Inhibition of Glucose 6-Phosphate Dehydrogenase by Palmitoyl Coenzyme A*

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SUMMARY

Low concentrations of palmitoyl coenzyme A inhibit yeast glucose-6-P dehydrogenase (TAKETA, K., AND POPELL, B. M. (1966) J. Biol. Chem. 241, 720). This inhibition is prevented or reversed by bovine serum albumin, mycobacterial polysaccharides, or alkylated cyclodextrins. Sephadex chromatography and sucrose density gradient centrifugation show that palmitoyl-CoA inhibits by dissociating the tetrameric dehydrogenase to dimeric, enzymatically inactive subunits. These structural changes are accompanied by firm binding of palmitoyl-CoA to the dimeric subunit and by release of NADP. After removal of the (nondialyzable) inhibitor from the palmitoyl-CoA-dimer complex by alkylated cyclodextrin the subunits reaggregate to enzymatically active tetramer. Reaggregation does not require NADP. In contrast to palmitoyl-CoA, sodium dodecyl sulfate dissociates yeast dehydrogenase to monomers. It is concluded that palmitoyl-CoA, unlike the synthetic anionic detergent, perturbs the dehydrogenase subunit structure in a controlled manner which may be important for regulating the activity of lipogenic enzymes.

The dimeric glucose-6-P dehydrogenase from Leuconostoc mesenteroides is inhibited only by high concentrations of palmitoyl-CoA. In this instance palmitoyl-CoA neither binds to the enzyme nor dissociates it. Torulopsis utilis glucose-6-P dehydrogenase, also dimeric, is irreversibly converted to inactive monomers by low concentrations of palmitoyl-CoA.

EXPERIMENTAL PROCEDURE

Materials—Materials were obtained from the following sources: glucose-6-P dehydrogenase (bakers' yeast, 270 units per mg of protein), laetic dehydrogenase (rabbit muscle, 750 units per mg of protein), hemoglobin (bovine), crystalline bovine serum albumin, and sodium glucose-6-P from Sigma; T. utilis glucose-6-P dehydrogenase (220 units per mg of protein) and palmitoyl-CoA from P-L Biochemicals; L. mesenteroides glucose-6-P dehydrogenase (207 units per mg of protein) from Worthington Biochemical Corp.; [1-14C]palmitoyl-CoA (60 mCi per mmole) from New England Nuclear; NADP from Calbiochem. Heptakis(2,6-di-O-methyl)-β-cyclodextrin (11), hereafter referred to as CH₃-cyclodextrin, and MMP, 3-O-methylmannose containing polysaccharide from Mycobacterium phlei (12), were prepared as described below.

1 The abbreviations used are: SDS, sodium dodecyl sulfate; CH₃-cyclodextrin, heptakis(2,6-di-O-methyl)-β-cyclodextrin; MMP, 3-O-methylmannose containing polysaccharide from Mycobacterium phlei.

2 P. Flick, unpublished results.
previously described. All other chemicals were of analytical grade.

Glucose-6-P Dehydrogenase Assay—Enzyme activity was measured spectrophotometrically by following the initial velocity of NADP reduction at 25°. The increase in absorbance at 340 nm was recorded on a Cary 118 spectrophotometer. The standard assay system for the yeast enzyme contained 0.05 mM Tris-HCl buffer, pH 7.4, 0.1 mM glucose-6-P, 0.15 mM NADP and 0.12 µg of enzyme in a total volume of 1.0 ml. Unless otherwise stated, reactions were started by addition of enzyme. Protein was determined by the method of Lowry et al. (13) with crystalline bovine serum albumin as a standard.

Sucrose Density Gradient Centrifugations—These were carried out essentially as described by Martin and Ames (14), except that 0.05 M potassium phosphate buffer, pH 7.4, was used routinely. Samples were layered on 5 to 20% sucrose gradients and centrifuged at 39,000 rpm for 15 hours at 4° using the SW 39 rotor in a Spinco model L ultracentrifuge. Each tube was then punctured at the bottom and drops were collected for the assay of protein concentration and radioactivity. Radioactive samples were dissolved in Bray's solution and counted in a Packard Tri-Carb scintillation spectrometer.

RESULTS

Inhibition of Glucose-6-P Dehydrogenase by Palmitoyl-CoA—The inhibition of yeast glucose-6-P dehydrogenase by palmitoyl-CoA is shown in Fig. 1. Under the conditions employed, 30% inhibition of the enzyme was obtained with 1.5 µM palmitoyl-CoA and almost complete inhibition with 10 µM palmitoyl-CoA. Palmitoyl pantetheine, palmitoyl carnitine, coenzyme A, or potassium palmitate in concentrations up to 0.1 M did not inhibit the enzyme (Table I). Lauryl-CoA at 25 µM gave 21% inhibition and 54% at 50 µM (Table I). In the experiments shown in Fig. 1A (upper panel), the reaction was started by the addition of enzyme (Curve a, upper panel), enzyme was preincubated with palmitoyl-CoA and either glucose-6-P or NADP and the reaction started by the addition of the other substrate. When the enzyme was preincubated with palmitoyl-CoA and NADP (Fig. 1B, Curve b, upper panel), the extent of inhibition was the same as that shown in Fig. 1A (upper panel). However, when enzyme and palmitoyl-CoA were preincubated with glucose-6-P alone or without any substrate (Fig. 1B, Curves c and d, upper panel), inhibition was more severe for the first 5 min. Thereafter, the extent of inhibition became nearly the same in the experiments in which the preincubation mixture contained NADP. These results show that NADP but not glucose-6-P protects the enzyme against palmitoyl-CoA inhibition.

Reversibility of Palmitoyl-CoA Inhibition—Under specified conditions bovine serum albumin reverses the inhibition of glucose-6-P dehydrogenase by palmitoyl-CoA (1). Equally effective in restoring reaction rates to normal were the mycobacterial polysaccharide MMP (65 µM) and CH3-cyclodextrin (30 µM) (Fig. 1, lower panel). SDS (130 µM) also inhibited the dehydrogenase (80%, Table I) but in this case inhibition was not reversed by the addition of either MMP, CH3-cyclodextrin, or bovine serum albumin.

TABLE I

Effect of palmitoyl-CoA and related compounds on glucose-6-P dehydrogenase activity

<table>
<thead>
<tr>
<th>Addition (µM)</th>
<th>Relative glucose-6-P dehydrogenase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Palmitoyl pantetheine (57)</td>
<td>106</td>
</tr>
<tr>
<td>Palmitoyl pantetheine (115)</td>
<td>109</td>
</tr>
<tr>
<td>Palmitoyl carnitine (10)</td>
<td>103</td>
</tr>
<tr>
<td>Palmitoyl carnitine (100)</td>
<td>104</td>
</tr>
<tr>
<td>Palmitoyl-CoA (10)</td>
<td>112</td>
</tr>
<tr>
<td>Coenzyme A (125)</td>
<td>147</td>
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<tr>
<td>Lauryl-CoA (25)</td>
<td>79</td>
</tr>
<tr>
<td>Lauryl-CoA (50)</td>
<td>46</td>
</tr>
<tr>
<td>Palmitoyl-CoA (10)</td>
<td>3.5</td>
</tr>
<tr>
<td>SDS (113)</td>
<td>20</td>
</tr>
</tbody>
</table>

1 In the course of these studies an apparent relief of palmitoyl-CoA inhibition by Mg²⁺ was noted. The effect is probably due to the formation of insoluble Mg salts of palmitoyl-CoA.
Fig. 2. Left, sucrose density gradient centrifugation of [14C]palmitoyl-CoA-treated yeast glucose-6-P dehydrogenase. Enzyme solution (0.1 ml) containing 120 μg of protein was layered over 5.0 ml of a 5 to 20% (w/v) linear sucrose density gradient in 0.05 m potassium phosphate buffer, pH 7.4. Centrifugation was carried out for 15 hours at 39,000 rpm at 4°. A, native enzyme in the presence of 0.15 mM NADP. B, enzyme treated with [14C]palmitoyl-CoA (1 mM, 60,000 cpm) in the presence of 0.15 mM NADP in a total volume of 0.1 ml was kept for 10 min at 25° before centrifugation. C, NADP-free enzyme prepared by the method of Chung and Langdon (15). Center, sucrose density gradient centrifugation of [14C]palmitoyl-CoA-treated yeast glucose-6-P dehydrogenase. Conditions of centrifugation were the same as described for left panel. A, enzyme (0.11 μg) treated with palmitoyl-CoA (3 μM, 10,000 cpm) in the presence of 0.15 mM NADP in a total volume of 0.1 ml. B, palmitoyl-CoA (3 μM, 10,000 cpm). C, enzyme (0.11 μg) treated with palmitoyl-CoA (9 μM, 30,000 cpm) in the presence of 0.15 mM NADP in a total volume of 0.1 ml. D, palmitoyl-CoA (9 μM, 30,000 cpm). Right, sucrose density gradient centrifugation of SDS-treated yeast glucose-6-P dehydrogenase. Conditions of centrifugation were the same as in left panel. A, native enzyme in the presence of 0.15 mM NADP. B, enzyme treated with palmitoyl-CoA (1 mM). C, enzyme treated with SDS (final concentration 0.1%) in the presence of 0.15 mM NADP in a total volume of 0.1 ml. D, enzyme treated first with [14C]palmitoyl-CoA (1 mM, 60,000 cpm) in the presence of 0.15 mM NADP for 10 min and then with SDS (final concentration 0.1%).

albumin. When a mixture of SDS (130 μM) and CH3-cyclodextrin (1.3 mM) was added to the assay mixture at the start, no inhibition was observed. These results indicate that SDS inactivates the enzyme irreversibly and that CH3-cyclodextrin protects against this inactivation by complexing the detergent.

Sucrose Density Gradient Centrifugation—The sedimentation pattern of the yeast dehydrogenase in the presence of palmitoyl-CoA was followed by sucrose density centrifugation. Fig. 2, left, shows the sedimentation pattern of native enzyme (A), enzyme treated with 1 mM [14C]palmitoyl-CoA (B), and NADP-free enzyme (C) prepared by treatment with acid ammonium sulfate (15). Under the conditions employed, free [14C]palmitoyl-CoA was located at the top of the gradient.

Native yeast glucose-6-P dehydrogenase is known to be a tetramer containing 4 moles of NADP (16). On removal of NADP by acid ammonium sulfate, the tetramer dissociates to dimers, molecular weight 102,400 ± 2,400 (17). It is clear from the results shown in Fig. 2, left, that palmitoyl-CoA converts the native enzyme to a protomer of the same size (dimer) and moreover, that palmitoyl-CoA binds to the dimer but not to the native, undissociated enzyme. This figure also shows that exposure of the dehydrogenase to palmitoyl-CoA fails to produce any monomeric subunits.

In the experiments that demonstrated dissociation of the native dehydrogenase to dimeric subunits (Fig. 2, left) the concentrations of palmitoyl-CoA were relatively high (1 mM and 120 μg of enzyme in 0.1 ml). Attempts were, therefore, made to ascertain whether partial enzyme inhibition by lower palmitoyl-CoA concentrations4 can also be attributed to changes in the quaternary enzyme structure. Such experiments are technically difficult because they have to be conducted with enzyme concentrations which are too low for assaying protein in the sucrose density gradient fractions. Two such experiments, involving only measurements of enzyme activity and radioactivity, are illustrated in Fig. 2, center. With 3 and 9 μM [14C]palmitoyl-CoA, the dehydrogenase (0.11 μg/0.1 ml) was inhibited 50% and 90%, respectively, as judged by enzymatic assay of the gradient fractions. Two such experiments, involving only measurements of enzyme activity and radioactivity, are illustrated in Fig. 2, center. With 3 and 9 μM [14C]palmitoyl-CoA, the dehydrogenase (0.11 μg/0.1 ml) was inhibited 50% and 90%, respectively, as judged by enzymatic assay of the gradient fractions corresponding to the tetramer region. No radioactivity was associated with enzymatically active fractions. However, the immediately following fractions, which would be expected to contain dimer, were significantly radioactive. From the results obtained by Sephadex G-200 chromatography (Fig. 3), it can be calculated that dissociated dehydrogenase produced by palmitoyl-CoA contains about 10 moles of palmitoyl-CoA per mole of dimer. From the data in Fig. 2, center, and on the assumption that the fraction of dimeric dehydrogenase is equal to the difference between total initial protein and catalytically active protein (tetrmeric), the estimated radioactivities for the dimer region are 170 cpm and 310 cpm, respectively, for the two experiments with 3 and 9 μM palmitoyl-CoA (A and C). Judging from the sedimentation pattern (Fig. 2, center), free [14C]palmitoyl-CoA
FIG. 3. Sephadex G-200 chromatography. Yeast enzyme (1 mg of protein) was incubated with [14C]palmitoyl-CoA (8 mM, 200,000 cpm) and NADP (7.5 mM) in a total volume of 0.2 ml for 10 min at 25°. The mixture was then applied to a column of Sephadex G-200 (0.9 × 46 cm) which had been previously equilibrated with 0.05 M potassium phosphate buffer, pH 7.4. Ten drops each (0.05 ml) were collected, and protein and radioactivity were determined in 0.1-ml aliquots.

expected at the top of the gradient (B and D) slightly overlapped with the fractions corresponding to dimer. Therefore, the total radioactivity bound to dimer cannot be accurately calculated. However, the radioactivities in the area corresponding to the dimer peaks (Fraction 11, (A and C)) contained 50 and 80 cpm, respectively. In spite of the uncertainty of these calculations these data provide qualitative evidence for the contention that even under conditions of partial inactivation, the enzyme dissociates, i.e. that dehydrogenase inhibition is a direct consequence of palmitoyl-CoA-induced changes in the quaternary structure of the enzyme.

Native enzyme treated with SDS shows a sedimentation pattern different from that obtained with palmitoyl-CoA. Smaller, presumably monomeric, subunits are formed (Fig. 2C, right). When native enzyme was treated first with [14C]palmitoyl-CoA and subsequently with SDS, dissociation proceeded all the way to the monomer with retention of radioactivity (Fig. 2D, right). The dimer-monomer transformation, therefore, does not change the affinity of protein for palmitoyl-CoA.

Gel Filtration on Sephadex G-200—Enzyme (1 mg/0.2 ml) treated with [14C]palmitoyl-CoA (8 mM) was applied to a column (0.9 × 46 cm) of Sephadex G-200 and elution carried out with 0.05 M potassium phosphate buffer, pH 7.4. The elution pattern (Fig. 3) showed a small nonradioactive peak (A), presumably native enzyme, a large protein peak with coincident radioactivity (B), and a third peak (C) containing radioactivity (palmitoyl-CoA) but no protein. The pattern confirms the results obtained by sucrose density gradient centrifugation. Palmitoyl-CoA dissociates the dehydrogenase and binds to the resulting dimer. When fractions from Peak B (Fig. 3) were dialyzed against 0.05 M phosphate buffer, pH 7.4, for 15 hours, about 95% of the radioactivity remained associated with protein indicating firm binding of palmitoyl-CoA to the dimeric enzyme.

For molecular weight determinations, palmitoyl-CoA-treated enzyme was co-chromatographed on Sephadex G-200 with hemoglobin and lactate dehydrogenase as standards (Fig. 4). The calculated molecular weight, 1.1 × 10⁵, is one-half of the value for the native dehydrogenase (16) and identical with that determined for NADP-free enzyme (17).

Reaggregation of Dimer to Tetramer—In attempts to regenerate native enzyme from the palmitoyl-CoA-dimer complex, advantage was taken of the ability of CH₄-cycloextrin to complex long chain fatty acyl-CoA (11). Solutions of enzymatically inactive, [14C]palmitoyl-CoA-treated enzyme (Peak B, Fig. 3) were concentrated to small volume with the aid of a Minicon B15 filter after the addition of either CH₄-cycloextrin, NADP, or both. Each sample was then placed on a sucrose density gradient (Fig. 5). Concentrating the [14C]palmitoyl-CoA-dimer in the presence of CH₄-cycloextrin completely removed the radioactive inhibitor from the protein and at the same time afforded reaggregated, enzymatically active tetramer (Fig.

FIG. 4. Molecular weight determination of palmitoyl-CoA-treated glucose-6-P dehydrogenase. Marker proteins were dissolved in 0.5 ml of 0.05 M potassium phosphate buffer, pH 7.4, and applied to a Sephadex G-200 column (0.9 × 46 cm) pre-equilibrated in the same buffer. Lactic dehydrogenase was assayed by absorption at 280 nm and hemoglobin at 555 nm.

FIG. 5. Reaggregation of dimer produced by palmitoyl-CoA to tetramer. Solutions of [14C]palmitoyl-CoA-dimer obtained from a Sephadex G-200 column (Fraction B, Fig. 3; 5 ml, 1 mg of protein, 150,000 cpm) were concentrated to 0.1 ml with a Minicon B15 filter either without any additions (A), or after addition of 15 mM NADP (B); after addition of 50 mM CH₄-cycloextrin (C) and after addition of both 15 mM NADP and 50 mM CH₄-cycloextrin (D). In Experiment D palmitoyl-CoA was nonradioactive. CD, CH₄-cycloextrin.
amine by difference spectroscopy whether or not enzyme that NADP. The differences in the 340~nm absorption of native and reaggregated formation of NADFH in the absence of added pyridine after removal of the inhibitor by CH₃-cyclodextrin, contained are shown in Fig. 6. Native enzyme catalyzes glucose-6-P-dehydrogenase contains 4 moles of tightly bound NADP (16). Therefore, we excluded that palmitoyl-CoA treatment removes NADP from the enzyme.

The aim of the present investigation was to resolve, if possible, the controversial question whether palmitoyl-CoA and related long chain acyl-CoA derivatives inhibit enzymes by a physiologically plausible mechanism or whether the effects are non-specific, of the type shown by synthetic detergents. Yeast glucose-6-P dehydrogenase was chosen for this purpose because it is readily available, well characterized (17,21), and highly sensitive to palmitoyl-CoA (1, 9).

Inhibition of Leuconostoc glucose-6-P dehydrogenase by palmitoyl-CoA and relief by CH₃-cyclodextrin

Assay mixtures contained 0.05 M Tris-HCl buffer, pH 7.4, 1.0 mM glucose-6-P, 0.15 mM NADP or 1.0 mM NAD as indicated, 0.08 μg of enzyme and palmitoyl-CoA as indicated, in a total volume of 1.0 ml. Reactions were started by the addition of enzyme and 2 min later 0.1 ml of 3 mM cyclodextrin was added.

TABLE II

Inhibition of Leuconostoc glucose-6-P dehydrogenase by palmitoyl-CoA and relief by CH₃-cyclodextrin

<table>
<thead>
<tr>
<th>Pyridine nucleotide</th>
<th>Palmitoyl-CoA</th>
<th>Glucose-6-P dehydrogenase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before addition of CH₃-cyclodextrin</td>
</tr>
<tr>
<td>NADP</td>
<td>0</td>
<td>0.0546 (100)</td>
</tr>
<tr>
<td>NADP</td>
<td>500</td>
<td>0.0400 (72.3)</td>
</tr>
<tr>
<td>NAD</td>
<td>0</td>
<td>0.0978 (100)</td>
</tr>
<tr>
<td>NAD</td>
<td>500</td>
<td>0.0300 (30.6)</td>
</tr>
</tbody>
</table>

The specific activity of Fraction 10, Fig. 5C, was about one-third of that of the original, native dehydrogenase. Treatment of palmitoyl-CoA-dimer with NADP alone neither removed palmitoyl-CoA nor did it produce enzymatically active tetramer (Fig. 5B). When both CH₃-cyclodextrin and NADP were added to palmitoyl-CoA-inactivated enzyme (Fig. 5D) the results were essentially the same as those obtained with CH₃-cyclodextrin alone. Thus, NADP does not appear to be necessary for reaggregating dimeric dehydrogenase to the tetramer.

Role of NADP during Reaggregation—Native yeast glucose-6-P dehydrogenase contains 4 moles of tightly bound NADP (16). On removal of the pyridine nucleotide by acid ammonium sulfate, the enzyme dissociates to the dimer (17). Therefore, we examined by difference spectroscopy whether or not enzyme that had first been treated with palmitoyl-CoA and then reaggregated after removal of the inhibitor by CH₃-cyclodextrin, contained NADP. The differences in the 340 nm absorption of native and reaggregated enzyme in the presence or absence of glucose-6-P are shown in Fig. 6. Native enzyme catalyzes glucose-6-P-dependent formation of NADPH in the absence of added pyridine nucleotide, but palmitoyl-CoA-treated and subsequently reaggregated enzyme does not. This evidence, although indirect, leads to the conclusion that enzyme reaggregated to an active tetramer as described above does not contain NADP, and by the same token, that palmitoyl-CoA treatment removes NADP from the enzyme.

Inhibition of Glucose-6-P Dehydrogenase from L. mesenteroides—According to Olive and Levy (18), Leuconostoc glucose 6-P dehydrogenase, an enzyme equally active with NAD and NADP, is insensitive to 10 μM palmitoyl-CoA. Recently, Coe and Hsu (10) reported that the NAD-dependent activity was inhibited by relatively high concentrations of oleoyl-CoA (Kᵣ = 0.15 mM). We have obtained similar results with palmitoyl-CoA as inhibitor (Table II). The NAD-dependent dehydrogenase activity was more sensitive to palmitoyl-CoA than the NADP-dependent reaction, and in both instances, inhibition was almost completely reversed by adding CH₃-cyclodextrin. Fig. 7, left, shows sucrase density gradient patterns of native Leuconostoc enzyme (A) and of enzyme treated with ¹⁴C-palmitoyl-CoA (B). The molecular weight of the enzyme did not change nor did radioactivity bind to protein. From SDS polyacrylamide gel electrophoresis a molecular weight of 54,000 was calculated for the monomeric Leuconostoc enzyme. Since a molecular weight of 103,700 has been reported for the Leuconostoc dehydrogenase (19), this enzyme must be a dimer in the native form.

Inhibition of Glucose-6-P Dehydrogenase from T. utilis—For comparative purposes, inhibition studies were extended to the glucose-6-P dehydrogenase from T. utilis. This dimeric enzyme (reported mol wt of 104,000 (20)) was nearly as sensitive to low palmitoyl-CoA concentrations as enzyme from bakers' yeast (Table III), but unexpectedly none of the palmitoyl-CoA-complexing agents (CH₃-cyclodextrin, bovine serum albumin, or MMP) reversed the inhibition. From the results obtained on sucrase density gradient centrifugation (Fig. 7, right), it is concluded that palmitoyl-CoA dissociates the dimeric Torulopsis enzyme to monomers and binds to the monomeric subunits.

DISCUSSION

The aim of the present investigation was to resolve, if possible, the controversial question whether palmitoyl-CoA and related long chain acyl-CoA derivatives inhibit enzymes by a physiologically plausible mechanism or whether the effects are non-specific, of the type shown by synthetic detergents. Yeast glucose-6-P dehydrogenase was chosen for this purpose because it is readily available, well characterized (17, 21), and highly sensitive to palmitoyl-CoA (1, 9).

The synthetic detergent SDS dissociates enzymes into monomeric, usually inactive subunits (22). It, therefore, seemed of interest to examine the effects of inhibitory concentrations of palmitoyl-CoA on the quaternary structure of yeast glucose-6-P dehydrogenase. The results obtained both by sucrose density gradient centrifugation and by Sephadex G-200 chromatography clearly demonstrate that palmitoyl-CoA affects the dehydrogenase subunit structure, converting the native tetramer (mol wt 205,000) to a smaller subunit. Enzyme dissociated in this manner has the same size (mol wt 1.1 x 10⁶) as the dimeric, NADP-free enzyme obtainable from native dehydrogenase by acid ammonium sulfate (17).

Two further events accompany the palmitoyl-CoA-induced dissociation of the dehydrogenase. First, the inhibitor binds to the subunit as shown by the coincidence of radioactivity (¹⁴C)-palmitoyl-CoA with the protein peak that corresponds to the dimer (Figs. 2 and 3). Dialysis does not remove the bound radioactivity. No such binding occurs or is detectable with the native, undissociated enzyme. Secondly, formation of the palmitoyl-CoA dimer complex appears to be associated with the release of bound NADP (Fig. 6). These results and earlier observations on the tetramer-dimer relationship (16) indicate that removal of the pyridine nucleotide from yeast glucose-6-P...
Fig. 7. Left, sucrose density gradient centrifugation of [14C]-palmitoyl-CoA-treated Leuconostoc glucose-6-P dehydrogenase. The conditions for centrifugation were the same as described in Fig. 2. A, native enzyme (390 μg). B, enzyme (390 μg) was treated with palmitoyl-CoA (50 mM, 20,000 cpm) in a total volume of 0.1 ml and kept for 10 min at 25° before centrifugation. Right, sucrose density gradient centrifugation of [14C]palmitoyl-CoA-treated glucose-6-P dehydrogenase from Torulopsis. Conditions for centrifugation were the same as described in Fig. 2. A, native enzyme (400 μg). B, enzyme (400 μg) was treated with [14C]palmitoyl-CoA (4 mM, 20,000 cpm) in a total volume of 0.1 ml for 10 min at 25° prior to centrifugation. Both samples were diluted with 0.05 M potassium phosphate buffer, pH 7.4, to 10 ml and then concentrated to 0.05 ml with a Minicon B15 filter. The concentrated samples were subjected to centrifugation.

Table III

Molecular properties of various glucose-6-P dehydrogenases and palmitoyl-CoA inhibition

<table>
<thead>
<tr>
<th></th>
<th>Mol wt x 10^4</th>
<th>No. of monomeric subunits</th>
<th>Palmitoyl-CoA inhibition[a]</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Bakers’ yeast</td>
<td>2.05</td>
<td>4</td>
<td>3</td>
<td>1, 9, 16, 21</td>
</tr>
<tr>
<td>Torulopsis utilis</td>
<td>1.04</td>
<td>2</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Leuconostoc mesenteroides</td>
<td>1.04</td>
<td>2</td>
<td>350 (NAD) 700 (NADP)</td>
<td>10, 18, 19</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>1.33 (2.44)</td>
<td>2(4)</td>
<td>+</td>
<td>18, 25</td>
</tr>
<tr>
<td>Rat liver</td>
<td>1.10</td>
<td>4</td>
<td>+</td>
<td>1, 26</td>
</tr>
<tr>
<td>Human erythrocytes</td>
<td>2.05</td>
<td>4</td>
<td>+</td>
<td>9, 27</td>
</tr>
</tbody>
</table>

[a] Concentration of palmitoyl-CoA, μM, which produces 50% inhibition.

[b] Palmitoyl-CoA was reported to inhibit enzyme activity but no quantitative data for pure enzyme are available.

dehydrogenase, whether by acid ammonium sulfate or by palmitoyl-CoA, weakens the native quaternary structure of the enzyme sufficiently to cause dissociation.

Since NADP protects the dehydrogenase against palmitoyl-CoA inhibition (Fig. 1), the binding sites for the two ligands cannot be identical. Moreover, the calculated number of palmitoyl-CoA molecules bound per mole of enzyme (about 10 moles per mole of dimer calculated from 3H data, Fig. 3) greatly exceeds the number of binding sites for NADP (four per tetramer (16)). We, therefore, envision the following events for the palmitoyl-CoA-induced enzyme dissociation (Fig. 8). Palmitoyl-CoA produces a conformationally altered dehydrogenase with lowered affinity for NADP. The destabilized, coenzyme-depleted tetramer then dissociates to the dimer with exposure of high affinity sites for palmitoyl-CoA.

As Taketa and Fogell have shown, palmitoyl-CoA inhibition of the yeast dehydrogenase can not only be prevented by simultaneous addition of bovine serum albumin to the reaction mixture but can also be reversed if bovine serum albumin is added a few minutes after reaction has started (1). We have confirmed these results and have shown further that the palmitoyl-CoA complexing agents, MMP and CH₃-cyclohexatin, similarly relieve palmitoyl-CoA inhibition. The reversibility of this process is now explainable in light of the demonstration that the inhibited and enzymatically inactive species of the dehydrogenase is a palmitoyl-CoA-dimer complex. Displacement of palmitoyl-CoA from the protein by reagents which have a high affinity for
the inhibitor—bovine serum albumin, MMP or CH$_2$-cyclodextrin—will regenerate a dimeric species that spontaneously reassociates to the dehydrogenase tetramer. Figs. 3 to 5 provide the following documentation for these events. Palmitoyl-CoA-containing dimer isolated by Sephadex chromatography, concentrated by Diaflo filtration in the presence of CH$_2$-cyclodextrin, and subsequently subjected to sucrose density gradient centrifugation, yielded a protein peak in a position corresponding to the dehydrogenase tetramer. This fraction had regained about one-third of the specific activity of the native enzyme. For reactivation, removal of palmitoyl-CoA from the protein is essential and this can be effected by CH$_2$-cyclodextrin—and presumably also by other palmitoyl-CoA-complexing agents—but not by NADP. It is also clear from these results that the dimer-tetramer reassociation takes place in the absence of pyridine nucleotide and that a NADP-free tetramer is capable of existence.

Inhibitory concentrations of SDS (150 $\mu$M) dissociate yeast glucose-6-P dehydrogenase completely to monomeric subunits (Fig. 2, right). Whether this tetramer-monomer transformation proceeds by way of dimeric intermediates is not known. Monomer obtained from the [14C]palmitoyl-CoA-dimer complex by treatment with SDS retains radioactivity indicating that the two depolymerizing agents bind to different protein sites. Thus, the effects of palmitoyl-CoA and SDS on dehydrogenase subunit structure are clearly distinguishable. Palmitoyl-CoA is the much more selective “denaturant,” and at least in the case of yeast glucose-6-P dehydrogenase, the more potent enzyme inhibitor. These differences as well as the reversibility of the palmitoyl-CoA-induced enzyme dissociation seem to us strong arguments in favor of a physiological role for long chain acyl-CoA in the regulation of glucose-6-P dehydrogenase activity. Reversibility of the process, i.e. reassociation of the dehydrogenase dimer to active tetramer will, of course, require the presence or availability in the cell of effective palmitoyl-CoA-complexing agents. Bovine serum albumin, the mycobacterial polysaccharides (11), CH$_2$-cyclodextrin (23), and phospholipid (24) have the requisite affinity for binding palmitoyl-CoA and for displacing it from inhibited enzyme. Whether molecules of any of these structural types regulate the yeast enzyme in the proposed manner remains to be established.

In their native form, glucose-6-P dehydrogenases of different origins appear to occur either as tetramers of an approximate molecular weight of 200,000 or as dimers of molecular weight 100,000 (Table III). With the apparent exception of the mammalian enzymes, the monomeric dehydrogenase subunits have a molecular weight of about 50,000. Palmitoyl-CoA inhibits all the dehydrogenases tested.

For ascertaining whether palmitoyl-CoA sensitivity is related to subunit structure, comparisons between the tetrameric yeast dehydrogenase and the dimeric enzymes from Leuconostoc, and from the aerobic yeast T. utilis seemed of interest. Leuconostoc dehydrogenase, an enzyme with dual pyridine nucleotide specificity (18), is inhibited only by relatively high palmitoyl-CoA concentrations (10). Moreover, it is more sensitive to palmitoyl-CoA in the presence of NAD than of NADP. This enzyme, when inhibited, neither binds palmitoyl-CoA nor dissociates to smaller subunits. The dimeric dehydrogenase from Torulopsis shows still another pattern. Palmitoyl-CoA causes a dimer- monomer transition, and in the process, binds to the monomeric subunit. In this instance, however, the various palmitoyl-CoA-complexing agents fail to reverse enzyme inhibition. A tentative conclusion to be drawn from these results is that those dehydrogenases which are highly sensitive to palmitoyl-CoA respond to the inhibitor by undergoing changes in quaternary structure, either from tetramer to dimer or from dimer to monomer, dissociation producing subunits that have high affinity for palmitoyl-CoA. That palmitoyl-CoA can also inhibit dehydrogenases without binding or without changing subunit interactions is exemplified by the Leuconostoc enzyme.

In yeast, palmitoyl-CoA is an end product of fatty acid synthesis, a process that requires NADPH. Thus, by interfering with NADPH production, palmitoyl-CoA conforms broadly with the definition of a negative feedback inhibitor. However, as an allosteric modifier, palmitoyl-CoA appears to be atypical in at least two respects. First, binding of this ligand to protein causes not only a conformational change but also alters the quaternary structure of the yeast glucose-6-P dehydrogenase. Secondly, the number of dehydrogenase binding sites for the effector molecule greatly exceeds the number of monomeric subunits and may not conform to a fixed strict geometry. Nevertheless, inhibition by palmitoyl-CoA is clearly not an indiscriminate process. Numerous enzymes, polymeric as well as monomeric, are totally resistant to palmitoyl-CoA concentrations as high as 10$^{-4}$ M (1). Therefore, concurring with Eger-Neufeldt et al. (9) and Srere (5), we are inclined to view long chain CoA derivatives as “metabolic detersents” or “natural denaturants” which regulate metabolism by controlled perturbation of quaternary enzyme structure.

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* We are aware of only one other example of enzyme inhibition resulting from product-induced oligomer dissociation. As shown by Shizuta et al. (28), $\alpha$-ketobutyrate causes dissociation of the AMP-dependent tetrameric threonine deaminase to dimeric or monomeric subunits.
Inhibition of Glucose 6-Phosphate Dehydrogenase by Palmitoyl Coenzyme A
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