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Structural Changes in Glyceraldehyde-3-Phosphate Dehyrogenase Isolated from Temperature-Acclimated Rainbow Trout (*Salmo gairdneri*)[1] (39549)

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It is well documented that the activity and kinetics of various enzymes are changed as a result of temperature acclimation (1). There is evidence that the differences reported may be due to the functional significance of isoenzymes in thermal acclimation (2, 3). We have previously reported kinetic and thermodynamic differences in glyceraldehyde-3-phosphate dehydrogenase (G3PDH) isolated from warm, 15° (G3PDH(W)), and cold, 5° (G3PDH(C)), acclimated rainbow trout, *Salmo gairdneri* (4).

There are also data which show that, although temperature-dependent interconversions may take place which result in altered kinetics, the components may not be separable by electrophoretic or electrofocusing analyses (5). It is assumed that the observed kinetic changes in our case are due to a temperature-dependent conformational change which is stable through enzyme isolation procedures.

In order to establish that stable conformational changes did occur as a result of temperature acclimation, we have measured several physical parameters of purified G3PDH. Our coenzyme binding studies establish a difference in the enzyme as a result of temperature acclimation. The spectroscopy data indicate a conformational difference.

Materials and methods. Rainbow trout (*Salmo gairdneri*) were obtained from the Federal Fish Hatchery at Leetown, W. Va. They were maintained in aquaria at either 15° (warm) or 5° (cold). They were fed every other day with food supplied by the hatchery.

After 3 weeks, fish were sacrificed and G3PDH was purified from the lateral muscle following the method of Cori *et al.* (6). All reagents contained 10^{-3} *M* dithiothreitol. The resulting product moved as a single band in polyacrylamide-gel electrophoresis using amido black as a stain.

The enzymes were assayed in a Beckman DB spectrophotometer at 340 nm with a recorder adjusted to 6×10^{-6} *M* NADH full scale. The reaction mixture contained 40 m*M* triethanolamine at pH 8.5, 5 m*M* Na_2HPO_4, 1.58 m*M* NAD, and glyceraldehyde phosphate (GAP) at concentrations ranging from 18.3 to 300 μM. The concentration of the D-isomer of GAP was determined by the reaction with triosephosphate isomerase and glycerolphosphate dehydrogenase which was measured spectrophotometrically. NAD was purchased from Pabst Laboratories, GAP from Sigma Chemical Company.

Electrophoresis was carried out in a Canalco Model 1200 in a 4° refrigerator. The procedure followed that of Davis (7), except that gel polymerization was catalyzed by ammonium persulfate, and phenol red was used as a tracking dye. Gels of 6 and 8.5% cross-linkage were used. The cathode buffer was at pH 8.4 and the anode buffer at pH 7.5. Samples were electrophoresed until the tracking dye was within 0.5 cm of the bottom of the gel tube. Gels were removed from the tubes and stained with amido black (0.5 mg/ml in 15% acetic acid) for 2 hr. The gels were destained in 15% acetic acid.

Measurements of coenzyme binding were carried out on a Perkin-Elmer MPF-2 spectrofluorimeter. The excitation wavelength was 280 nm; the emission was measured at 350 nm (8). Fluorescence was measured with successive 10-μl additions of a 1 m*M* solution of NAD to 2.00 ml of a 2.4 μM solution of G3PDH in 0.01 *M* Tris-acetate buffer at pH 8.5. The procedure of Stockell (9) was used to analyze the NAD concentra-

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tion and the fraction bound to give the number of binding sites and the intrinsic dissociation constant.

Ultraviolet spectra of three warm and two cold preparations were recorded on a Cary 14 spectrophotometer at room temperature in glass-distilled water. Values of optical density were read from the spectra every 5 nm between 200 and 350 nm. The extinction was calculated at each of these wavelengths using a molecular weight of 140,000. The extinctions at each wavelength were averaged for the three warm and two cold preparations. The resulting spectra were plotted on a Calcomp X–Y recorder. A difference spectrum was obtained by subtracting the average cold from the average warm extinction.

The two average spectra were each fitted to a model which assumed that the absorption at each wavelength was a linear combination of the absorption of tyrosine, phenylalanine, and tryptophan.

A matrix was set up using the following equation:

$$E_\lambda = \mathrm{Tyr}_\lambda\,(x_1) + \mathrm{Phe}_\lambda\,(x_2) + \mathrm{Trp}_\lambda\,(x_3) + e_\lambda,$$

where at wavelength λ: E_λ = total extinction; Tyr_λ = extinction of tyrosine; Phe_λ = extinction of phenylalanine; Trp_λ = extinction of tryptophan; x_1, x_2, and x_3 = mole fractions of Tyr, Phe, and Trp, respectively, in G3PDH; and e_λ = error of estimate. The matrix was solved by least squares, so as to give a best average solution of x_1, x_2, and x_3 over the spectrum and to minimize e_λ. The difference between G3PDH(W) and G3PDH(C) was obtained by subtracting the values of the number of moles of each amino acid in the cold enzyme from those in the warm enzyme. The value obtained corresponds to the difference in number of amino acids in a position to absorb radiation.

Circular dichroism (CD) spectra for each of the enzyme preparations were measured on a Cary Model 60 spectropolarimeter fitted with a Model 6001 CD attachment. The conformation of the enzymes was determined by assuming that the mean residue ellipticity at a given wavelength was due to a linear combination of the mean residue ellipticities of the mole fractions of α-helix, β-sheet, and random coil forms (10). A matrix was set up with the equation:

$$\theta = x_1A_\lambda + x_2B_\lambda + x_3R_\lambda + e_\lambda,$$

where at wavelength λ: θ = mean residue ellipticity in degrees · square centimeters per decimole; A_λ, B_λ and R_λ = mean residue ellipticities of 100% α-helix, β-sheet, and random coil forms, respectively; e_λ = error in estimates. The best values for x_1, x_2, and x_3 were obtained by minimizing the error over a range of discrete wavelengths (10) which best indicate the α-helix, β-sheet, or random coil structures.

Protein concentration was determined by the method of Lowry *et al.* (11).

Results and discussion. Cold acclimation has no effect on the electrophoretic properties of G3PDH. Coelectrophoresis of a sample of G3PDH(W) and G3PDH(C) shows that the enzymes move as a single band in various gel cross-linkages. The single band indicates that both species have approximately the same size and molecular weight per charge ratio. G3PDH is an oligomeric enzyme (12) and from several sources has been found to contain four identical subunits in the active form (13), and there is no evidence showing that G3PDH contains any number other than four identical subunits. From electrophoresis data, it follows that temperature acclimation does not change the number of subunits.

The results of the NAD-binding experiments are shown in Fig. 1. Both the 15° enzyme and the 5° enzyme have three binding sites. This corresponds to the number of binding sites found in the rabbit muscle enzyme (8). The intrinsic association constants for NAD are $6.68 \times 10^4\ M^{-1}$ for G3PDH(W) and $12.3 \times 10^4\ M^{-1}$ for G3PDH(C). These values are averages of those obtained by duplicate measurements on each of two G3PDH preparations. It can be concluded that temperature acclimation affects NAD binding.

The interaction of G3PDH with NAD appears to be affected by the thermal history of the animal from which the enzyme was obtained. Greene and Feeney (14) have reported that the K_m values of NAD are affected by "cold-adaptation." They found the K_m to be $1.80 \times 10^{-5}\ M$ for the rabbit

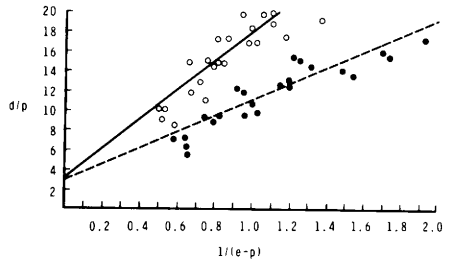

FIG. 1. NAD binding: d = concentration of NAD, e = enzyme concentration, p = fraction of sites bound times enzyme concentration. There are three NAD-binding sites on each enzyme. For the 15° enzyme $K°_{NAD} = 6.68 \times 10^4\ M^{-1} \pm 13\%$; for the 5° enzyme $K°_{NAD} = 12.3 \times 10^4\ M^{-1} \pm 10\%$.

muscle G3PDH and $7.62 \times 10^{-5}\ M$ for the "cold-adapted" fish muscle enzyme (*Dissostichus mawsoni*).

The difference in the uv spectra of cold and warm G3PDH measured between 350 and 200 nm is shown in Fig. 2. This plot represents the difference between the average cold and warm spectra. Protein absorption in this portion of the spectrum is due to absorption of aromatic amino acids (15). Differences in this region can be correlated with differences in the positioning of tryptophan, phenylalanine, and tyrosine. Based on the difference spectrum, it can be seen that the cold enzyme has a greater absorption than the warm. This is probably due to the aromatic amino acids in the cold enzyme being in a better position to absorb radiation. The results of the calculated difference, shown in Table I, show the change in the number of each of the residues in a position to absorb radiation for each of the studied enzymes.

These results are compatible with published amino acid analyses of various species of G3PDH. Greene and Feeney (14) reported that for both the rabbit and the halibut G3PDH there are 40 tyrosines, 60 phenylalanines, and 16 tryptophans/mole. The spectral analysis in the present study shows that about one-half of the tyrosines and phenylalanines and one-fourth of the tryptophans are affected by temperature acclimation. These data, then, are consistent with the idea that a stable conformational change has occurred *in vivo* in the G3PDH as a result of temperature acclimation.

The CD spectra are shown in Fig. 3. The

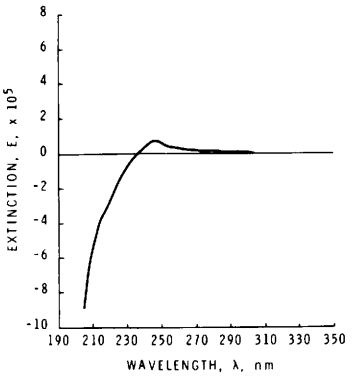

FIG. 2. G3PDH difference spectrum: Curve illustrates the difference when the extinction coefficient of cold enzyme is subtracted from the extinction coefficient of warm enzyme.

TABLE I. DIFFERENCE IN UV-ABSORBING RESIDUES FOLLOWING THERMAL ACCLIMATION.

	(Moles of warm) − (Moles of cold)
Tyrosine	19
Phenylalanine	26
Tryptophan	4

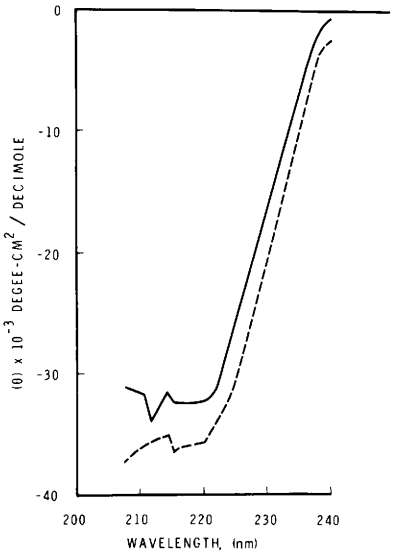

FIG. 3. CD spectra. (- - -), Average of 15° enzymes; (——), average of 5° enzymes.

TABLE II. SECONDARY STRUCTURE OF G3PDH FOLLOWING THERMAL ACCLIMATION.

	α (%)	β (%)	Random coil (%)
15° enzyme	9.1	14.0	76.9
5° enzyme	11.1	13.0	75.9

results of the analysis are shown in Table II. The spectra obtained closely resemble those of carboxypeptidase A, chymotrypsin, and chymotrypsinogen (10). The 15 and 5° enzymes have approximately the same distribution of α helix, β sheet, and random coil. The similarity of the CD spectra of the 15 and 5° enzymes shows that there is probably not a large difference in the secondary structure of the two enzymes. The change is more likely a difference in the higher-order structure of the holoenzyme rather than a transition of helix to β structure or random coil.

The activity of G3PDH changes with temperature acclimation. We are concerned in this study with the mechanism by which this activity is affected. Changes in subunit structure are ruled out by electrophoretic studies. The similarity of the CD spectra, as well as the relative invariance in amino acid composition of the enzyme isolated from various species, further weakens the hypothesis of synthesis, *de novo,* of subunits with significantly different secondary structure.

It may be concluded, then, that temperature acclimation results in a change in the higher-order structure of the G3PDH which is stable through isolation procedures. This is supported by a difference in uv-absorption characteristics and NAD-binding properties between the warm and cold enzyme. In addition, immunological data from our laboratories (16) to be published elsewhere also support the idea of altered tertiary or quaternary structure.

Summary. Glyceraldehyde-3-phosphate dehydrogenase has been purified from rainbow trout acclimated to 15° (warm) and 5° (cold) and compared with respect to their electrophoretic properties, NAD-binding behavior, and ultraviolet absorption. Both the warm and cold species of G3PDH show the same electrophoretic properties. They differ, however, in NAD-binding behavior and in ultraviolet-absorption properties. It was concluded that temperature acclimation brings about a conformational change in the higher-order structure of G3PDH which is stable through purification procedures.

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