The effect of dimethylformamide on phosphoglyceraldehyde dehydrogenase

In earlier communications we have reported that the steric structure of phosphoglyceraldehyde dehydrogenase is comparatively labile. Relative small alterations in the steric structure (changes in the state of H-bonds, of sulfhydryl and polar groups, displacement of the firmly bound coenzyme) leads to striking changes in its enzymic activity as well as in its structure[1,2]. We believe that the reason for this lability is that the enzyme contains no disulfide linkages[3], which are particulary important for the stabilisation of the steric structure of other protein molecules.

The present paper reports some data on the importance of the apolar amino acid side chains for the steric structure and biological activity. According to YANG AND DOTY[4] and BRESSLER *et al.*[5] the water-miscible N,N'-dimethyl formamide is able to change the conformation of proteins by solvation of their apolar amino acid residues. Changes in the enzymic activity as well as in the optical rotation and viscosity of pig-muscle phosphoglyceraldehyde dehydrogenase by treatment with dimethylformamide have been studied.

(1) Physico-chemical measurements (Table I) revealed that progressive solvation of the apolar groups results mainly in swelling of the protein, which causes an increase in the hydrodynamic volume. The changes in the viscosity are more pronounced than those in the optical rotation. 25 % or more dimethylformamide suddenly and irreversibly denatures the protein.

TABLE I

THE EFFECT OF DIMETHYLFORMAMIDE ON THE SPECIFIC OPTICAL ROTATION AND VISCOSITY OF PIG-MUSCLE PHOSPHOGLYCERALDEHYDE DEHYDROGENASE

Dimethylformamide (%, v/v)	*Specific optical rotation* $[\alpha]_D^{20}$		*Intrinsic viscosity** $[\eta] \times 10^2$, *dl/g*	
	Time of preincubation (min)		*Buffers*	
	10	*30*	*0.2 M phosphate, pH 6.5*	*0.2 M glycine, pH 8.4*
0	−31.3	−31.3	2.95	3.35
5	—	—	3.45	4.28
10	−32.8	−33.0	5.10	5.90
15	−36.0	−36.9	6.40	7.95
20	−38.4	—	7.55	6.75

* Time of preincubation 15 min. The data are averages of measurements carried out between 15 to 40 min after addition of dimethylformamide.

In contrast to serum albumin[5] the swelling and unfolding of phosphoglyceraldehyde dehydrogenase following apolar solvation of the hydrophobic groups causes a rapid and irreversible breakdown of its steric structure. Serum albumin when treated with hydrophobic solvents responds by "accommodation", *i.e.* the molecule turns with its hydrophobic amino acid residues towards the solvent.

(2) Two phases can be distinguished in the effect of DMF on the enzymic activity (Fig. 1):

Abbreviations: DPN, diphosphopyridine nucleotide.

(a) When dimethylformamide is added immediately before determination of the activity by Warburg's optical method the velocity of DPN reduction increases with increasing dimethylformamide concentration up to 15 % (v/v). At this point the activity is about 50 % higher than that of the control sample. Further increases in concentration of dimethylformamide (from 15 to 25 %) causes a decrease of the activity to the original value.

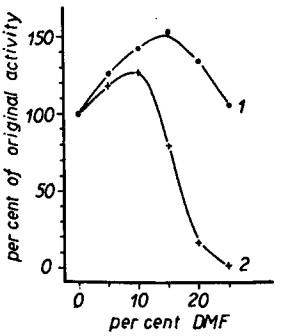

Fig. 1. The effect of dimethylformamide on the enzymic activity of pig- muscle phosphoglyceraldehyde dehydrogenase. Reaction mixture: D-glyceraldehyde 3-phosphate 0.1 μmole; DPN, 0.08 μmoles; arsenate 0.1 μmole; phosphoglyceraldehyde dehydrogenase, $2 \cdot 10^{-5}$ μmole; cysteine 0.5 μmole; dimethylformamide (DMF) and 0.2 *M* glycine buffer up to an and volume of 3.0 ml. Final pH 8.4. Curve 1, no preincubation of enzyme and dimethylformamide; curve 2, pre-incubation for 60 min at room temperature.

(b) When the enzyme is preincubated with dimethylformamide at room temperature for 30 to 60 min, activation is observed only up to about 10 % dimethylformamide. Treatment for 60 min with 25 % dimethylformamide causes almost complete inactivation.

We conclude that an aqueous medium may not represent the optimal conditions for maximal activity of phosphoglyceraldehyde dehydrogenase. It seems that up to a point, a small swelling of the protein molecule increases the catalytic function.

Detailed experimental data on the effect of dimethylformamide on phosphoglyceraldehyde dehydrogenase and other enzymes will be published elsewhere.

Institute of Biochemistry, Hungarian Academy of Sciences, Budapest (Hungary) PÀL ELÖDI

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Received September 17th, 1960