PHOSPHOGLYCERATE MUTASE HAS ESSENTIAL ARGINYL RESIDUES

C. L. Borders, Jr.[1] and Brett A. Wilson[2]

Department of Chemistry, College of Wooster
Wooster, Ohio 44691

Received September 29, 1976

Phosphoglycerate mutase is inactivated by butanedione in borate buffer. Inactivation by 0.13 mM reagent correlates with the modification of one arginyl residue per subunit, and is prevented by either 2,3-diphosphoglycerate or 3-phosphoglycerate. With 0.50 mM butanedione, inactivation is accompanied by the modification of three arginyl residues per subunit, two of which are protected by the combined presence of cofactor and substrate.

Arginyl residues play a general role in the functional binding of anionic cofactors and substrates to enzyme active sites. Thus, chemical modification with reagents that specifically modify arginyl residues reveals that at least five kinases (1), two synthetases (2), an ATPase (3), and a number of dehydrogenases (4-8) -- all enzymes utilizing phosphate-containing coenzymes -- contain essential arginyl residues. Similarly, several enzymes associated with nucleic acid metabolism (9-11), as well as carboxypeptidases A (12) and B (13), aspartate aminotransferase (14), alkaline phosphatase (15), aldolase (16), and aspartate transcarbamylase (17), all enzymes utilizing anionic substrates, contain essential arginine.

The generality of this role for arginine is emphasized by a recent report which strongly suggests that all but one of fourteen enzymes associated with the glycolytic pathway have essential arginyl residues. In this study phosphoglycerate mutase was found to be inactivated more rapidly by butanedione than any of the glycolytic enzymes (18). The work reported in the present paper is a further characterization of the role of the essential arginyl residues in phosphoglycerate mutase.

---

[1] To whom inquiries should be addressed.

[2] Taken in part from the Senior Independent Study thesis of Brett A. Wilson, The College of Wooster, 1976.

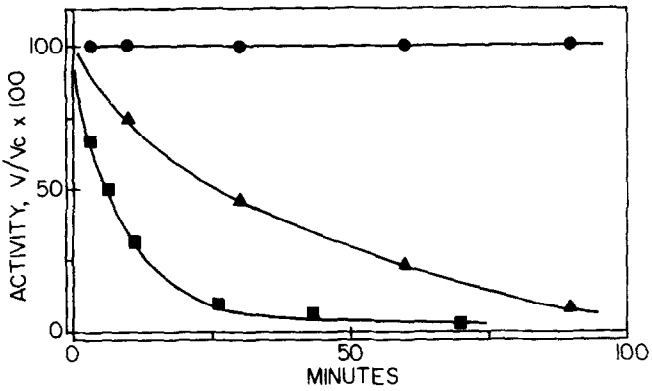

FIGURE 1: Phosphoglycerate mutase inactivation by butanedione. The enzyme was modified with either 0.13 mM (▲) or 0.50 mM (■) butanedione in 50 mM borate, pH 8.3. Activities are expressed relative to a control (●) subjected to the same conditions but in the absence of butanedione.

MATERIALS AND METHODS

3-Phosphoglycerate and 2,3-diphosphoglycerate were obtained from Sigma Chemical Company, as was yeast enolase. 2,3-Butanedione (redistilled before use) was purchased from Aldrich Chemical Company. All other chemicals were reagent grade. Crystalline rabbit muscle phosphoglycerate mutase was obtained from Sigma, and on polyacrylamide gels containing sodium dodecyl sulfate (19) gave a single band with a molecular weight of about 26,000. Enzyme activity was determined at 25° by a modification of published procedures (20). A standard assay mixture (1.0 ml) contained the following: 100 mM Tris[3] chloride, pH 7.0, 10 mM 3-PGA, 0.1 mM 2,3-DPGA, 5 mM $MgSO_4$, and 5 units of enolase. The assay was initiated by the addition of phosphoglycerate mutase, and the increase in absorbance at 240 nm was measured as a function of time.

Chemical modification of enzyme was carried out at 25° under conditions given in the figure and table legends. Activity is expressed as the ratio of the activity of the modified enzyme, V, to that of the control subjected to the same conditions but in the absence of butanedione, Vc, multiplied by 100. Arginine modification was determined by analysis on a Beckman 120C amino acid analyzer after workup analogous to published procedures (1). The total number of arginyl residues per phosphoglycerate mutase subunit of molecule weight 27,000 (21) was determined by averaging duplicate amino acid analyses of the control enzyme after hydrolysis at 110° with 6 N HCl for 24, 48, and 72 hours.

RESULTS

The time course for the inactivation of phosphoglycerate mutase by butanedione in 50 mM borate, pH 8.3, depends on the concentration of reagent used

[3] Abbreviations used are: Tris, tris-(hydroxymethyl)aminomethane; 2,3-DPGA, 2,3-diphosphoglycerate; 3-PGA, 3-phosphoglycerate; 2-PGA, 2-phosphoglycerate; PGA, phosphoglycerate; EP, phosphoenzyme; BD, 2,3-butanedione.

TABLE I: Effect of Substrate and Cofactor on the Inactivation of Phosphoglycerate Mutase by Butanedione-Borate

Addition	Activity on Modification with	
	[a]0.13 mM BD	[b]0.50 mM BD
None	10	8
2,3-DPGA, 0.2 mM	80	45
3-PGA, 10 mM	95	78
2,3-DPGA, 0.2 mM + 3-PGA, 10 mM	100	98

[a]Modification of phosphoglycerate mutase, 2 μM, was carried out for 90 minutes with 0.13 mM butanedione in 50 mM borate, pH 8.3.

[b]Modification of phosphoglycerate mutase, 2 μM, was carried out for 25 minutes with 0.50 mM butanedione in 50 mM borate, pH 8.3.

(Figure 1). With 0.13 mM butanedione the activity is reduced to 10% of the control after 90 minutes. When 0.50 mM butanedione is used, 8% activity remains after 25 minutes. The rate of inactivation is markedly reduced if modification is carried out in the absence of borate. Under conditions where the activity is reduced to 10% in the presence of 50 mM borate, 90% activity remains if the borate is replaced by HEPES. Inactivation by butanedione-borate is also reversible. If the enzyme is modified to 10% of the control activity by butanedione-borate, and then gel filtered through a Sephadex G-25 column equilibrated with 50 mM Tris chloride, pH 8.3, 90% of the native activity is restored after three hours. If gel filtration is performed in borate, however, no reactivation is observed. This data strongly suggests that inactivation is due to the modification of essential arginyl residues.

Protection against inactivation is provided by either cofactor or substrate, or both combined, with the degree of protection related to the concentration of butanedione used for modification (Table I). When 0.13 mM butanedione is used for modification, the presence of either 2,3-DPGA, 0.2 mM, or 3-PGA, 10 mM, provides nearly complete protection against inactivation. With 0.50 mM butanedione, either 2,3-DPGA alone or 3-PGA alone is less efficient in protecting against inactivation, but full protection is observed when cofactor and substrate are present simultaneously.

TABLE II: Correlation of Phosphoglycerate Mutase Inactivation by 0.13 mM Butanedione with Loss of Arginine, and Protection by Substrate and Cofactor[a]

Enzyme	V/Vc x 100	Arg per Subunit	Arg Modified per Subunit
Control	100	16.3	-
+ Butanedione	8	15.1	1.2
+ Butanedione + 2,3-DPGA	85	16.6	-
+ Butanedione + 3-PGA	95	16.5	-
+ Butanedione + 2,3-DPGA + 3-PGA	100	16.4	-

[a]Modification of phosphoglycerate mutase, 21 μM, was carried out with 0.13 mM butanedione in 50 mM borate, pH 8.3, in the absence of substrate or cofactor or in the presence of 2,3-DPGA, 0.2 mM, or 3-PGA, 10 mM, or both simultaneously. After 2.0 hours samples were subjected to gel filtration of a column (0.9 x 20 cm) of Sephadex G-25 equilibrated with 50 mM borate, pH 8.3, aliquots were assayed for enzymatic activity, and the balance was hydrolyzed with 6 N HCl for 20 hours at 110° and subjected to amino acid analysis as described in the text.

Inactivation of phosphoglycerate mutase by 0.13 mM butanedione correlates with the modification of one arginyl residue per subunit (Table II). Amino acid analysis of a sample treated with butanedione for 2.0 hours indicated 15.1 arginyl residues per subunit compared to 16.3 for the control. Neither lysine, histidine, nor any other amino acid is modified. The presence of either 2,3-DPGA or 3-PGA, or both combined, provides nearly full protection against inactivation and no loss of arginine is observed.

When inactivation is carried out for 1.0 hour with 0.50 mM butanedione, activity is reduced to 5% and 3.0 arginyl residues are modified per subunit of phosphoglycerate mutase (Table III). The simultaneous presence of 2,3-DPGA and 3-PGA provides full protection against inactivation and only 1.0 arginyl residue per subunit is modified. Again, no other residue was found to be modified

DISCUSSION

A number of amino acid residues have been implicated in the mechanism of action of phosphoglycerate mutase (22). A single histidyl residue per subunit of the muscle enzyme is phosphorylated by 2,3-DPGA to form a catalytically

TABLE III: Correlation of Phosphoglycerate Mutase Inactivation by 0.50 mM Butanedione with Loss of Arginine, and Protection by Substrate plus Cofactor[a]

Enzyme	V/Vc x 100	Arg per Subunit	Arg Modified per Subunit
Control	100	16.4	-
+ Butanedione	5	13.4	3.0
+ Butanedione + 3-PGA + 2, 3-DPGA	100	15.4	1.0

[a]Modification of phosphoglycerate mutase, 22 μM, was carried out with 0.50 mM butanedione in 50 mM borate, pH 8.3, in the absence of substrate and cofactor, or in the simultaneous presence of 3-PGA, 10 mM, and 2, 3-DPGA, 0.2 mM. After 1.0 hour samples were subjected to analysis as described in Table II.

significant phosphoenzyme (21, 23), and a similar intermediate is suggested to occur in the yeast enzyme (24). In addition, it has long been known that the mammalian mutases are inactivated by sulfhydryl reagents (25, 26), and recent reports suggest that one sulfhydryl per subunit is in close proximity to the active site (27, 28). In addition, yeast phosphoglycerate mutase is known to have 4 essential lysyl residues per tetramer which are a part of the substrate binding sites (29).

The data presented here indicate that arginyl residues are critical to the mechanism of action of phosphoglycerate mutase. Amino acid analysis of phosphoglycerate mutase modified with 0.13 mM butanedione indicates that only arginine is modified and that inactivation can be correlated with loss of one arginyl residue per subunit. When 0.50 mM butanedione is utilized, inactivation is accompanied by the modification of up to three arginyl residues per subunit, two of which can be protected by the combined presence of cofactor and substrate.

The protection against inactivation provided by 2, 3-DPGA is quite informative, for under these conditions the predominant enzyme species is most likely the phosphoenzyme rather than the E·2, 3-DPGA $\rightleftharpoons$ EP·PGA complex (30). Since formation of a phosphoenzyme apparently prevents modification of the single arginyl residue, it is quite likely that this arginine interacts with

the transferable phosphate. A recent report demonstrates that an arginyl residue is immediately adjacent to the active site phosphohistidine (23), and it is tempting to suggest that this may be the residue being modified by 0.13 mM butanedione. However, confirmation will have to await sequence studies on the modified arginyl peptide.

Arginine modification is less selective when 0.50 mM butanedione is used, for up to three arginyl residues per subunit are modified. Protection against inactivation by either 2,3-DPGA or 3-PGA is less efficient, but when both 2,3-DPGA and 3-PGA are present simultaneously, full protection is observed and 2 arginyl residues per subunit are protected. This suggests that at least two arginyl residues are involved in formation of the E·2,3-DPGA $\rightleftharpoons$ EP·PGA complex. As indicated above, the first essential arginyl residue to be modified likely interacts with the transferable phosphate, while a second residue could serve as a recognition site for either the carboxylate moiety or the remaining phosphate of substrates. Thus, the present study emphasizes the ever more apparent occurrence of essential arginyl residues at the active sites of enzymes acting on anionic substrates.

ACKNOWLEDGMENT

This work was supported by a Cottrell College Science Grant from the Research Corporation. We thank Jackie A. Zurcher for running the electrophoresis.

REFERENCES

1. Borders, C. L., Jr, and Riordan, J. F. (1975), Biochemistry 14, 4699-4704.
2. Powers, S. G. and Riordan, J. F. (1975), Proc. Nat. Acad. Sci. U.S.A. 72, 2616-2620.
3. Marcus, F., Schuster, S. M. and Lardy, H. A. (1976), J. Biol. Chem. 251, 1775-1780.
4. Lange, L. G., III, Riordan, J. F. and Vallee, B. L. (1974), Biochemistry 13, 4361-4370.
5. Foster, M. and Harrison, J. H. (1974), Biochem. Biophys. Res. Commun. 58, 263-267.
6. Yang, P. C. and Schwert, G. W. (1972), Biochemistry 11, 2218-2224.
7. Blumenthal, K. M. and Smith, E. L. (1975), J. Biol. Chem. 250, 6555-6559.
8. Domagk, G. F., Domschke, W. and von Hinuber, C. (1970), Hoppe-Seyler's Z. Physiol. Chem. 351, 718-720.
9. Borders, C. L., Jr., Riordan, J. F. and Auld, D. S. (1975), Biochem. Biophys. Res. Commun. 66, 490-495.
0. Takahashi, K. (1968), J. Biol. Chem. 243, 6171-6179.

11. Salvo, R. A., Serio, G. F., Evans, J. E. and Kimball, A. P. (1976), Biochemistry 15, 493-497.
12. Riordan, J. F. (1973), Biochemistry 12, 3915-3923.
13. Werber, M. M. , Moldovan, M. and Sokolovsky, M. (1975), Eur. J. Biochem. 53, 207-216.
14. Riordan, J. F. and Scandurra, R. (1975), Biochem. Biophys. Res. Commun. 66, 417-424.
15. Daemen, F. J. M. and Riordan, J. F. (1974), Biochemistry 13, 2865-2871.
16. Lobb, R. R., Stokes, A. M., Hill, H.A.O. and Riordan, J. F. (1975), F.E.B.S. Lett. 54, 70-72.
17. Kantrowitz, E. R. and Lipscomb, W. N. (1976), J. Biol. Chem. 251, 2688-2695.
18. Riordan, J. F., McElvany, K. D. and Borders, C. L., Jr. (1976), submitted.
19. Weber, K. and Osborn, M. (1969), J. Biol. Chem. 244, 4406-4412.
20. Grisolia, S. (1962), Meth. Enzymol. 5 , 236-242.
21. Rose, Z. B. (1970), Arch. Biochem. Biophys. 140, 508-513.
22. Ray, W. J. and Peck, E. J. (1972), The Enzymes, 3rd Ed., 6, 459-475.
23. Rose, Z. B., Hamasaki, N. and Dube, S. (1975), J. Biol. Chem. 250, 7939-7942.
24. Rose, Z. B. (1971), Arch. Biochem. Biophys. 146, 359-360.
25. Cowgill, R. W. and Pizer, L. I. (1956), J. Biol. Chem. 223, 885-895.
26. Pizer, L. I. (1960), J. Biol. Chem. 235, 895-901.
27. Carne, T. J., McKay, D. J. and Flynn, T. G. (1976), Can. J. Biochem. 54, 307-320.
28. Hartman, F. C. and Norton, I. L. (1976), J. Biol. Chem. 251, 4565-4569
29. Sasaki, R., Sugimoto, E. and Chiba, H. (1971), Biochim. Biophys. Acta 227, 584-594.
30. Britton, H. G., Carreras, J. and Grisolia, S. (1972), Biochim. Biophys. Acta 289, 311-322.