BBA 63415

Stabilization of lactate and malate dehydrogenase by organic solvents

Many enzymes are rather unstable and exhibit a tendency to denature upon storage, even at low temperature. There is thus a great need to develop methods for preserving enzymes in a stable and fully active form. Much work has been done on the preparation of insoluble derivatives of various enzymes, beginning with the work of NELSON AND GRIFFIN[1], who adsorbed invertase on charcoal and alumina and found that the bound enzyme was active. KAY[2] has recently reviewed the methods of enzyme insolubilization which have been thus far used. Observations on the stabilizing effects of various additives have been published from time to time. A number of enzymes can be stabilized by adding reducing agents, chelating compounds, competitive inhibitors, co-enzymes and substrates or their analogues. Recently, TAKEMORI *et al.*[3] reported that benzoyl alcohol dehydrogenase from Pseudomonas sp., homogentisicase from bovine liver and alcohol dehydrogenase from yeast are stabilized by low concentrations of acetone and ethanol. The present communication describes a phase of our current work on the stabilization of enzymes in which organic additives have been used in an effort to stabilize lactate dehydrogenase (EC 1.1.1.27) and malate dehydrogenase (EC 1.1.1.37).

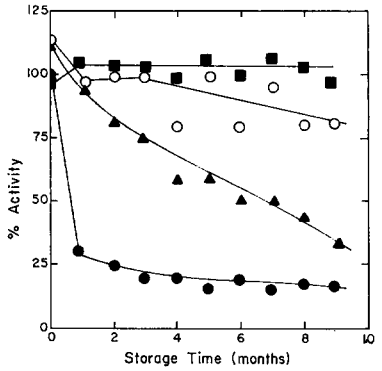
Lactate dehydrogenase (beef heart) was observed to have a half-life of from 2 to 3 weeks when stored in the cold ($4 \pm 2°$) at an enzyme concentration of 1.5 μg/ml[4].

TABLE I

EFFECT OF ORGANIC COMPOUNDS ON STABILITY OF BEEF HEART LACTATE DEHYDROGENASE

Values expressed are for beef heart lactate dehydrogenase after long-term storage in the cold ($4 \pm 2°$). After storage for the time periods indicated, 0.20-ml aliquots were transferred to the assay reaction mixtures and assayed as described in Fig. 1. Higher enzyme concentrations (*e.g.* 50–100-fold) also have a stabilizing activity, providing approximately a 50% increase in enzyme stability over the time intervals shown. All values are $\pm$ 5% error. Methanol and ethanol concentrations; % (v/v).

Compound	*Concn. (%)*	*% Activity after storage time (weeks)*					
		0	*1*	*4*	*12*	*24*	*36*
Control		100	65	29	18	18	16
Gelatin	0.25	104	118	86	84	81	63
	0.5	106	118	88	86	76	70
	1.0	100	97	60	60	59	60
Bovine serum albumin	0.025	96	76	42	29	30	11
	0.05	95	76	37	28	28	18
	0.10	100	72	28	22	24	18
Methanol	2.5	100	70	50	44	37	26
	5.0	100	80	55	53	35	16
	10	100	65	51	51	48	28
Ethanol	2.5	100	72	50	39	30	8
	5.0	96	80	60	46	48	36
	10	93	93	60	51	52	37



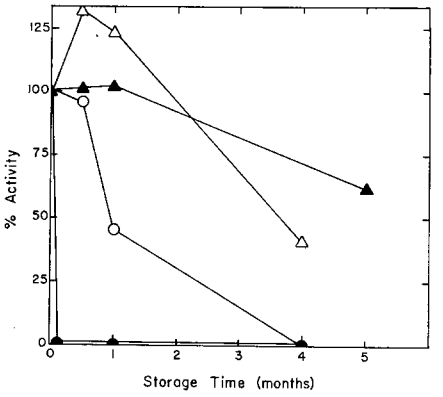


Fig. 1. Stability of lactate dehydrogenase in organic solvents. Enzyme (1.5 μg/ml) was stored at 4 $\pm$ 2° in 0.002 M potassium phosphate buffer (pH 7.0) containing different concentrations of additive. At the intervals indicated, 0.20-ml aliquots were transferred to the assay reaction mixture. The assay reaction mixtures contained: pyruvate (1 μmole); NADH (0.2 μmole); potassium phosphate buffer (80 μmoles; pH 7.4) and enzyme (0.3 μg) in a final volume of 3.0 ml. The reaction was at room temperature (25 $\pm$ 2°) and was followed by measuring the decrease in absorbance at 340 mμ as a function of time for 1 min. Dimethylsulfoxide concentrations (v/v): ■—■, 40%; ○—○, 30%; ▲—▲, 20%; ●—●, none.

Fig. 2. Stability of malate dehydrogenase in organic solvents. Enzyme (1.2 μg/ml) was stored at 4 $\pm$ 2° in 0.005 M potassium phosphate buffer (pH 7.4), which contained different concentrations of additive. At the times indicated, 0.06-ml aliquots were transferred to the assay reaction mixtures and assayed as described in Table II. The malate dehydrogenase samples were made up as follows: ▲—▲, 0.1% gelatin *plus* 30% ethanol; △—△, 0.1% gelatin *plus* 70% glycerol; ○—○, 70% glycerol; ●—●, none.

The addition of dimethyl sulfoxide, ethanol or methanol caused significant stabilization of lactate dehydrogenase to long-term storage in the cold (4 $\pm$ 2°)[4]. Thus, in the presence of 40% dimethylsulfoxide, the enzyme retained 100% of its original activity after storage for 9 months. Experiments shown in Fig. 1 indicate that after 1-month storage, lactate dehydrogenase (1.5 μg/ml) at 4 $\pm$ 2° and pH 7.0, lost 50% of its original activity but that no inactivation occurred in the presence of 40% dimethyl sulfoxide.

As shown in Table I, whereas gelatin protects against inactivation for 7 months, bovine serum albumin does not; in addition, methanol and ethanol also offer some stabilization.

Pig heart malate dehydrogenase had a half-life of about 30 min when stored in the cold (4 $\pm$ 2°) in a final concentration of 0.12 μg/ml[5]. Of the compounds tested, ethanol, *i*-propanol, *n*-butanol, *i*-butanol, acetone, dioxane and glycerol were the most effective in partially stabilizing this enzyme (Table II). Mixtures of glycerol and gelatin were found to result in significant stabilization of malate dehydrogenase not only in short-term studies but also during long-term storage in the cold (4 $\pm$ 2°)[5]. In the presence of 70% glycerol and 0.1% gelatin, the enzyme (1.2 μg/ml) was found to possess 100% of its original activity after storage for 7 weeks as shown in Fig. 2. The control (no additive) had a half-life of 5–10 h.

The mechanism by which these enzymes are stabilized by solvents and added proteins requires study. It seems possible that stabilization is associated with mainte-

TABLE II

EFFECT OF ORGANIC SOLVENTS ON STABILITY OF PIG HEART MALATE DEHYDROGENASE

Enzyme activities are expressed as 50% activity for samples containing zero concentration of organic compound. Values expressed are for pig heart malate dehydrogenase after 30-min incubation. The reaction mixtures contained: oxaloacetate (0.3 μmole); NADH (0.2 μmole); potassium phosphate buffer (280 μmoles; pH 7.4) and enzyme (0.24 μg) in a final volume of 3.0 ml. The reaction was run at room temperature (approx. 25°) and was followed by measuring the decrease in absorbance at 340 mμ (absorption maximum for NADH) as a function of time. All values are ± 5% error.

Compound	*% of activity at solvent concentration (v/v)*								
	0%	*5%*	*10%*	*20%*	*30%*	*40%*	*50%*	*60%*	*70%*
Methanol	50	51	63	62	59	63	66	40	20
Ethanol	50	75	72	87	87	76	79	68	62
n-Propanol	50	74	78	77	31	11	—	—	—
i-Propanol	50	85	—	99	105	—	—	—	—
n-Butanol	50	84*	67**	—	—	—	—	—	—
i-Butanol	50	79*	88**	—	—	—	—	—	—
Acetone	50	76	77	95	99	109	101	81	—
Dioxane	50	86	94	77	77	—	—	—	—
Dimethylsulfoxide	50	50	32	42	38	—	—	—	—
Dimethylsulfone	50	48	—	83***	—	—	—	—	—
Glycerol	50	59	57	82	96	109	94	107	109
Tetrahydrofuran	50	59	63	73	78	21	—	—	—

* 1.5% (v/v).
** 2.5% (v/v).
*** 15% (v/v).

nance of the enzymes in the "native" or "active" conformational state, by exclusion or replacement of water molecules.

We thank Dr. Alton Meister and Dr. Orrie M. Friedman for their helpful advice and guidance. The investigation was supported by a contract from the U.S. National Aeronautics and Space Administration.

Biochemical Laboratories,
Collaborative Research, Inc.
Waltham, Mass. (U.S.A.)

H. GEORGE
J. MCMAHAN
K. BOWLER
M. ELLIOTT

1 J. M. NELSON AND E. G. GRIFFIN, *J. Am. Chem. Soc.*, 38 (1961) 1109.
2 G. KAY, *Process Biochem.*, 3 (1968) 36.
3 S. TAKEMORI, E. FURUYA, H. SUZUKI AND M. KATAGIRI, *Nature*, 215 (1967) 417.
4 H. GEORGE, J. MCMAHAN AND K. A. HERRINGTON, *Abstr. 156th Nat. Meeting Am. Chem. Soc., Atlantic City, N.J., 1968.*
5 H. GEORGE, M. E. ELLIOTT AND J. MCMAHAN, *Abstr. 1st Northeast Regional Meeting Am. Chem. Soc., Boston, Mass., 1968.*

Received May 16th, 1969