The Reaction of Epoxides with Yeast Glyceraldehyde-3-Phosphate Dehydrogenase

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SUMMARY: Yeast glyceraldehyde-3-phosphate dehydrogenase was found to react irreversibly with several epoxides. The reactivity, relative to model thiols, follows the order: glycidol phosphate > glycidol > propylene oxide. The substituent effect on the C-3 carbon parallels the effect of substituents on the reactivity of aldehyde substrates. Four sulfhydryl residues in the tetramer must be modified by the epoxides to completely inactivate the enzyme. Since glycidol phosphate does not show saturation kinetics most of the binding energy of the phosphate moiety is utilized to induce a conformation change which makes the subunit more reactive toward the epoxide. This conformation change is not transmitted across intersubunit domains.

Yeast glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.12) is a tetramer, composed of four identical polypepetide chains (1). NAD binding and chemical modification studies indicate ligand-dependent subunit interactions in this enzyme (2). Furthermore, this enzyme displays the interesting property of half-of-the-sites reactivity (3) with a variety of sulfhydryl reagents (4). Thus, when two of the four active-site sulfhydryl groups (cys-149) are modified the enzyme is catalytically inactive and the remaining two SH groups have diminished reactivity toward the modifier.

The presence of the phosphate substituent on the aldehyde substrate enhances its reactivity with the enzyme (4). To further clarify the relationship between subunit modification and the role of subunit interactions in the catalytic mechanism of action of glyceraldehyde-3-phosphate dehydrogenase, as well as the role of substituent effects on reactivity, we investigated a series of irreversible inhibitors which are structurally similar to the substrate glyceraldehyde-3-phosphate.

MATERIALS AND METHODS

Glyceraldehyde-3-phosphate dehydrogenase from Saccaromyces cerevisae was prepared and assayed as previously described (2). D,L-Glycidol was distilled prior to use. D,L-Glycidol phosphate was prepared as described by Rose and O'Connell (5).

The number of sulfhydryl residues modified were determined by incubating the enzyme with the epoxides and removing aliquots at various times. The aliquots were diluted into 6M guanidine-HCl (pH 8.5) and titrated with DTNB[1] (6) to determine the number of free sulfhydryl groups remaining. The concentration of the epoxides were in excess over the concentration of sulfhydryl groups by more than a factor of 100.

RESULTS AND DISCUSSIONS

The reactions of propylene oxide, glycidol and glycidol phosphate with glyceraldehyde-3-phosphate dehydrogenase were followed by the loss of enzymic activity. Incubation of the enzyme with 0.5M cyclohexylammonium phosphate (pH 8.5) for periods up to two hours resulted in no irreversible loss of enzymic activity. The reactions with the three epoxides follows pseudo first-order kinetics for over 90% of the reaction. The observed pseudo first-order rate constant for inactivation by glycidol phosphate is linearly dependent on the epoxide concentration (Figure 1). Glycidol and propylene oxide also show second-order kinetics up to the highest concentrations with these epoxides. The substrate, glyceraldehyde-3-phosphate competitively protects against inhibition by the epoxides.

The site of modification on the enzyme by the epoxides was determined to be a cysteine residue by titration with DTNB. The stoichiometry of sulfhydryl group loss indicates complete activity loss when four sulfhydryl residues are modified (Figure 2). In this respect the epoxides differ from the other alkylating reagents[iodoacetate, iodoacetamide, 1-fluoro-2, 4-dinitrobenzene, 2-bromoacetamido-4-nitrophenol, 4-bromoacetamido-2-nitrophenol, 5-bromoacetamido naphthalene-1-sulfonate, trifluoromethyl acrylonitrile, 3-(2-iodoacetamido)-2,2,5,5-tetramethyl 1-pyrrolidinyloxyl,

[1]Abbreviations used are: bicine, N,N-bis(2-hydroxyethyl) glycine; DTNB, 5,5′-dithiobis (2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; GSH, glutathione.

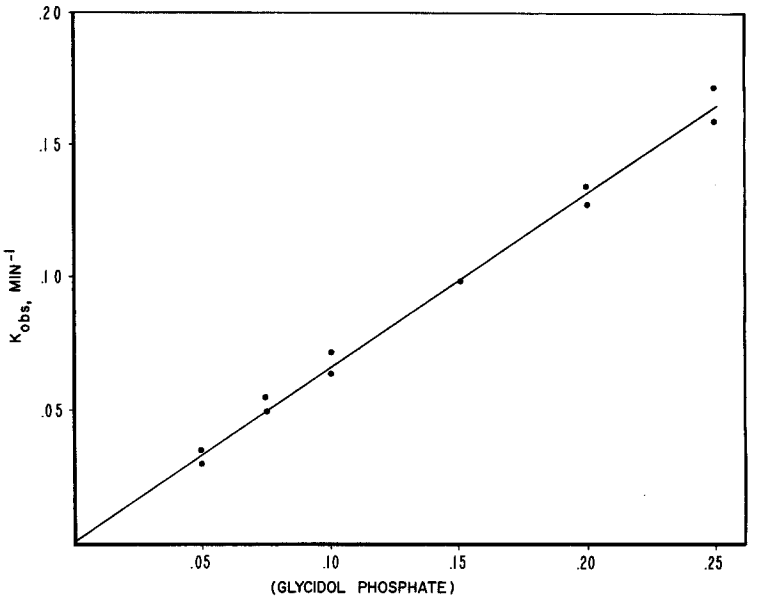

Figure 1. Dependence of the pseudo first-order rate constant (k_{obs}) for reaction of the epoxide with glyceraldehyde-3-phosphate dehydrogenase (0.05M bicine, 1mM EDTA, pH 8.5, 25°) on the concentration of glycidol phosphate (M). Ionic strength maintained at μ = 0.8M with Na_2SO_4. The enzyme concentration in the incubation mixture was 0.5 - 3.2 μM.

4,4´-difluoro-3,3´-dinitrophenyl sulfone, 1,5-difluoro-2,4-dinitrobenzene (7), 2-nitro-5-thiocyanobenzoate (8) and N-ethylmaleamide (9)] which are half-of-the-site reagents with the yeast enzyme.

The second-order rate constants for most of the alkylating reagents with the thiolate anion (k_2^{lim}) are larger with the enzyme than with model thiols by factors varying from 2 to 20. However, for propylene oxide and glycidol the reaction with the enzyme is slower than with model thiols while for glycidol phosphate a rate enhancement with the enzyme is observed (Table 1). NAD reduces the second-order rate constant for reaction of the epoxides with the enzyme. At high concentrations of NAD the enzyme is completely protected against inhibition by the epoxides. In contrast to NAD, NADH enhances reactivity of the enzyme towards glycidol phosphate. Thus, in the presence of 3.2 mM NADH the rate constant for inhibition by glycidol phosphate is increased 180%.

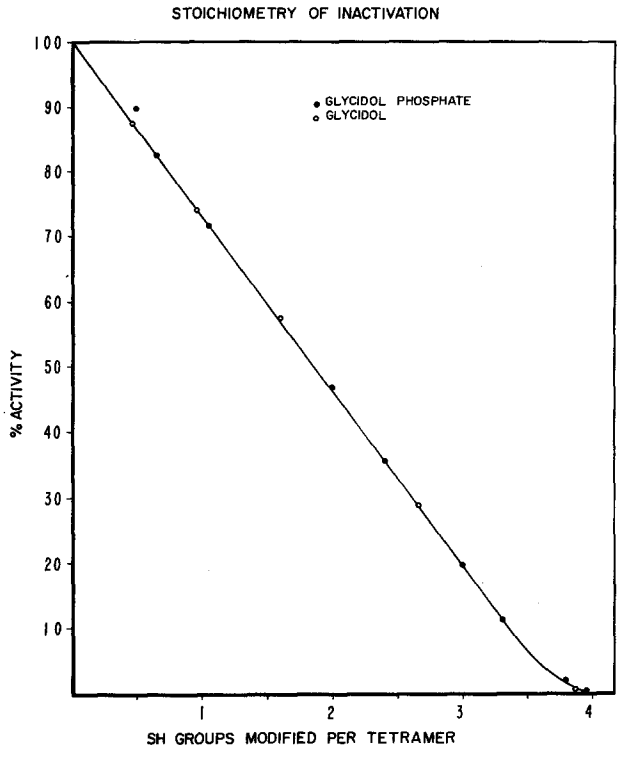

Figure 2. Stoichiometry of inactivation of glyceraldehyde-3-phosphate dehydrogenase by glycidol phosphate (●) and by glycidol (0). Conditions are the same as in Figure 1. The number of modified sulfhydryl groups were determined by titration with DTNB.

The high rate of reactivity of glycidol phosphate with the enzyme and the inhibition of the reaction by the substrate glyceraldehyde-3-phosphate indicates that the reactive residue is the active-site cys-149. It is unlikely that the SH group attacks glycidol phosphate at the C-2 position rather than the C-1 position because 1) at pH values greater than neutrality nucleophilic attack on epoxides occur at the least substituted carbon (10), 2) the phosphate residue is a major binding component of the substrate and this fixes the SH group and glycidol phosphate in an orientation favoring attack at C-1 and 3) both D-and L-glycidol phosphate react with a glutamate residue in triose phosphate isomerase (an enzyme for which glyceraldehyde-3-phosphate is a substrate) via nucleophilic attack at C-1 (11).

TABLE I: Substituent Effects

	$X-CH_2\overset{O}{\frown}CH-CH_2$		$X-CH_2CH_2CHO$	
X	k_2^a ($M^{-1}Min^{-1}$)	$\left[\frac{k_2^{lim}\ (enzyme)}{k_2^{lim}\ (GSH)}\right]^b_{rel}$	$V^c_{Max,rel}$	$\left(\frac{V_{Max}}{K_M}\right)^c_{rel}$
H-	0.17	1.0	1.0	1.0
HO-	0.29	4.7	2.0	7.1
$^{=}O_3PO-$	0.66	271	12	688

[a]pH 8.5, 25°, μ = 0.8M maintained with Na_2SO_4. [b]Ratio of limiting second-order rate constant for reaction of the epoxide with the enzyme (pK_a' = 8.5, ref. 8) to the limiting second-order rate constant for the reaction with glutathione (pK_a' = 8.1) normalized to the propylene oxide reaction. [c]Data for the rabbit muscle enzyme from Orsi and Cleland (14), saturating amounts of NAD and phosphate (pH 8.6, 30°).

Glycidol phosphate, then, most likely reacts with the enzyme to produce a tetra-(3-phospho-2,3-dihydroxy-n-propylated) enzyme. This enzyme has a very similar electronic spectra to that of the unmodified enzyme. From gel filtration studies both the modified and unmodified enzymes remain as tetramers. Thus, there is little detectable change in the gross conformation of the enzyme accompanying reaction with glycidol phosphate. Furthermore glycidol phosphate is not a half-of-the-sites reagent. Thus, modification of one of the subunits has no effect on the catalytic reactivity of the unmodified subunits nor is the reactivity of the unmodified subunit toward glycidol phosphate altered.

The lack of half-of-the-sites reactivity of glycidol phosphate suggests that the 3-phospho-2,3-dihydroxy-n-propylated enzyme more closely resembles the thiohemiacetal adduct than the phosphoglyceroylated enzyme. Glyceraldehyde-3-phosphate can simultaneously react with all four subunits of the enzyme (8,12) while only the diphosphoglyceroylated enzyme is stable (8). Furthermore, reaction of the diacylated enzyme with glyceraldehyde-3-phosphate has no effect on the rate of deacylation of the diacylated enzyme whereas reaction with 1,3-diphosphoglyceric acid increase the deacylation rate of the diacylated enzyme (13). Thus, the effect of the triose substrate on the subunit interactions is dependent on the hydridization state of the C-1 carbon adduct with the enzyme.

The effect of substituents on C-3 on the reactivity of the epoxides is interesting. The reactivity of $X\text{-}CH_2\overset{\diagup O \diagdown}{C}H\text{-}CH_2$ with glyceraldehyde-3-phosphate dehydrogenase relative to glutathione follows the order X $=^{=}O_3PO\text{->}$ X = HO-> X = H-. This is the same order observed by Orsi and Cleland (14) for reactivity with the rabbit muscle enzyme for the aldehyde substrates $X\text{-}CH_2CH_2CHO$. The fact that Vmax as well as V_{max}/K_M is affected by the substituent at C-3 (Table I) indicates that this substituent does more than just facilitate aldehyde binding. Indeed, none of the epoxides tested show saturation kinetics with the yeast enzyme (Ki>1M for glycidol phosphate). It is interesting, therefore, that glycidol phosphate is 20 times more reactive toward the enzyme than toward glutathione.

Since glycidol phosphate is more reactive toward the enzyme relative to model compounds than are the other epoxides and the glycidol phosphate reaction is not preceeded by a reversible binding step it is likely that the inherent phosphate binding energy is utilized to induce a conformation change which makes the enzyme more reactive toward the epoxide.

These results indicate the importance of conformation changes induced by ligands on the catalytic activity and chemical reactivity of glyceraldehyde-3-phosphate dehydrogenase.

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REFERENCES

1. Jones, G. M. and Harris, J. I. (1972) FEBS Lett., 22, 185-189.
2. Mockrin, S. C., Byers, L. D. and Koshland, D. E., Jr. (1975) Biochemistry, 14, 5428-5437.
3. Levitzki, A. and Koshland, D. E., Jr. (1976), Current Topics in Cellular Regulation, 10, 1-40.
4. Chance, B. and Park, J. H. (1967) J. Biol. Chem., 242, 5093-5105.
5. Rose, I. A. and O'Connell, E. L. (1969) J. Biol. Chem., 244, 6548-6550.
6. Ellman, G., Arch. Biochem. Biophys. (1959) 82, 70-77.
7. Stallcup, W. B. and Koshland, D. E., Jr. (1973) J. Mol. Biol., 80, 41-62.
8. Byers, L. D. and Koshland, D. E., Jr. (1975) Biochemistry, 14, 3661-3669.
9. Holland, M. J. and Westhead, E. W. (1973) Biochemistry, 12, 2276-2281.
10. a) Rosowsky, A. (1964) in "Heterocyclic Compounds" Weissberger, A. ed., 1 (part 1), 1-523.
    b) Danehy, J. P. and Noel, C. J. (1960) J. Am. Chem. Soc., 82, 2511-2515.
    c) Wilson, C. E. and Lucas, H. J., (1936) J. Am. Chem. Soc., 58, 2396-2402.
11. a) Miller, J. C. and Waley, S. G. (1971) Biochem. J., 123, 163-170.
    b) Schray, K. K., O'Connell, E. L. and Rose, I. A. (1973) J. Biol. Chem., 248, 2214-2218.
12. Kirschner, K. (1971) J. Mol. Biol., 58, 51-68.
13. Stallcup, W. B. and Koshland, D. E., Jr. (1973) J. Mol. Biol., 80, 77-91.
14. Orsi, B. A. and Cleland, W. W. (1972) Biochemistry, 11, 102-109.