EFFECT OF POLYHYDRIC ALCOHOLS ON KINETIC PARAMETERS OF ENZYMES

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SUMMARY: The effect of glycerol and other polyhydric compounds on the kinetic parameters of sixteen enzymes has been investigated. Substantial changes in K_m and turnover rate were observed. Kinetic values obtained depended on pH as well as on the specific polyhydric compound added and its concentration. No pattern in direction or extent of change in kinetic constants was evident.

Although polyhydric alcohols have been widely used as stabilizing agents for biological systems, the means by which stabilization is brought about is unknown and knowledge of the effect on kinetic parameters is equally limited. Recently, an aldehyde dehydrogenase was found to have a higher affinity for substrate in the presence of glycerol, an effect ascribed to a change in enzyme conformation (1), and several reports have noted changes in the kinetic constants of other enzymes (2-4). If such properties are widespread, caution should be exercised in interpreting the kinetics of enzyme systems in which polyhydric compounds are included.

The present communication describes the results of an investigation of the action of several polyhydric compounds, particularly glycerol, on the kinetic parameters of a number of enzymes.

MATERIALS AND METHODS: The following sources were used for reagents and enzymes: reagent and spectral grade glycerol (Fisher Scientific); "Chromatoquality" ethylene glycol (Matheson, Coleman, and Bell); Enzyme-grade sucrose (Mann Research Laboratories); alkaline phosphatase from Escherichia coli, alcohol dehydrogenase from yeast, diaphorase and xanthine oxidase (Sigma); aldolase, glyceraldehyde-3-phosphate dehydrogenase from rabbit

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muscle, glucose-6-phosphate dehydrogenase from yeast, sorbitol dehydrogenase, glutamate dehydrogenase and glyoxylate reductase (Boehringer-Mannheim); phosphodiesterase I, β-galactosidase, trypsin, and α-chymotrypsin (Worthington). Micrococcal nuclease and deoxythymidine-5′-p-nitrophenylphosphate-3′-phosphate were gifts of Dr. Benjamin Dunn and hydroxypyruvate reductase was a gift from Dr. Leonard Kohn.

All assays were performed with either a Gilford Model 2000 or a Cary 15 recording spectrophotometer, each equipped with an expanded scale and a constant temperature device at 25°.

RESULTS AND DISCUSSION: The Michaelis constant and turnover rate for sixteen enzymes were measured in the presence and absence of 30% glycerol (v/v); a concentration of 30% was selected because it appears to be in the range found to stabilize proteins. From the data presented in Table I it is clear that each of the sixteen enzymes tested underwent a change in either K_m or turnover number or both as the result of the presence of this reagent. At the extremes are the K_m for o-nitrophenyl-β-galactoside, which increased 270-fold in the presence of glycerol and the K_m for FDP, which decreased 6-fold. With two exceptions, phosphodiesterase and trypsin, the turnover rate in the presence of glycerol is substantially decreased.

With many enzymes the addition of glycerol resulted in a decreased turnover rate and an unaltered or increased K_m, a situation suggesting the role of glycerol as a classical inhibitor. Kinetic studies, in which glycerol concentration was varied with substrate, revealed a pattern of mixed inhibition in the cases of alkaline phosphatase, β-galactosidase, and hydroxypyruvate reductase. In the diaphorase system glycerol appeared to be noncompetitive with DPNH. However, sorbitol dehydrogenase did not exhibit a classical inhibition pattern. In this instance the concentration of the substrate, fructose, was sufficiently high to suggest that it also may have acted as a polyhydric compound.

The addition of glycerol does not always cause inhibition. For example,

TABLE I. Effect of 30% glycerol on kinetic constants of enzymes

Enzyme	Substrate[a]	Assay ref.	Normal		With glycerol	
			K_m	Turnover[b]	K_m	Turnover[b]
			mM		mM	
Alcohol dehydrogenase	DPN	5	0.23	49,000	0.063	8,000
Alcohol dehydrogenase	Ethanol	5	11	37,000	2.8	13,000
Aldolase	FDP	6[c]	0.62	750[d]	0.098	160[d]
Alkaline phosphatase	NPP	7	0.028	2,400	0.12	640
α-Chymotrypsin	ATEE	8	1.4	3,700	1.6	1,400
Diaphorase	DPNH	9	0.018	730	0.016	150
β-Galactosidase	ONPG	10	0.25	17,000	67	3,800
Glucose-6-P dehydrogenase	G-6-P	11	0.013	1,900	0.014	980
Glutamate dehydrogenase	L-Glutamate	12	1.9	250	0.44	75
Glyceraldehyde-3-P dehydrogenase	PGAL	13	0.17	3,500	0.20	1,100
Glyoxylate reductase	Glyoxylate	14	24	67[e]	26	13[e]
Hydroxypyruvate reductase	HP	15[f]	19	1,500	36	320
Micrococcal nuclease	NPpdTp	16	0.023	4.2	0.019	1.8
Phosphodiesterase I	bisNPP	17	0.49	0.042[e]	3.3	0.14[e]
Sorbitol dehydrogenase	Fructose	18	300	1,000[e]	1,430	430[e]
Trypsin	BAA	19[g]	1.8	3.5	8.0	16
Xanthine oxidase	Benzaldehyde	20	0.50	1,000	0.67	780

[a] The following abbreviations are used: FDP, fructose-1,6-diphosphate; NPP, p-nitrophenyl phosphate; ATEE, N-acetyltyrosine ethyl ester; ONPG, o-nitrophenyl-β-galactoside; G-6-P, glucose-6-phosphate; PGAL, glyceraldehyde-3-phosphate; HP, hydroxypyruvate; NPpdTp, deoxythymidine-5′-p-nitrophenyl-phosphate-3′-phosphate; bisNPP, bis(p-nitrophenyl)phosphate; BAA, benzoyl-L-arginine amide.

[b] Expressed as μmoles of product formed per minute per μmole of protein.

[c] Assayed in .05 M 3,6-endomethylene-1,2,3,6-tetrahydrophthalate, pH 6.6.

[d] Expressed as absorbance change per minute per μmole of protein.

[e] Expressed as μmoles of product formed per minute per mg of protein.

[f] Assayed at pH 6.6.

[g] Assayed in .05 M Tris-chloride, pH 7.6.

with trypsin and phosphodiesterase the overall reaction rate is stimulated by its addition, although the K_m is also increased; replacement of water by polyhydroxy compounds represents a possible mechanism (21). With aldolase, glutamate dehydrogenase, and alcohol dehydrogenase the opposite is the case; both the K_m and turnover are decreased.

TABLE II. Effect of pH on the kinetic constants of alcohol dehydrogenase as a function of glycerol

Buffer[a]	pH	Glycerol	DPN		Ethanol	
			K_m	Turnover[b]	K_m	Turnover[b]
		% (v/v)	mM	X 10^{-3}	mM	X 10^{-3}
EMTA[c], 50 mM	6.6	0	0.27	13	—	—
EMTA[c], 50 mM	6.6	30	0.25	5	—	—
EMTA[c], 50 mM	7.5	0	0.20	32	13	21
EMTA[c], 50 mM	7.5	30	0.16	15	10	13
Tris chloride, 50 mM	7.5	0	0.22	38	12	16
Tris chloride, 50 mM	7.5	30	0.22	19	10	5
Triethanolamine, 200 mM	8.0	0	0.21	45	11	36
Triethanolamine, 200 mM	8.0	30	0.22	20	7	17
Pyrophosphate, 32 mM	8.8	0	0.23	49	11	37
Pyrophosphate, 32 mM	8.8	30	0.06	8	3	13
Diethanolamine, 200 mM	9.2	0	0.21	19	—	—
Diethanolamine, 200 mM	9.2	30	0.05	3	—	—

[a] Ionic strength adjusted to 0.25 with KCl.

[b] Expressed as μmoles of product formed per minute per μmole of protein.

[c] EMTA is an abbreviation for 3,6-endomethylene-1,2,3,6-tetrahydrophthalate.

To characterize the effects of glycerol further, its interaction with alcohol dehydrogenase was studied as a function of pH (Table II). In the absence of glycerol, the constants for both substrates are independent of pH in the range from 6.6 to 9.0. In the presence of glycerol and at constant ionic strength, the K_m for both substrates is unchanged at pH 7.5, but at pH 8.8 and above the K_m is substantially decreased.

Since other polyhydric compounds, e.g. sucrose and ethylene glycol, have also been used for stabilization of enzymes, their effect on the kinetics of alcohol dehydrogenase was also tested (Table III). It is apparent that 30% ethylene glycol, sucrose, and glycerol do not affect the kinetic parameters identically. At pH 7.5, for example, the K_m with glycerol added is approximately the same as without the reagent, whereas sucrose or ethylene glycol increase K_m.

These results indicate that the presence of polyhydric compounds can

TABLE III. Effect of polyhydric alcohols on the kinetics of alcohol dehydrogenase

Solvent	DPN	
	K_m	Turnover[a]
	mM	X 10^{-3}
1. Pyrophosphate, 32 mM at pH 8.8	0.23	49
plus 10% (v/v) glycerol	0.21	35
plus 20% (v/v) glycerol	0.18	17
plus 30% (v/v) glycerol	0.06	8
plus 30% (v/v) ethylene glycol	0.14	19
plus 30% (w/v) sucrose	0.12	28
2. EMTA[b], 50 mM at pH 7.5	0.20	32
plus 30% (v/v) glycerol	0.16	15
plus 30% (v/v) ethylene glycol	0.31	16
plus 30% (w/v) sucrose	0.48	15

[a] Expressed as μmoles of product formed per minute per μmole of protein.

[b] EMTA is an abbreviation for 3,6-endomethylene-1,2,3,6--tetrahydrophthalate.

change enzyme kinetic parameters substantially, and that the extent and direction of change is not a readily predictable function of pH or of the concentration and nature of the polyhydric compound. Thus, caution is necessary in interpreting data from protein systems in which polyhydric compounds have been added as stabilizing agents. The fact is, little is known about the effect of these reagents, although there are indications that glycerol may induce protein conformational changes (1). Experiments in this direction are now in progress.

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