Sulphydryl Groups of Yeast Phosphofructokinase-specific Localization on β Subunits of Fructose 6-Phosphate Binding Sites as Demonstrated by a Differential Chemical Labeling Study*

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Yeast phosphofructokinase contains 83 ± 2 cysteinyl residues/enzyme oligomer. On the basis of their reaction toward 5,5'-dithiobis(2-nitrobenzoic acid), the accessible cysteinyl residues of the native enzyme may be classified into three groups. For titrations performed with N-ethylmaleimide, subdivisional classes of reactivity are evidenced. In each case, the 6 to 8 most reactive cysteines are not protected by fructose 6-phosphate from chemical labeling and do not seem involved in subsequent enzyme inactivation. Differential labeling studies as well as direct protection experiments in the presence of fructose 6-phosphate, indicate that 12 —SH groups/oligomer (i.e. three —SH groups per binding site) are protected by the allosteric substrate from the chemical modification. Specific labeling by the differential method of the cysteinyl residues protected by fructose 6-phosphate and further separation of the two types of subunits constituting yeast phosphofructokinase, show that the substrate binding sites are localized exclusively on subunits of β type. Thus, α subunits are not implicated directly in the catalytic mechanism of yeast phosphofructokinase reaction.

Phosphofructokinase (EC 2.7.1.11) is a complex regulatory enzyme which plays a central role in the initiation of the Pasteur effect (1) and in the triggering of glycolytic oscillations (2, 3). Modeling of these biological phenomena requires a precise knowledge of the molecular properties of this enzyme, at both structural and functional levels.

Yeast phosphofructokinase is an octamer (4-6) built up of an equal number of two types of subunits α and β which differ slightly in their molecular weight (7, 8) and are further differentiated by their immunological properties (9).

We have recently presented (10, 11) binding data showing that the stoichiometries obtained for each functional ligand (fructose 6-phosphate, ATP-substrate, ATP-effector, and AMP) are equal to only half the number of total subunits constituting the enzyme oligomer. These results are well correlated with our functional studies (11, 12) which indicate the existence of a small number (three or four) of interacting catalytic protomers in the enzyme oligomer. However, our hypothesis (10) on the occurrence of distinct catalytic and regulatory subunits in yeast phosphofructokinase must be substantiated by new additional evidence, particularly by the localization of each type of ligand binding sites on α and β subunits.

In this paper, we present an investigation of the reactivity of the sulphydryl groups of the protein and we study the protecting effect of fructose 6-phosphate on the chemical modification of the enzyme. Moreover, we describe the ability to separate partially α and β subunits by means of ion-exchange chromatography on a DEAE-52 cellulose column under denaturing conditions. This technique is used to demonstrate that the 12 sulphydryl groups/oligomer, protected differentially by fructose 6-phosphate from the chemical modification of yeast phosphofructokinase with N-ethylmaleimide or 5,5'-dithiobis(2-nitrobenzoic acid), are localized exclusively on subunits of β type. The respective roles of α and β subunits in the expression of the catalytic and regulatory properties of the enzyme are discussed.

EXPERIMENTAL PROCEDURES

Materials—Phosphofructokinase was purified from commercial bakers' yeast (Springer) according to the slightly modified procedure of Diezel et al. (7). The enzyme was stored at 0°C as a suspension in 4 M ammonium sulfate in the presence of 1 mM ATP. Before use, aliquots were treated by the procedure previously described (11), except that dithiothreitol was omitted from buffers. An extinction coefficient of 0.97 mg⁻¹ cm⁻¹ at 280 nm (10) was used for the evaluation of the phosphofructokinase concentration. Alternatively, protein concentrations might be estimated with the Lowry method using bovine serum albumin as a reference standard. Both procedures gave consistent results within 5%. A revised molecular weight of 630,000 (5) was assumed for the calculation of enzyme moieties. Enzymatic activities were determined spectrophotometrically with the coupled assay previously described (12). Specific activity of native yeast phosphofructokinase was 60 units/mg when assayed under these conditions. Except where otherwise stated, experiments were performed in 50 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (pH 6.8) containing 25 mM K₂HPO₄, 1 mM EDTA, and 5 mM MgCl₂. This buffer is referred to later as standard Tes/phosphate buffer.

The 5,5'-dithiobis(2-nitrobenzoic acid) was an Aldrich product. Before use, it was recrystallized from acetic acid. Radioactive N-(ethyl-2',3'H)ethylmaleimide (600 μCi/mmol) was purchased from New England Nuclear. Unlabeled MalNEt was obtained from Mann Research Laboratories. Urea (Merck) was recrystallized twice from ethanol. Coupling enzymes and all other biochemicals were obtained from Sigma.

Reaction of Yeast Phosphofructokinase with NbSz—Determined

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1 The abbreviations used are: MalNEt, N-ethylmaleimide; NbSz, 5,5'-dithiobis(2-nitrobenzoic acid); Tes, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.
tion of the thiol content of yeast phosphofructokinase and modification of reactive sulfhydryl groups with NbS₂ were carried out by Ellman’s procedure (13). The reagent was prepared fresh each day by dilution in standard Tes/phosphate buffer and kept on ice. Its concentration was determined by titration with an excess of diithiothreitol. The increase of absorbance was measured at 412 nm and 25°C on a Cary 16 spectrophotometer and the number of —SH groups titrated was calculated using a molar extinction coefficient of 13,600 cm⁻¹ M⁻¹ (19) for the thionitrobenzoate ion of NbS₂. For data analysis, the output signal of the spectrophotometer was stored on a punched tape with previous analogies to digital conversion. The kinetic data were then transferred into a Wang 2200 B computer and stored on a punched tape with previous analogies to digital conversion programs presented in the basic version of the calculator.

For inactivation experiments, the NbS₂ reaction was stopped by dilution of aliquots of the reaction mixture in 10 volumes of ice-cold standard Tes/phosphate buffer (pH 6.8). Under these conditions, MalNEt reaction is highly specific for protein sulfhydryl groups (15) as verified by differential titration with NbS₂ of the denatured enzyme, after complete reaction with diithiothreitol. The kinetics of incorporation of radioactive MalNEt in phosphofructokinase was measured routinely with the filter paper disc technique (16, 17). Filters were preincubated in 400 mM dithiothreitol before spotting of 50-μl aliquots of the reaction mixture. The paper disc method was calibrated by titration of the reaction mixture with 0.8 M SePhadex C-25 column (0.8 x 13 cm), at the end of the reaction. Efficiency of the filter counting was estimated to 80%.

For inactivation experiments, reaction was stopped by addition of an excess of diithiothreitol of 100 times the molar concentration of the reagent.

For differential labeling studies, phosphofructokinase (3 to 4 mg/ml) was first incubated with unlabeled MalNEt (0.9 mM) for 120 min. The reaction mixture was then filtered on the small Sephadex G-50 column and reincubated with radioactive MalNEt or with NbS₂.

Separation of Yeast Phosphofructokinase Subunits under Denaturing Conditions—Yeast phosphofructokinase ranging from 4 to 9 nM mg was denatured by incubation in 25 mM Tris/sulfonic acid buffer (pH 8.6) containing 50 mM glycine, 1 mM EDTA, 100 mM 2-mercaptoethanol, and 8 M urea (standard Tris/glycine buffer). For heating for 5 min in a boiling water bath, the enzyme was dialyzed overnight against two changes of 50 ml of the same buffer. The dialyzed enzyme subunits were applied to a DEAE-52 cellulose (Whatman) column (0.95 x 26 cm) which had been equilibrated with standard Tris/glycine buffer in which the 2-mercaptoethanol concentration was 20 mM. The subunits were eluted with a linear gradient of KCl (0 to 150 mM) present in the equilibration buffer. Before elution, the gel was washed with 30 ml of the equilibration buffer. Elution was followed in a MPF 44 B Perkin Elmer spectrofluorimeter, after calibration of the fluorescence intensity scale with a phosphofructokinase solution of known concentration. The excitation and emission wavelengths were 295 and 350 nm, respectively. No discrepancy should be noted if protein concentrations are measured directly in the enzyme-containing fractions by the Lowry method, even in the case of the chemically modified enzyme. A new gel sample was packed before each ion-exchange chromatography experiment. About 80% of the total amount of the protein applied to the column was recovered after chromatography.

For experiments performed on labeled phosphofructokinase, radioactivity was measured on 0.5-ml aliquot of each eluted fraction which was added to 5 ml of Instagel (Packard) as scintillation liquid. Under our experimental conditions, the excess of labeled MalNEt which did not react with the enzyme was thus eliminated before the KCl gradient was applied.

Before electrophoresis, the peak fractions were dialyzed extensively against 10 mM K₂HPO₄ buffer (pH 7) containing 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol, to remove urea.

MalNEt—Electrophoresis methodology was adapted from that of Laemmli (18) using a 7.5% polyacrylamide lower gel and a 3.5% polyacrylamide stacking gel. Electrophoresis was performed on slabs (15 x 15 cm). Radioactivity incorporated into α and β subunits of yeast phosphofructokinase was counted from gel slices which were incubated overnight at 25°C in 1 ml of perhydrol containing 30% H₂O₂ and 50 μl of 1 M ammonia (19). Before addition of 5 ml of Instagel as scintillation liquid, each sample was adjusted to pH 7 and 1 μg of catalase was added to prevent chemiluminescence resulting from the excess of H₂O₂.

RESULTS

Titration of Native and Denatured Yeast Phosphofructokinase with NbS₂—Protection of Sulfhydryl Groups by Fructose 6-Phosphate—Upon denaturation of the enzyme in 8 M urea, 83 ± 2 thiol groups/oligomer can be titrated with NbS₂ in agreement with previous amino acid analysis data of Kopperschläger et al. (5). Hence, the active form of yeast phosphofructokinase contains few, if any, disulfide bonds.

Fig. 1 shows the kinetics of titration of native phosphofructokinase with NbS₂ in the presence and absence of fructose 6-phosphate, respectively. In absence of ligand, about 7 ± 1 cysteines/oligomer react very rapidly (Class I-NbS₂) and a second class of about 8 less reactive cysteines can be titrated (Class II-NbS₂). Finally, a further quasi linear increase of absorbance is observed but the enzyme solution becomes turbid after 30 min of reaction under our experimental conditions. Although the inactivation process resulting from chemical modification of the enzyme seems to involve at least two classes of reactive cysteines, a substantial part of the phenomenon may be attributed to the reaction of the second class of residues. In the presence of 1 mM fructose 6-phosphate, the first class of 7 ± 1 cysteines reacts still rapidly with NbS₂ but the number of cysteines titrated in the second class is reduced to about four —SH groups per enzyme oligomer. Furthermore, the final slope corresponding to the reaction of less reactive cysteines is reduced by a factor of two in the presence of fructose 6-phosphate. It is important to note that phosphofructokinase remains fully active after titration with NbS₂ in the presence of fructose 6-phosphate. This indicates that the first class of reactive cysteines is not involved in enzyme inactivation which can be related to the modification of a large number of slowly reacting cysteines.

Reaction of Yeast Phosphofructokinase with N-Ethylmaleimide—The kinetics of incorporation of MalNEt in native

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**Fig. 1. Reaction of native yeast phosphofructokinase with NbS₂ in the presence and absence of fructose 6-phosphate.** Reactions were carried out at 25°C in standard Tes/phosphate buffer (pH 6.8) as described under "Experimental Procedures." Protein concentration was 1 μM enzyme oligomer and NbS₂ concentration was 0.2 mM. Kinetics of reagent incorporation (solid curves) were analyzed according to Equation 1, where rₐ represents the number of —SH groups modified per enzyme oligomer.

\[ rₐ = r₁ + r₂(1 - e^{-kt}) + kₐt \]

The following values of the parameters were used: Curve a; in absence of fructose 6-phosphate, r₁ = 6.9, r₂ = 7.4, k₁ = 0.32 min⁻¹, k₂ = 0.49 min⁻¹. Curve b; in presence of 2 mM fructose 6-phosphate, r₁ = 7.25, r₂ = 0.8, k₁ = 0.41 min⁻¹, k₂ = 0.20 min⁻¹. The dashed line corresponds to enzyme inactivation observed upon chemical modification of yeast phosphofructokinase with NbS₂, in absence of fructose 6-phosphate.
phosphofructokinase is shown in Fig. 2. The incorporation of MalNEt levels off after 2 h of incubation to 36 ± 1 residues modified/oligomer. The number of residual sulfhydryl groups which had not reacted with MalNEt was determined under conditions (8 M urea) which have been shown to denature the enzyme and to expose all of its —SH groups to reaction with NbS2. As expected, 56 ± 1 sulfhydryl groups/oligomer can be titrated with NbS2, indicating that the N-ethylmaleimide reaction is specific for —SH groups under our experimental conditions (pH 6.8). As in the case of NbS2, a first class of about 6 ± 1 cysteines/oligomer reacts very rapidly with MalNEt and is not involved in subsequent enzyme inactivation. As shown in the inset of Fig. 2, the overall reaction of less reactive sulfhydryl groups is at least triphasic. The number of residues titrated in each class of reactivity is 2.9, 9.4, and 8.2 with the respective pseudo-first order rate constants equal to 0.06 min⁻¹, 0.03 min⁻¹, and 0.015 min⁻¹. Fig. 2 shows that enzyme inactivation may be described satisfactorily by assuming a monoexponential decay with a first order rate constant of inactivation equal to 0.026 min⁻¹. In this case, however, a small part (about 20%) of the total amplitude of the phenomenon must be attributed to the modification of more reactive sulfhydryl groups (b parameter in Equation 3). Fructose 6-phosphate, which completely prevents enzyme inactivation, reduces the maximum level of MalNEt incorporation to 14 residues modified/oligomer. As in the case of NbS2, the most reactive sulfhydryl groups remains titrable with MalNEt in the presence of fructose 6-phosphate. By considering the existence of this class of 6 to 8 fast-reacting cysteines which are not protected by the substrate and which do not seem to be implicated in the expression of enzymatic activity, the protecting effect of fructose 6-phosphate was investigated further by means of differential labeling.

**Differential Labeling of Yeast Phosphofructokinase in the Presence of Fructose 6-Phosphate**—After complete reaction of native phosphofructokinase with unlabeled MalNEt in the presence of 5 mM fructose 6-phosphate, the protecting ligand and the excess of unlabeled reagent were eliminated by filtration on a Sephadex G-50 column. The enzyme was then reincubated either with radioactive MalNEt (Fig. 3) or with NbS2 (Fig. 4). The kinetics of chemical modification of —SH groups labeled in the second step of the differential reaction and the kinetics of subsequent enzyme inactivation were followed simultaneously, in each case. Sedimentation velocity measurements performed on the modified enzyme revealed only one species (s = 20 S) which attests the absence of enzyme dissociation upon chemical modification with MalNEt or NbS2. Moreover, the enzyme remains fully active after the

**Fig. 2.** Reaction of native yeast phosphofructokinase with MalNEt. Experimental conditions: standard TES/phosphate buffer, pH 6.8, 25°C, 1.24 μM enzyme oligomer, 0.9 mM MalNEt. The semilogarithmic plot (inset) relative to MalNEt incorporation in phosphofructokinase indicates the existence of three distinct classes of kinetically titrable —SH groups, under our experimental conditions. Accordingly, the kinetics of chemical modification (○) of reactive sulfhydryl groups were fitted to the following equation:

\[ r_m = n_1 + n_2(1 - e^{-k_1t}) + n_3(1 - e^{-k_2t}) + n_4(1 - e^{-k_3t}) \]  

with \( n_1 = 5.7 \), \( n_2 = 2.9 \), \( n_3 = 9.4 \), \( n_4 = 8.2 \), \( k_1 = 0.06 \) min⁻¹, \( k_2 = 0.03 \) min⁻¹, \( k_3 = 0.015 \) min⁻¹. Inactivation data (□) were fitted to the following empirical equation:

\[ A_{10} - F = e^{-(k_r t + b)} \]  

where \( A_{10} / A_0 \) is the percentage of enzymatic activity remaining at time \( t \) and \( F \) is the residual activity after complete reaction. Residual activity was estimated experimentally to 5% of initial enzyme activity. Apparent first order rate constant of inactivation (\( k_r \)) was adjusted to 0.026 min⁻¹ and \( b \) parameter (see text) was equal to 0.267.

**Fig. 3.** Differential reaction of yeast phosphofructokinase with MalNEt and fructose 6-phosphate as protecting ligand. The first step of the differential reaction of 4.7 μM enzyme oligomer with 0.9 mM unlabeled MalNEt was performed at 25°C in standard TES/phosphate buffer (pH 6.8) in the presence of 5 mM fructose 6-phosphate. At the end of the reaction (120 min), the reaction mixture was filtered on a Sephadex G-50 column (0.8 x 13 cm) and the enzyme-containing fractions were pooled (1.5 μM enzyme oligomer) and reincubated with 0.9 mM N-ethyl-3-{'H}ethylmaleimide (8.5 x 10⁸ cpm/mmol). Kinetics of chemical modification (○) and enzyme inactivation (□) were then followed simultaneously, as described under "Experimental Procedures." The open circles (○) correspond to the kinetics of modification of —SH groups specifically localized on β subunits, as estimated from direct counting of gel slices obtained from sodium dodecyl sulfate polyacrylamide gel electrophoresis, after differential reaction of the enzyme (see text).

**Fig. 4.** Labeling of —SH groups localized in fructose 6-phosphate binding sites with NbS2. Reversibility of the chemical modification. The first step of the differential reaction of reactive sulfhydryl groups was performed with MalNEt, as described in the legend of Fig. 3. In the second step of the differential reaction, NbS2 modification was substituted for radioactive labeling with MalNEt and NbS2 incorporation (○) and enzyme inactivation (□) were then followed simultaneously. The inset shows the kinetics of enzyme reactivation observed by incubation of the differentially NbS2-modified enzyme with 100 mM dithiothreitol, for different extent of initial enzyme inactivation.
Thiol Groups of Yeast Phosphofructokinase

Fig. 5. Separation of yeast phosphofructokinase subunits by ion exchange chromatography on DEAE-52 cellulose. Specific localization on α and β subunits of –SH groups protected by fructose 6-phosphate from MalNEt modification. The detailed procedure is as described under "Experimental Procedures." The volume of each fraction was 1 ml. Separation of α and β subunits of chemically unmodified phosphofructokinase. About 4.5 mg of the urea-denatured enzyme were applied to the column. Chromatography of a solution of radioactive MalNEt (0.9 mM) in standard Tris/glycine buffer (pH 8.6), in the absence of protein. The area of the small peak of radioactive contaminant centered around Fraction 58 corresponds to less than 0.2% of the total amount of radioactive reagent (not shown on the figure) which was eluted before the KCl gradient was applied. Separation of α and β subunits of yeast phosphofructokinase differentially labeled with MalNEt. About 3.5 mg of denatured protein specifically labeled with N-[ethyl-2-H]ethylmaleimide on –SH groups protected by fructose 6-phosphate, were applied to the column. In each case, radioactivity was counted from 0.5-ml aliquot of each fraction and sodium dodecyl sulfate gel electrophoresis of the peak fractions of upper (upper photography) and lower (lower photography) chromatographies were performed as described under "Experimental Procedures." Reference samples correspond to uncromatographed yeast phosphofructokinase. The partially proteolyzed material is referenced as α' and β' subunits, as previously described (7, 10).

first step of the differential reaction. Whatever the reagent used in the second step may be, the number of residues which are able to react differentially is equal to 12 –SH groups/enzyme oligomer (i.e. three sulfhydryl groups per fructose 6-phosphate binding site). For both experiments, however, the kinetics of incorporation of the reagent cannot be described by assuming a monoeponential process but they require a more complex reaction scheme involving at least two classes
of reactive —SH groups. We note that enzyme inactivation is complete for differential experiments performed with NbS2 (Fig. 4), whereas a slight residual activity is observed when the second step of the differential reaction is performed with MalNEt (Fig. 3).

Although peptide mapping would be necessary to correlate kinetic experiments carried out under distinct conditions, we note that 12 —SH groups are differentially titrated with MalNEt, as expected from direct labeling experiments performed with this reagent. In the case of NbS2 labeling, the agreement between differential and direct protection experiments is more ambiguous, as a result of the existence of an apparent zero order process following the direct titration of the most reactive sulphydryl groups (Fig. 1). However, we observe that for differential labeling experiments where —SH groups protected by fructose 6-phosphate are modified with NbS2, enzymatic activity may be recovered completely by reversing NbS2 labeling with an excess of dithiothreitol, even when the inactivation is complete (Fig. 4, inset). Thus, the occurrence of a large unfolding of the protein as a consequence of the chemical modification of one of its —SH groups, appears to be very unlikely.

Specific Localization of —SH Groups Protected by Fructose 6-Phosphate on β Subunits in Yeast Phosphofructokinase—In order to localize the fructose 6-phosphate-binding sites on α and/or β subunits, phosphofructokinase labeled with MalNEt by the differential method was denatured further in 8 M urea and its constituent subunits were partially separated by ion-exchange chromatography on a DEAE-52 cellulose column. As shown in Fig. 5, the labeled modified residues were found localized exclusively in β subunits. The same result is obtained if, instead of the ion-exchange chromatography step, we directly perform a sodium dodecyl sulfate gel electrophoresis. Furthermore, this last technique was used to follow the kinetics of incorporation of labeled reagent in each of the α and β bands. Whatever the time of the radioactive labeling may be, no labeled modified residue is detected in α subunits. On the other hand, the kinetics of incorporation in β subunits is well correlated with previous measurements performed on the denatured enzyme oligomer (Fig. 3) and levels off to three —SH groups modified per β subunit (i.e. 12 sulphydryl groups modified/αβαβ oligomer). The 12 —SH groups/oligomer protected by fructose 6-phosphate from the chemical modification of the enzyme with N-ethylmaleimide are thus localized exclusively on β subunits.

**DISCUSSION**

In the present work, we have shown, by means of differential labeling of yeast phosphofructokinase in the presence and absence of fructose 6-phosphate, that there are three —SH groups per subunit associated with the binding site of the allosteric substrate. These —SH groups may be modified specifically with NbS2 by means of a differential labeling experiment in which the first reaction is performed with MalNEt, in the presence of fructose 6-phosphate. In these conditions, primary effects of inactivation resulting from NbS2 modification can be reversed completely by incubation of the inactivated enzyme with an excess of dithiothreitol. Phosphofructokinase specifically labeled with radioactive MalNEt in its fructose 6-phosphate binding sites was denatured further and its constituent α and β subunits were separated either by ion-exchange chromatography in 8 M urea or on sodium dodecyl sulfate polyacrylamide gel. In both cases, radioactivity was found to be incorporated exclusively in β subunits. The kinetics of chemical modification measured directly from the incorporation of labeled reagent in β subunits is well correlated with the kinetics of modification studied on the undenatured enzyme and three —SH groups can be titrated finally per β subunit. These experiments show that the 12 —SH groups/oligomer which are protected by fructose 6-phosphate from the chemical modification of yeast phosphofructokinase with thiol reagents are localized exclusively on subunits of β type.

These data have to be compared with our previous binding studies showing the existence of only three or four binding sites for fructose 6-phosphate (11) as for all the other functional ligands (10). Probably, the value of four has to be considered, taking into account the revised molecular weight of the enzyme oligomer (5) and the demonstration of its octameric structure (6). The present results permit us to conclude that these substrate binding sites are localized exclusively on β subunits, in accord with our hypothesis (10) on the existence of distinct catalytic and regulatory subunits in yeast phosphofructokinase.

However, taking into account higher binding stoichiometries obtained elsewhere for fructose 6-phosphate (21), the alternative interpretation of the present data would be that there are two types of fructose 6-phosphate binding sites in yeast phosphofructokinase: the binding sites localized on β subunits which would have three —SH groups per site, protected by the substrate from the chemical modification with thiol reagents, and the binding sites localized on α subunits which would have no —SH group protected by fructose 6-phosphate. In such a case, however, the structural difference between these two types of fructose 6-phosphate binding sites should cause some heterogeneity in their substrate binding abilities and in the functional properties of the enzyme. Our kinetic studies (11, 12) as well as those of Freyer et al. (22) and Reuter et al. (23) do not suggest such an occurrence. Moreover, binding isotherms obtained for fructose 6-phosphate (11, 21) cannot be interpreted on the basis of the existence of two classes of binding sites for the allosteric substrate. Similarly, the occurrence of half-site reactivity in the chemical modification of yeast phosphofructokinase appears to be very unlikely by considering that kinetics of the catalyzed reaction exhibits strongly positive cooperativity (i.e. positive interactions between subunits containing the substrate binding sites). Moreover, such a phenomenon should not be restricted to the chemical modification of the enzyme but should also be involved for the binding of all ligands since only four binding sites are evidenced for each of them (10, 11). Such a situation is rather unlikely in reference to the well-known mechanism of half-site reactivity (24). In addition, the extent of enzyme inactivation observed upon differential chemical modification of the protected —SH groups would be hardly explained in such a case, since phosphofructokinase remains fully active upon sulphydryl modification in the presence of fructose 6-phosphate.

Although the final demonstration of the existence of distinct catalytic and regulatory subunits in yeast phosphofructokinase requires the localization of ATP and AMP binding sites on each type of subunits, the present results constitute strong evidence for the absence of direct role of α subunits in the catalytic mechanism of the yeast phosphofructokinase reaction. Although human erythrocytes phosphofructokinase is also known to be constituted of two types of subunits (25), the yeast enzyme is the first one for which the structural heterogeneity is correlated with its functional significance.
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REFERENCES