing conditions (0.067 M phosphate + 1 mM DTT + 2 mM ATP) cannot unambiguously be correlated with subunit dissociation since the main peak of the elution profile apparently belongs to the slightly modified tetramer while the trailing edge points to partial dissociation. The decrease of the sedimentation coefficient by $\Delta s \approx 0.4$ S in the presence of ATP seems to corroborate this result (Table 1). On the other hand high-speed sedimentation equilibria at 4° C yield a weight average molecular weight of $M_w = 134\,000 \pm 7\,000$. There are no measurable deviations from this value over a concentration range of 0.001 - 1 mg/ml. This suggests that even under conditions of maximum deactivation the deviation from $M_{tetramer}$ results either from a change in the partial specific volume or inaccuracy in molecular weight determination (16), rather than from significant dissociation of the enzyme.

The Arrhenius plot of the deactivation reaction in the presence of ATP (Fig. 6) does not show a characteristic transition. Instead, a smooth curve

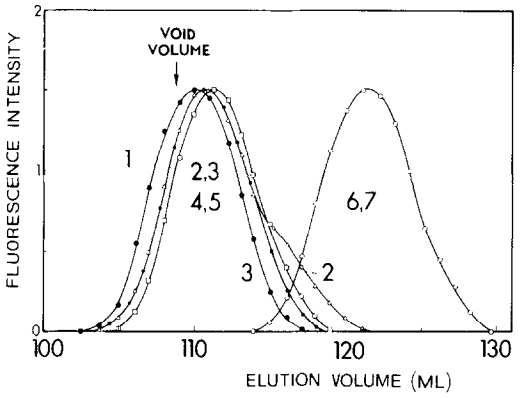

Fig. 5. Elution pattern of Y-GAPDH (c=0.05 mg/ml)[a] in different incubation media: Gel chromatography on Sephadex G 100, pH 7.0

1 0.1 M imidazole + 0.1 M MEt (K_{av} = 0.011)
2 0.06 M phosphate buffer + 1 mM DTT + 2 mM ATP (K_{av} = 0.013)
3 0.1 M imidazole buffer + 1 mM DTT (K_{av} = 0.013)
4 0.06 M phosphate buffer + 1 mM DTT (K_{av} = 0.015)
5 0.06 M phosphate buffer + 0.1 M MEt (K_{av} = 0.015)
6 0.1 M imidazole buffer + 0.1 M MEt + 2 mM ATP (K_{av} = 0.925)
7 0.1 M imidazole buffer + 1 mM DTT, incubation 70 hrs (K_{av} = 0.925)

a K_{av}-values remain unchanged at c=0.01-0.7 mg/ml, standard deviation $\pm$ 6%. Incubation time in the respective media before application: 1-2 hrs. Excitation at 280 nm, emission at 350 nm.

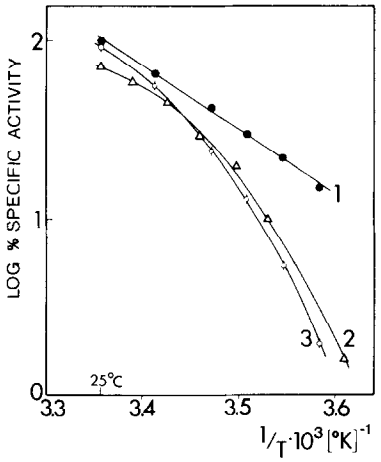

Fig. 6. Temperature dependence of the specific activity of Y-GAPDH. (0.025 M phosphate buffer pH 7.0 + 0.2 mM DTT + 1 mM EDTA)
1, c = 0.2 mg/ml, no ATP ; 2, c = 0.2 mg/ml + 2 mM ATP ;
3, c = 0.002 mg/ml + 2 mM ATP.
Measurements after 2 hours incubation, starting from 4°C.

is obtained which again is in contrast to typical temperature dependent dissociation-association reactions (e.g. 17, 18). The only conditions in the gel chromatographic experiments (Fig. 5) which cause complete dissociation to the dimer (K_{av} = 0.925) are either imidazole + 2-mercaptoethanol + ATP (at 0° C) or long incubation in imidazole + DTT (70 hours at 0° C). In both cases deactivation turns out to be irreversible towards increasing temperature.

CONCLUSION. Contrary to earlier hypotheses (4-6) ATP or KCl-induced dissociation and/or deactivation does not appear to play an important role in the regulation of Y-GAPDH in vivo. Although the effective ATP concentration is similar to the ATP level reported for yeast cells under normal conditions (15), the unspecific effect of high concentrations of 2-mercaptoethanol in connection with low temperature, high pH and the low concentration of the enzyme do not correspond to physiological conditions. Useful as the investigation of interactions of electrolytes and other ligands with a specific enzyme may be regarding the general features of subunit interactions, the biological implications have to be discussed with caution considering the in vivo situation.

ACKNOWLEDGEMENTS. Work was supported by grants of the Deutsche Forschungsgemeinschaft (to R. J.) and the Fonds der Chemie. Excellent technical assistance of Mrs. M. Laepple and Miss E. Kraus is gratefully acknowledged. We extend our special thanks to Dr. H. and Mrs. G. Durchschlag for the donation of the enzyme used in this study.

REFERENCES.

1. Krebs, E.G. and Fischer, E.H. (1962) Advan. Enzymol. 24, 263-290
2. Taketa, K. and Pogell, B.M. (1963) Biochem. Biophys. Res. Commun. 12, 229-235
3. Bonsignore, A. et al. (1963) J. Biol. Chem. 238, 3151-3154
4. Stancel, G.M. and Deal, W.C. jr. (1968) Biochem. Biophys. Res. Commun. 31, 398-403
5. Stancel, G.M. and Deal, W.C. jr. (1969) Biochemistry 8, 4005-4011
6. Yang, S.T. and Deal, W.C. jr. (1969) Biochemistry 8, 2806-2820
7. Oguchi, M. et al. (1973) J. Biol. Chem. 248, 5571-5576
8. Kirschner, K. et al. (1966) Proc. Natl. Acad. Sci(US) 56, 1661-1667
9. Jaenicke, R. and Gratzer, W.B. (1969) Eur. J. Bioch. 10, 158-164
10. Fuller Noel, J.K. and Schumaker, V.N. (1972) J. Mol. Biol. 68, 523-532
11. Constantinides, S.M. and Deal, W.C. (1970) J. Biol. Chem. 245, 246-253

12. Kirschner, K. and Voigt, B. (1968)Hoppe-Seylers Z. Physiol. Chem. 349, 632-644
13. Beisenherz, G. et al. (1953) Z. Naturforsch. 8b, 555-577
14. Shaltiel, S. et al. (1965) Biochemistry 5, 2108-2116
15. Betz, A. and Moore, C. (1957)Arch. Biochem. Biophys. 120, 268-273
16. Jaenicke, R. et al. (1968) Biochemistry 7, 919-926
17. Lauffer, M. A., Entropy-driven processes in biology, (Mol. Biol., Biochem. and Biophys. Vol. 20), Berlin, Heidelberg, New York, 1975
18. Adiarte, A. L. et al. Biochem. Biophys. Res. Commun., in press