Identification of an arginine residue important for catalytic activity in the primary structure of d-glyceraldehyde 3-phosphate dehydrogenase

Studies with the rat skeletal-muscle enzyme

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(Received 14 July 1981/Accepted 21 July 1981)

The reaction of holo-(d-glyceraldehyde 3-phosphate dehydrogenase) (EC 1.2.1.12) from rat skeletal muscle with [14C]butanedione in 0.05 M NH4HCO3, pH 8.0, resulted in modification (*) of two arginine residues per subunit with a concomitant loss of catalytic activity. From a tryptic digest of the modified protein two radiolabelled peptides were isolated, with the following sequences:

(1) Val-Ile-Ile-Asn-Pro-Thr-Ala-Asp-Ala(Glx, Met, Leu, Phe, Met)Gly-Val-Asx-Arg-Glx(His, Tyr)Ser-Lys

and

(2) Asp-Ala-Gly-Ala-Thr-Ile-Ala-Leu(Asx, Glx, Arg, Phe, Val)Lys.

By comparison of the data with the known sequences of homologous enzymes, the localization of the modified residues was established. The first peptide was identified as corresponding to residues 116–139, the second to residues 293–306. Experimental evidence from this and previous studies suggests that arginine-134 is important for the catalytic activity of the rat muscle enzyme, being involved in structural rearrangements accompanying the organization of the active centre on the binding of coenzyme and substrate.

D-Glyceraldehyde 3-phosphate dehydrogenase, a tetrameric enzyme composed of identical subunits (Harris & Perham, 1965; Harrington & Karr, 1965), was previously shown to be inactivated by an arginine-specific reagent, butane-2,3-dione (Nagradova & Asryants, 1975; Nagradova et al., 1976). Two arginine residues per subunit were found to be modified concomitant with the loss of the dehydrogenase activity in both yeast and rat muscle enzymes. Kinetic analysis of the data revealed that modification of a single arginine residue per subunit was responsible for the effect (Nagradova et al., 1978a).

The microenvironment of the essential arginine residue was found to change on transition from apo- to holo-enzyme conformation; this was evidenced by a marked increase in the rate constant of butanedione-induced inactivation in the presence of NAD+.

The effect was suggested to be caused by some movement of the essential arginine with respect to its position in the apoenzyme, introducing a positively charged guanidinium group in the active centre (Nagradova et al., 1978a). This suggestion was in line with the hypothesis of Garavito et al. (1977) that a similarity exists between conformational changes induced by the binding of NAD+ to lactate dehydrogenase, liver alcohol dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase. On the basis of crystallographic data, arginine-231 was suggested to be involved in structural rearrangements characteristic of the transition from the apo to holo conformation of the latter enzyme (Garavito et al., 1977; Nagradova et al., 1978a).

The present work was initiated to verify this suggestion. Our purpose was to reveal the localization of the butanedione-modified arginine residue in the primary structure of rat muscle...
glyceraldehyde 3-phosphate dehydrogenase. The complete amino acid sequence of this protein is not known, but the structure of the N-terminal region of the molecule (Nagradova et al., 1978b) and of the peptide containing the essential cysteine-149 (Baibakov et al., 1977) closely resemble the corresponding parts of the polypeptide chain of the pig muscle enzyme (Harris & Perham, 1968). These facts, together with a high degree of homology which is known to exist between glyceraldehyde 3-phosphate dehydrogenases of different origin (Olsen et al., 1975), justified our attempts to locate arginine residues modified with [14C]butanedione within the covalent structure of the protein.

Materials and methods

Enzyme

D-Glyceraldehyde 3-phosphate dehydrogenase was isolated from rat skeletal muscle (Nagradova & Guseva, 1971). The enzyme was shown to be pure by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate and by analysis of the N-terminal amino acid. Specific activity of the enzyme preparations assayed as previously described (Nagradova et al., 1975) corresponded to 100 μmol of NAD⁺ reduced/min per mg of protein. Protein concentrations were determined spectrophotometrically by using A₂₈₀ 0.83 for the apoenzyme.

Reagents

NAD⁺ and EDTA were purchased from Reanal (Budapest, Hungary), and 2-mercaptoethanol, butane-2,3-dione, trypsin (1-chloro-4-phenyl-3-L-tosylamidobutan-2-one-treated) and chymotrypsin from Sigma (St. Louis, MO, U.S.A.). Formic acid was from Czech (Warsaw, Poland), Sephadex G-50 from Pharmacia (Uppsala, Sweden), ninhydrin from Chemapol (Prague, Czechoslovakia), Cecilx from Bio-Rad (Richmond, CA, U.S.A.). All other reagents were of analytical grade. Pyridine was distilled over KOH, and acetic acid over Cr₂O₃. HCl was thrice distilled after boiling with SnCl₂. [2,3-¹⁴C]Butanedione was synthesized (Snell & McIlvain, 1931) at a specific radioactivity of 0.6 μCi/mm from [14C]acetic acid (specific radioactivity 120 μCi/mmol; Izotop, Leningrad, U.S.S.R.).

Enzyme modification by [¹⁴C]butanedione

Modification of the enzyme (100 mg; 10 mg/ml) was carried out in a medium containing 0.05 M-NH₄HCO₃, 5 mM-EDTA, 4 mM-2-mercaptoethanol, 150 μM-NAD⁺, pH 8.1–8.2, at 22°C. [¹⁴C]-Butanedione was added to a final concentration of 30 mM. The stock solution of [¹⁴C]butanedione (250 μCi/ml) was diluted before use with unlabelled reagent to give a ratio of 100 μCi/100 mg of protein. The time course of the reaction was followed by the decrease in enzymic activity. The reaction was stopped at different time intervals (5 or 20 min in various experiments) by gel filtration of the mixture on a column (2 cm x 40 cm) of Sephadex G-50 (coarse grade) equilibrated and eluted with 0.5% formic acid or acetic acid. The concentration of the acid in the resultant solution was then raised to 30%, after which the mixture was frozen and freeze-dried.

Enzyme oxidation

Performic acid oxidation of the modified enzyme was carried out as described by Hirs (1956). Performic acid (1.5–2.0 ml) was added to 60 mg of freeze-dried protein. The mixture was kept for 60 min in the cold, then diluted 50-fold with water, frozen and freeze-dried.

Tryptic hydrolysis

A sample of freeze-dried butanedione-modified oxidized protein (80 mg) was suspended in 0.2 M-borate buffer, pHe 8.2, to a final protein concentration of 6–8 mg/ml. Digestion was performed with a 1:100 (w/w) ratio of trypsin to glyceraldehyde 3-phosphate dehydrogenase, for 3.5 h at 37°C, with continuous stirring. A new portion of trypsin was then added in the ratio of 1:100 (trypsin/substrate) and the incubation continued for another 3.5 h. The completely soluble hydrolysate thus obtained was freeze-dried.

Chymotryptic hydrolysis

Freeze-dried peptide T-1 (see below) (about 50 nmol) was suspended in 100 ml of 0.05 M-NH₄HCO₃, pH 8.0. Chymotrypsin was added in a ratio of 1:100 (enzyme/substrate) and the mixture was incubated at 37°C for 6h with continuous stirring. The resultant solution was freeze-dried.

Amino acid analyses

Protein and peptides were hydrolysed at 110°C for 24 h in 5.7 M-HCl in evacuated sealed glass tubes. The analyses were performed with a Hitachi amino acid analyser model KLA-5 (protein samples), or with a LKB amino acid analyser model 3201 (peptide samples).

Peptide mapping

Two-dimensional peptide patterns of trypptic digests and purified peptides from trypptic digests were obtained as previously described (Harris & Perham, 1965; Vospelnikova et al., 1977).

Localization of peptides on paper was determined with the use of ninhydrin. Arginine-containing peptides were detected by the Sakaguchi reaction. Radiolabelled peptides were identified by autoradiographs by using an Uran-1 apparatus in the Department of Analysis of Radiolabelled Materials,
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The chymotrypsin digest of peptide T-1 was analysed on 20 cm x 20 cm microcrystalline cellulose plates (Avicel 250 μm; Analtech, Wilmington, DE, U.S.A.). Electrophoresis was performed in pyridine/acetic acid/water (1:10:89, by vol.) at pH 3.5 for 20 min at 200 V and then for 100 min at 900 V, with subsequent chromatography in butan-1-ol/pyridine/water (1:1:1, by vol.) adjusted to pH 5.4 with acetic acid. The peptides were detected as fluorescent spots after the plate was sprayed with 3% pyridine in acetone and then with 0.001% fluorescamine (Fluram; Roche Diagnostics, Basel, Switzerland) in acetone. They were recovered from the plate by extraction with concentrated formic acid, vacuum-dried and subjected to peptide analysis.

Isolation of peptides containing modified arginine residues

Peptides of a tryptic digest (60–70 mg) were separated on a column (2 cm x 150 cm) of Sephadex G-50 (fine grade) equilibrated and eluted with 30% acetic acid. The effluent was monitored for absorbance at 280 nm and for radioactivity. Radioactivity was measured with a Nuclear Chicago mark 2 counter using a dioxan-based scintillator [containing, per litre, 0.2 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene, 4 g of 2,5-diphenyloxazole, 60 g of naphthalene and 200 ml of ethanol]. Each vial contained 10 ml of scintillator and 0.1 ml of the sample to be analysed.

Column electrophoresis

Electrophoresis of the radiolabelled fractions was carried out on a 0.9 cm x 90 cm column packed with Cellex (Frykland & Eaker, 1975) at 1000 V and 4 mA for 18 h in formic acid/acetic acid/water (1:4:4.5, by vol.), pH 1.9. The sample applied to the top of the column was allowed to penetrate the carrier until the distance from the surface to the upper front of the sample reached about 3 cm. Electrophoresis started at this moment. At the end of electrophoresis, elution with 30% acetic acid was performed, the rate being 0.5 ml/min; 1 ml fractions were collected. Samples (100 μl) were analysed for radioactivity and for the ninhydrin-positive material measured as absorbance at 570 nm (Hirs, 1967).

Amino acid sequence analysis

Automated sequential degradation was performed with a Beckman 890C sequencer with program no. 050972 and a solid-phase Rank Hilger sequenator, model APS 24. Amino acid phenylthiohydantoins and their trimethylsilyl derivatives were quantified by g.l.c. (Pisano et al., 1972) on a Beckman GC-45 gas chromatograph. Occasionally, the identification of amino acid phenylthiohydantoins was confirmed by t.l.c. analysis (Jeppsson & Sjoquist, 1972) of the material on 20 cm x 20 cm plates covered with silica gel (Merck, Darmstadt, West Germany). The amino acid sequence of chymotryptic peptides was determined by manual sequential analysis (Ambler, 1967). N-Terminal amino acids were identified by the dansyl (5-dimethylaminonaphthalene-1-sulphonyl) method (Gray, 1972).

Results

Identification of peptides containing modified arginine residues

Table 1 summarizes the results obtained by amino acid analysis of the butane-2,3-dione-modified enzyme at different steps of the treatment needed to make the analysis. Modification affected two of the ten arginine residues per subunit, which is in agreement with our previous findings (Nagradova et al., 1976). The difference in the arginine content of control and modified enzyme samples persists throughout the procedure of tryptic-hydrolysis preparation. The product of modification appears to be sufficiently stable under the experimental conditions described.

Assuming that trypsin does not hydrolyse peptide bonds formed by the modified arginine residues (Yang & Schwert, 1972), we hoped to detect differences between the peptide maps of the modified and control enzyme preparations. Modifications of two arginine residues per subunit was expected to cause the disappearance of four peptides and the emergence of two new ones. Fig. 1 shows the results obtained by comparing the tryptic digests of control and modified enzyme preparations under strictly identical conditions. The differences are evident in the fractions of ‘acidic’ and ‘neutral’ peptides. A

<table>
<thead>
<tr>
<th>Sample</th>
<th>Arginine content (mol/mol of tetramer)</th>
<th>Modified arginine residues (mol/mol of protein subunit)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Modified</td>
</tr>
<tr>
<td>I</td>
<td>40.8</td>
<td>32.0</td>
</tr>
<tr>
<td>II</td>
<td>41.2</td>
<td>34.5</td>
</tr>
<tr>
<td>III</td>
<td>40.5</td>
<td>33.7</td>
</tr>
</tbody>
</table>
Isolation of peptides containing [14C]butanedione-modified arginine residues

Fig. 2 shows the elution profile obtained by chromatography of the tryptic digest of the modified enzyme on Sephadex G-50. The fractions containing the main radioactivity (70% of the total) were pooled to give fraction T, which was subjected to further analysis by paper electrophoresis at pH 1.9. As shown in Fig. 3, two radioactive peaks were detected in this fraction. The presence of several radioactivity-containing peaks in Fig. 2 can probably be explained by a partial splitting of the product of arginine modification (which is unstable), the radioactive moiety being adsorbed on peptide material. The non-specific character of this binding is demonstrated by the fact that only two radioactivity-containing peptides were detected on peptide maps and after the analysis of fraction T by paper electrophoresis at pH 1.9.

An attempt was made to compare the rates of accumulation of the two fractions in Fig. 3 in the course of enzyme inactivation. The ratio of total radioactivity in peak F1 to that in peak F2 was found to be 3:4 in the preparations treated with [14C]butanedione for 5 min; after 20 min incubation, the radioactivity became equally distributed between the two fractions. This result indicates that the most reactive arginine residue is localized in the peptide fraction which moves faster to the cathode under the conditions of Fig. 3. The data also suggest that the loss of enzymic activity, which was observed to reach 60% during 5 min of incubation, should be associated with modification of an arginine residue in this peptide fraction.

Fig. 4 shows the results of the chromatographic analysis of peptide fractions containing radioactivity. In addition to two radioactive peptides, fraction T contains two non-labelled peptides. None of the four spots was stained with the Sakaguchi reagent, which suggests that lysine residues are terminal residues in these peptides.
Arginine residue in glyceraldehyde 3-phosphate dehydrogenase

Fig. 3. Electrophoresis of fraction T (Fig. 2) on paper at pH 1.9
Freeze-dried fraction T was dissolved in 5 ml of formic acid/acetic acid/water (1:4:45, by vol.), and 0.05 ml of the resulting solution was applied on a sheet of paper (Whatman 3MM, 57 cm x 10 cm). Electrophoresis was carried out in the above formic acid/acetic acid/water mixture at 5000 V for 90 min. The sheet of paper was then cut into pieces (1.5 cm x 1 cm), which were analysed for radioactivity in scintillation fluid [a 1:1 (v/v) mixture of Triton X-100 (Sigma) and a toluene solution containing 5.0 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene per litre].

Fig. 4. Two-dimensional analysis of fraction T
The sample was treated as described in Fig. 3. After electrophoresis on a paper sheet (57 cm x 45 cm), chromatography was performed in butan-1-ol/acetic acid/water/pyridine (15:3:12:10, by vol.). The arrows indicate radioactive peptides.

Preparative separation of the peptides was performed by electrophoresis on a column packed with Cellex in formate buffer, pH 1.9 (Fig. 5). Four peaks were revealed (T-1, T-2, T-3 and T-4), the radioactivity being localized in fractions T-1 and T-2.

Table 2. Amino acid compositions of peptides T-1 and T-2
The values for valine and isoleucine are underestimated, since the samples were hydrolysed only for 24 h. The values for glycine and serine are overestimated, probably owing to the presence of some amounts of these amino acids in reagents (Gray, 1972). The values given in parentheses are calculated on the basis of amino acid sequence determinations (see below).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>T-1</th>
<th>T-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>2.8 (3)</td>
<td>2.3 (2)</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.2 (1)</td>
<td>1.0 (1)</td>
</tr>
<tr>
<td>Serine</td>
<td>1.9 (1)</td>
<td>1.05 (0)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.6 (2)</td>
<td>0.85 (1)</td>
</tr>
<tr>
<td>Proline</td>
<td>1.2 (1)</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>2.5 (1)</td>
<td>1.85 (1)</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.8 (3)</td>
<td>3.2 (3)</td>
</tr>
<tr>
<td>Valine</td>
<td>1.2 (2)</td>
<td>1.3 (1)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.0 (2)</td>
<td>0.7 (1)</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.4 (1)</td>
<td>0.9 (1)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.4 (1)</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.0 (1)</td>
<td>0.6 (1)</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.8 (1)</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>1.0 (1)</td>
<td>0.85 (1)</td>
</tr>
<tr>
<td>Arginine</td>
<td>Traces (1)</td>
<td>Traces (1)</td>
</tr>
<tr>
<td>Methionine sulphone</td>
<td>1.6 (2)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32.4 (24)</td>
<td>14.6 (14)</td>
</tr>
</tbody>
</table>
These fractions were demonstrated to be individual peptides by N-terminal amino acid determination. At the N-terminus of peptide T-1 valine was identified, and aspartic acid at the N-terminus of peptide T-2. The amino acid compositions of the two peptides containing modified arginine residues are given in Table 2.

Determination of amino acid sequences of peptides T-1 and T-2

The sequence of ten out of 24 residues of peptide T-1 was determined by automatic Edman degradation, and the presence of arginine in position 19 was established by a colour reaction with phenantrenequinone. The following structure was obtained:

\[
\text{Val-Ile-Ile-Asn-Ala-Pro-Thr-Ala-Asp-Ala-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Arg}
\]

The analysis of the sequence between residues 11 and 19 was hindered, owing to a marked decrease in the yield of phenylthiohydantoin derivatives. The attempts to detect the location of the modified arginine residue by radioactivity were unsuccessful in these experiments. We suppose that the product of modification is unstable under the conditions of the Edman degradation procedure, and its splitting results in partial regeneration of arginine.

Further analysis was performed with a chymotryptic digest of peptide T-1. Seven peptide fragments were detected in the digest, and three of them were obtained in a pure form. N-Terminal residues of these peptide fragments (c₁, c₂ and c₃) were determined as valine, glycine and serine respectively. The analysis of the amino acid compositions of peptide fragments c₁, c₂ and c₃ (Table 3) prompted us to suggest that fragment c₁ might derive from the N-terminal region of peptide T-1, fragment c₂ contains the radiolabelled arginine residue, and fragment c₃ is the C-terminal dipeptide Ser-Lys. This was confirmed by sequence determination. The structure of the N-terminal parts of peptide fragments c₁ and c₂ was found to be:

\[
\begin{align*}
c₁ & : \text{Val-Ile-Ile-Asx} \\
c₂ & : \text{Gly-Val-Asx-Arg-Glx}
\end{align*}
\]

The localization of arginine was established by the phenanthrenequinone method. Taken together, the results enable us to propose the amino acid sequence for peptide T-1 as shown in Fig. 6.

The structure of peptide T-2, which contains the second arginine residue modified with \([14C]\)-butanedione, was established by means of automatic Edman degradation and on the basis of its amino acid composition; it proves to be the following:

\[
\text{Asp-Ala-Gly-Ala-Thr-Ile-Ala-Leu(Asx,Glx,Arg,Phe,Val)Lys}
\]

Table 3. Amino acid compositions of peptides isolated from the chymotryptic digest of peptide T-1

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Peptide fragments</th>
<th>c₁</th>
<th>c₂</th>
<th>c₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td></td>
<td>2</td>
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</tr>
<tr>
<td>Threonine</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td></td>
<td></td>
<td>1</td>
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<tr>
<td>Alanine</td>
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<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td></td>
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<td>Leucine</td>
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<td>Tyrosine</td>
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<tr>
<td>Phenylalanine</td>
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<td>Histidine</td>
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<tr>
<td>Lysine</td>
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<td></td>
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<td>1</td>
</tr>
<tr>
<td>Arginine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine sulphone</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Total</td>
<td></td>
<td>14</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

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Arginine residue in glyceraldehyde 3-phosphate dehydrogenase

Discussion

A close homology between the amino acid sequences of glyceraldehyde 3-phosphate dehydrogenases isolated from different sources (Olson et al., 1975; Harris & Walker, 1977) makes it possible to determine the localization of peptides containing modified arginine residues in the covalent structure of the rat muscle enzyme. As shown in Fig. 7, the amino acid sequence of peptide T-1 corresponds very well to residues 116–139 in the polypeptide chain. The similarity between the structure of this peptide and the corresponding part of the molecule of several homologous enzymes indicates that the modified arginine residue is most probably situated in position 134 in the amino acid sequence of the rat muscle dehydrogenase.

According to the X-ray-diffraction studies of the lobster (Buehner et al., 1974) and Bacillus stearothermophilus (Biesecker et al., 1977) enzymes, residues 120–141 constitute a part of the coenzyme-binding domain of an enzyme subunit and are involved in the orientation of the nicotinamide nucleotide portion of the NAD⁺ molecule. The chain in this region forms an irregular structure with two flexible loops (residues 119–125 and 130–141).

Our data suggest that the microenvironment of arginine-134 is altered on transition from apo to holo conformation in rat muscle glyceraldehyde 3-phosphate dehydrogenase. Another conformational change affecting the reactivity of this residue is associated with the formation of a productive ternary complex, enzyme–NAD⁺–substrate (Nagradova et al., 1978a). The whole body of evidence points to the involvement of the part of the polypeptide chain comprising arginine-134 in the structural rearrangements accompanying catalysis in the rat muscle enzyme.

The localization of the second arginine residue, which becomes modified concomitantly with arginine-134, was established by comparing the amino acid sequences of five homologous enzymes with the structure of peptide T-2. As shown in Fig. 8, the sequence of this peptide corresponds rather well to the part of the polypeptide chain comprising residues 293–306. The modified arginine residue should be located in the sequence between residues 301 and 305.

The inspection of the stereo view of the Cα backbone atoms of the enzyme subunit (Buehner et al., 1974) shows that this segment of the polypeptide chain is situated far away from the active centre, and it seems unlikely that the modification of a residue in this part of the molecule can be responsible for the effects observed in our studies. This lends further support to the assumption that it is arginine-134 that is functionally important.

As far as arginine-231 is concerned, this residue appears to be unaffected by modification under our experimental conditions, and this precludes the analysis of its possible involvement in the conformational changes accompanying the apo–holo transition. It should be noted, however, that crystallographic studies revealed no alterations in the position of arginine-231 when the apo- and holo-enzyme structures were compared at 0.3 nm (3.0 Å) resolution (Murthy et al., 1980).

Comparison of amino acid sequences of glyceraldehyde 3-phosphate dehydrogenases isolated from five species (Harris & Walker, 1977) demonstrates that although the total number of arginine residues varies from 9 in the lobster enzyme to 16 in the Thermus aquaticus dehydrogenase, seven of them occupy fixed positions. The presence of arginine residues in positions 10, 13, 17, 194, 197, 231 and 320 appears to be a characteristic feature of this enzyme. It is interesting, in this connection, that both reactive arginine residues modified in our study are located in the variable regions of the polypeptide chain. Further, rat muscle dehydrogenase is the only enzyme as yet sequenced that has an arginine residue in position 134, and this means that butanedione-modified arginine residues of the yeast and probably rabbit muscle enzymes must be located elsewhere in the amino acid sequence. Since each of the three enzymes was found to have an essential arginine residue sensitive to NAD⁺-induced conformational changes, we suppose that different portions of the polypeptide chain of the rat, yeast

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### Fig. 7. Comparison of the amino acid sequences of residues 116–139 of glyceroldehyde 3-phosphate dehydrogenases from B. stearothermophilus (a), T. aquaticus (b), baker's yeast (c), lobster (d) and pig (e) with the structure of peptide T-1 derived from a tryptic digest of the rat muscle enzyme (f).

The data for (a)–(e) are taken from Harris & Walker (1977) and from Holland & Holland (1979). Sequence differences in enzymes from mesophilic organisms are shown in the boxed regions.
Arginine residue in glyceraldehyde 3-phosphate dehydrogenase

![Image](image-url)

Fig. 8. Amino acid sequence of peptide T-2 derived from a tryptic digest of rat muscle glyceraldehyde 3-phosphate dehydrogenase compared with the sequence of residues 293–306 in homologous enzymes from B. stearothermophilus (a), T. aquaticus (b), baker's yeast (c), lobster (d) and pig (e). Peptide T-2 is designated (f). The data for (a)–(e) are taken from Harris & Walker (1977) and from Holland & Holland (1979).

and possibly rabbit enzymes are involved in these changes. This may reflect the specificities of conformational transitions in enzymes from different sources. A functionally important structural rearrangement (i.e. introduction of a positive charge close to the substrate and nicotinamide ring) may be an obligatory step in the catalytic process, but its realization seems to have peculiar features in homologous enzymes.

We are grateful to Dr. Yu. B. Alachov, Dr. L. Vinokurov and Dr. N. Dovgas from the Laboratory of Protein Chemistry, Institute of Protein, Pushchino, for help in peptide analysis.

References


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