Identification of the Highly Reactive Sulphydryl Group of Pig Kidney Fructose 1,6-Bisphosphatase at Cysteine 128*

(Received for publication, November 8, 1983)

Tapati Chatterjee, Ida Edelstein, and Frank Marcus§
From the Department of Biological Chemistry and Structure, University of Health Sciences/The Chicago Medical School, North Chicago, Illinois 60064

John Eby, Ilene Reardon, and Robert L. Heinrikson
From the Department of Biochemistry, University of Chicago, Chicago, Illinois 60637

Fructose 1,6-bisphosphatases contain a highly reactive cysteine residue, the reactivity of which is influenced by ligands that bind at the catalytic and at the allosteric AMP sites of the enzyme. Nevertheless, the sulphydryl group appears to be proximal to these sites and not a functional component of either. Modification of pig kidney fructose 1,6-bisphosphatase with three reagents, 5,5'-dithiobis-(2-nitrobenzoic acid), iodoacetamide, and phenacyl bromide, yields derivatives with similar properties, thus suggesting that the same residue was modified in each case. The modified enzymes exhibited: (a) higher $V_{max}$ when Mn$^{2+}$ was used as the activating cation; (b) decreased activity in the presence of nonsaturating Mg$^{2+}$ concentrations; (c) no change in sensitivity toward AMP inhibition. Automated Edman degradation of a tryptic peptide containing radioactive carboxamidomethylcysteine showed the sequence of residues Gly-111-Arg-140 of pig kidney fructose 1,6-bisphosphatase. The modified residue was shown to be cysteine-128, and the same cysteine residue was alkylated when the enzyme was reacted with phenacyl bromide. Cysteine-128 is also present in rat and sheep liver fructose 1,6-bisphosphatase and a long stretch of the sequence around this reactive cysteine residue is highly conserved.

Fructose 1,6-bisphosphatase (d-fructose-1,6-bisphosphate 1-phosphohydrolase, EC 3.1.3.11) is a key enzyme of gluconeogenesis. The native "neutral pH optimum" enzyme isolated from different sources is a tetramer composed of identical subunits of molecular weights ranging from 36,000 to 40,000 (1, 2). A common feature of fructose 1,6-bisphosphatases appears to be the presence of a single highly reactive sulphydryl group/mol of enzyme subunit (3-7). The reactivity of this cysteine residue toward several sulphydryl reagents is decreased by the presence of the substrate fructose 1,6-bisphosphate (3, 5, 7), while the allosteric inhibitor AMP markedly increases its reactivity (3, 6, 7). Studies of the properties of several fructose 1,6-bisphosphatases modified at the reactive sulphydryl group suggest that the cysteine residue is not located at the active or at the allosteric site of the enzyme (3, 4, 7). The reactivity of this group, however, has provided the means to test whether compounds bind at one or the other of these sites in fructose 1,6-bisphosphatases (6, 8, 9). Distance measurements by NMR and EPR relaxation rates show that the reactive cysteine residue is located relatively close to the active site and a model has been proposed to depict the spatial relationships among the catalytic, allosteric, metal ion, and reactive sulphydryl sites of fructose 1,6-bisphosphatase (10). The present study has established the location of the highly reactive cysteine of pig kidney fructose 1,6-bisphosphatase within the recently determined primary structure of this enzyme (11).

EXPERIMENTAL PROCEDURES AND RESULTS

The amino acid sequence of a 30-residue tryptic peptide isolated from $[^{14}C]$carboxamidomethyl pig kidney fructose 1,6-bisphosphatase is shown in Fig. 1. This peptide corresponds to the sequence of residues Gly-111-Arg-140 of the enzyme (11) and contains the highly reactive cysteine residue of fructose 1,6-bisphosphatase. This residue has been identified as cysteine-128. The rationale for the methods used to isolate the peptide containing the reactive Cys came from our work on the elucidation of the complete covalent structure of pig kidney fructose 1,6-bisphosphatase (11), a sequence which shows cysteine residues located at positions 38, 92, 116, 128, 183, and 281. S-Carboxamidomethyl fructose 1,6-bisphosphatase labeled only at the most reactive cysteine residue was first cleaved with subtilisin into the S-peptide (residues 1-60) and the S-subunit (residues 61-335) components. The highly conserved Cys-38 (22) was ruled out as the reactive sulphydryl of the enzyme because radioactivity was associated only with the S-subunit. This conclusion is in agreement with our previous observations (14) with NBS-modified enzyme. Analysis of CNBr fragments from the S-subunit (Fig. 6) showed that the radioactivity was associated

---

1 Portions of this paper (including "Experimental Procedures," "Results," Figs. 2-7, and Table I) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-3193, cite the authors, and include a check or money order for $4.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

2 The abbreviation used is: NBS$_2$, 5,5'-dithiobis(2-nitrobenzoic acid).

3834
with the peak of highest molecular weight, corresponding to the peptide containing residues Gly-61-Met-172. This result clearly showed that neither Cys-183, present in the CNBr peak eluting at fractions 156-169, contained the carboxamidotetamethyl label and restricted our search to Cys residues 92, 116, and 128. Finally, the label was located in the tryptic peptide Gly-111-Arg-140 (Fig. 7) and the reactive sulphydryl was identified by sequence analysis as being that of Cys-128 (Table 1). The identification of this residue as the reactive cysteine explained the presence of the minor radioactive peak eluting at about fraction 138 in the separation of CNBr fragments shown in Fig. 6. Minor cleavage at Asp-Pro peptide bonds occurring during exposure to low pH values such as were encountered in CNBr treatment and peptide separations (23) results in cleavage at Asp-118-Pro-119 leading to the formation of a radioactive fragment Pro-119-Met-182 containing only 1 cysteine residue, the reactive Cys-128.

The region containing the most reactive Cys residue appears to be highly conserved in fructose 1,6-bisphosphatases. Our sequence analysis of this region in rat liver fructose 1,6-bisphosphatase showed identical sequences in the tryptic peptide Gly-111-Arg-140, except for the replacement of Val-130 of the pig kidney enzyme by alanine in rat liver fructose 1,6-bisphosphatase. A recent report on the complete sequence of sheep liver fructose 1,6-bisphosphatase (24) reveals full identity from residue 102 through 139 between the sheep liver enzyme and pig kidney fructose 1,6-bisphosphatase. Cysteine-128 also appears to react rapidly and specifically with NBS, like that of NBS2, was much more rapid than reaction with iodoacetamide suggesting that Cys-128 is located in an hydrophobic environment. Dinitrofluorobenzene also changes the properties of fructose 1,6-bisphosphatase in a fashion similar to that reported here, that is, activity at pH 7.5 measured in the presence of Mn2+ was increased, and that measured in the presence of Mg2+ was decreased (26, 27). It appears likely therefore that Cys-128 is also the site of reaction with dinitrofluorobenzene, although this has not been proven as yet.

The specific reaction of Cys-128 of fructose 1,6-bisphosphatase with phenacyl bromide may permit the exploration of the environment of that cysteine residue using the related bifunctional reagent, p-azidophenacyl bromide (28). Indeed, preliminary experiments in our laboratory indicate that modification of fructose 1,6-bisphosphatase with this reagent produces a derivative with properties similar to those of the phenacyl bromide-modified enzyme. Irradiation of the covalently bound reagent should give a highly reactive nitrene likely to generate cross-links in the vicinity of the catalytic and allosteric sites of the enzyme. Analysis of the cross-linked regions may provide three-dimensional structure information for fructose 1,6-bisphosphatases based upon chemical modification. Cysteine 128 also appears to react rapidly and specifically with either p-hydroxymercuribenzoate or p-hydroxymercuriophenylsulfonate, thus opening possibilities for preparing heavy Metal 128 derivatives of fructose 1,6-bisphosphatase suitable for x-ray diffraction. These experiments are now in progress.

Acknowledgment—We thank Dr. B. L. Horecker for providing us with unpublished information on the amino acid sequence of rabbit liver fructose 1,6-bisphosphatase.

REFERENCES

© 1983 The Rockefeller University Press 0021-9525/83/0385-0335$01.00/0

FIG. 1. Amino acid sequence of the tryptic peptide containing the reactive sulphydryl of pig kidney fructose 1,6-bisphosphatase. Numbers above residues indicate their position in the established amino acid sequence of pig kidney fructose 1,6-bisphosphatase (11). The reactive Cys-128 is shown underlined.
The Reactive Sulfhydryl of Fructose 1,6-Bisphosphatase

EXPERIMENTAL PROCEDURES

Renal Fructose 1,6-Bisphosphatase. In a typical experiment, Fructose 1,6-Bisphosphatase was incubated with 0.2 ml of 0.1 M Tris-HCl buffer (pH 7.5), 1.2 ml of water, 0.1 ml of the enzyme solution, and 0.4 ml of 5-10 mM Cysteine solution. The mixture was incubated at 37°C for 2 hours, and then the reaction was stopped by the addition of 0.2 ml of 2 N HCl. The Cysteine solution was added to the enzyme solution in a molar ratio of 1:1. The concentration of the Cysteine solution was determined spectrophotometrically at 420 nm from the absorbance of the Cysteine-Cystine pair. The absorption spectra of the enzyme-Cysteine complex were determined by using a Cary Model 14 spectrophotometer.

RESULTS

Modification of the Kidney Fructose 1,6-Bisphosphatase. The results of the modification experiments indicated that the modification of the enzyme with Cysteine was highly specific. The reaction was highly specific and the modification of the enzyme was almost complete. The reaction was carried out in a molar ratio of 1:1. The reaction was highly specific and the modification of the enzyme was almost complete. The reaction was carried out in a molar ratio of 1:1.

The radiolabeled Cysteine-Cystine pair was added to the enzyme solution in a molar ratio of 1:1. The reaction was highly specific and the modification of the enzyme was almost complete. The reaction was carried out in a molar ratio of 1:1.

The reaction was carried out in a molar ratio of 1:1. The reaction was highly specific and the modification of the enzyme was almost complete. The reaction was carried out in a molar ratio of 1:1.
Identification of the highly reactive sulphydryl group of pig kidney fructose 1,6-bisphosphatase at cysteine 128.

T Chatterjee, I Edelstein, F Marcus, J Eby, I Reardon and R L Heinrikson


Access the most updated version of this article at http://www.jbc.org/content/259/6/3834

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/259/6/3834.full.html#ref-list-1