A Multilocus System for Studying Tissue and Subcellular Specialization*

THE pH AND TEMPERATURE DEPENDENCE OF THE TWO MAJOR NADP-DEPENDENT ISOCITRATE DEHYDROGENASE ISOZYMES OF THE FISH FUNDULUS HETEROCLITUS

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In the teleost fish Fundulus heteroclitus, there are three NADP-dependent isocitrate dehydrogenase isozymes. IDH-B2 is the only cytoplasmic isozyme, and IDH-C2 dominates the mitochondria of all tissues other than liver, where IDH-A2 is expressed. Since fish are ectotherms, their intracellular temperature and pH change directly with environmental temperature. In order to evaluate the influence of these environmental parameters on a model fish NADP-isocitrate dehydrogenase system, the major cytoplasmic (IDH-B2) and mitochondrial (IDH-C2) isozymes were kinetically evaluated as a function of pH and temperature. Whereas $V_{\text{max}}$ and $K_{\text{m,IC}}$ (where ISOC is isocitrate) were pH-independent, the $K_m$ for NADP was pH-dependent for both isozymes. The cytoplasmic isozyme (IDH-B2) had smaller $K_{\text{m,NADP}}$ values between pH 7.0 and 8.0 than the mitochondrial form (IDH-C2). $V_{\text{max}}$ and $K_m$ for substrate and coenzyme were temperature-dependent. Energy of activation for IDH-B2 and IDH-C2 was 10.6 and 12.8 kcal/mol, respectively. Both proteins had $\Delta G^\circ$ values of about 15.8 kcal/mol, with significantly different distributions between $\Delta H^\circ$ and $\Delta S^\circ$. The cytoplasmic isozyme (IDH-B2) appears to have a greater rate of catalysis than the mitochondrial enzyme (IDH-C2) at temperatures less than 30°C. Moreover, the IDH-B2 isozyme had lower $K_{\text{m,NAD}}$ values than the IDH-C2 isozyme at all temperatures, whereas $K_m$ values for the two isozymes were indistinguishable. Our data suggest that the two major NADP-dependent isocitrate dehydrogenase isozymes have unique physiological and metabolic functions that are adapted to the tissues and cellular compartments in which they are expressed.

In the teleost fish Fundulus heteroclitus, there are three NADP-dependent isocitrate dehydrogenase isozymes (isocitrate:NADP$^+$ oxidoreductase (decarboxylating), EC 1.1.1.42) that are coded for by three independent loci (1). The three isozymes, IDH-A2, IDH-B2, and IDH-C2, are thought to have been derived from gene duplication and subsequent evolutionary divergence and specialization of gene function (1). The isocitrate dehydrogenase isozymes exhibit differences in tissue and subcellular distribution (1), suggesting that they have significantly diverged and adapted through evolutionary processes toward the specific metabolic needs of the tissues in which they are expressed. Recently, these enzymes were purifed to homogeneity and characterized in order to assess their potential physiological roles in different tissues and cell compartments (2). The results of those studies have shown that these multilocus enzymes are structurally and functionally non-equivalent. Whereas kinetic studies need to be done in the reverse direction, the differences observed between the kinetic parameters of the liver cytoplasmic isozyme (IDH-B2) and mitochondrial isozyme (IDH-A2) for the forward reaction were consistent with the shuttling of reducing equivalents (NADPH) into the liver cytoplasm, as suggested by Kaplan (3). However, the role of the mitochondrial isozyme found in all other tissues (IDH-C2) is less well defined. Since the IDH-B2 cytoplasmic isozyme is present in most tissues and the dominant mitochondrial form in all tissues other than liver is IDH-C2 (4), they were selected as models for further studies to examine the physiological significance of tissue specificity and subcellular specialization.

Fish are cold-blooded organisms (i.e. ectotherms) subjected to external environmental temperature fluctuations that directly affect their internal temperature and pH (5-7). These parameters have a profound effect on protein structure and function (5-7). Since the fish F. heteroclitus is subjected to large environmental temperature fluctuations (8) and the kinetics of many enzymes are affected by changes in both temperature and pH (9), the effects on these variables on the rate of isocitrate oxidative decarboxylation (forward reaction) were studied as a model to probe the role of these environmentally important parameters for the two predominant multilocus NADP-isocitrate dehydrogenase isozymes (IDH-B2 and IDH-C2). Whereas the liver mitochondrial isozyme (IDH-A2) may be involved in the shuttling of reducing equivalents into the cytoplasm (see Ref. 3) as suggested by Kaplan (3), the role of the other mitochondrial NADP-isocitrate dehydrogenase (IDH-C2) appears to be primarily involved in supplying...

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1 In keeping with the IUPAC-IUB recommendations for multiple enzyme nomenclature (IUPAC-IUB Commission on Biochemical Nomenclature, 1977, J. Biol. Chem. Vol. 252, 5939-5941) and a previous report on the NADP-dependent isocitrate dehydrogenase isozymes (1), they are designated IDH-A2, IDH-B2, and IDH-C2 respectively.
EXPERIMENTAL PROCEDURES

The purification, physical characterization, and general kinetic procedures for the forward reaction at 25 °C and pH 7.4 for the IDH-B and IDH-C isozymes are described in a previous paper (2).

Kinetic Procedures—The following buffers were used for pH studies at 25 °C and constant ionic strength: 50 mM triethanolamine chloride (concentration in the anion) was used for kinetic studies at pH 7.4-8.0; and 30 mM Tris chloride was used for kinetic studies at pH 8.3-9.0. Temperature studies were carried out with 30 mM triethanolamine chloride at pH 8.0.

Buffers were chosen with a Kd that would allow for any ΔpKd that might occur due to temperature changes and were prepared at constant ionic strength (10) (see Table 1). Appropriate standardizations of buffers, substrate, and coenzyme were carried out (see Miniprint).

The isozymes were diluted in 30 mM triethanolamine chloride buffered pH 7.4-7.9 and 33% (w/v) bovine serum albumin. No loss in activity was observed for dilutions kept on ice for 8-10 h.

Velocity measurements (ΔAmin/min) for the forward reaction were made by steady-state kinetics using a Beckman Acta cII UV-visible, dual-beam spectrophotometer with an automatic sampling system which monitors four reactions sequentially. The change in absorbance at 340 nm for each 0.01 ml cuvette was recorded every 15 s and converted to digital output by a Beckman 52700 teletype intercoupler interfaced to the spectrophotometer. The enzyme reactions were kept at constant temperature (+0.5 °C) with a Haake T92-T33 circulating constant temperature bath connected to the water jacket of the spectrophotometer sample compartment.Velocities were obtained by an unweighted least square analysis. Quadruplicate assays were averaged for each velocity. The kinetic parameters, K and Vmax, were calculated using the parametric weighted regression method of Cleland (11). Statistical tests and analysis of the kinetic constants were performed by standard procedures (12, 13).

pH Studies—For kinetic studies at constant temperature (25 °C) and varying pH, Vmax and K values for isocitrate and NADP were determined by two different methods: the matrix approach and the single variable method. For the matrix method, five isocitrate and four NADP concentrations increased in a geometric sequence at pH 6.5, 7.0, 7.4, 8.0, 8.5, and 9.0 were used. The concentration of the divalent metal ion was held constant (1.5 mM MnSO4).

Data obtained from this approach were fitted to Equation 1,

\[ v = \frac{V_{\text{max}}[A][B]}{K_a + [A][B]} \]  

(1)

where v is the initial rate, Vmax is the maximum velocity for the forward reactions, [A] is the concentration of coenzyme, [B] is the concentration of the substrate, K is the true equilibrium dissociation constant for the isocitrate dehydrogenase-NADP complex when the isocitrate concentration tends to zero. K and K are the limiting Michaelis constants for the coenzyme and substrate, respectively. This equation is valid for ordered mechanisms such as Bi Uni, Bi Bi, or Bi Ter and also for rapid equilibrium random mechanisms such as Bi Bi or Bi Ter in which all steps other than the central conversion of EAB → EPQR are in rapid equilibrium (14-16).

For the single variable method, each K and Vmax value was obtained at saturating concentration of the other substrate, i.e. either NADP (100 μM) or isocitrate (2 mM). The concentration of metal was held constant (1.5 mM MnSO4) at saturating levels (see Figs. 1 and 2). Data collected by this approach were fitted to Equation 2,

\[ v = \frac{V_{\text{max}}[A]}{K_a + [A]} \]  

(2)

where v is the initial rate, Vmax is the maximum velocity, [A] is the concentration of isocitrate or NADP in the forward direction, and K is the Michaelis constant for either NADP or isocitrate.

Temperature Studies—For kinetic studies at constant pH 8.0 and variable temperature, K and Vmax values for isocitrate and NADP were obtained at saturating concentrations of either coenzyme or substrate while changing the concentration of the other variable at 10, 15, 20, 25, and 30 °C. Reaction mixtures contained 30 mM triethanolamine chloride buffer, 1.5 mM MnSO4, and 2 mM isocitrate when NADP was varied and 100 μM NADP when isocitrate was varied. In all cases, the reactions were initiated by addition of enzyme.

Data obtained from temperature studies were fitted in Equation 2 above.

The energy of activation (Ea) was determined from an Arrhenius plot, i.e. log Vmax versus 1/T (K-1). The apparent Ea value was calculated from the slope of the plot (i.e. slope = -Ea/2.303R) by linear regression analysis (15).

The thermodynamic quantities, ΔH* (enthalpy) and ΔS* (entropy), of the activated state were calculated from a modification of the linear Arrhenius equation (16),

\[ \log \frac{V_{\text{max}}}{T} = -\frac{\Delta H^*}{2.303R} + \frac{\Delta S^*}{2.303R} \]  

(3)

where h is the Boltzman's constant and T is the Plank's constant.

Data from the plot Vmax/T (K-1) versus 1/T (K-1) were analyzed by the least square method. The free energy of activation (ΔG*) was estimated from Equation 4.

\[ \Delta G^* = \Delta H^* - T \Delta S^* \]  

(4)

In a rapid equilibrium system, like the NADP-dependent isocitrate dehydrogenases (17-22), K values may approximate the dissociation constants between enzyme and substrate (i.e. K = K) (15). Under such conditions, the apparent dissociation constants (K) for isocitrate or NADP can be used to calculate apparent free energies, enthalpies, and entropies of substrate binding (9). If the Arrhenius plots are linear, the following equation can be employed,

\[ \log K_a = -\frac{\Delta H^*}{2.303R} + \frac{\Delta S^*}{2.303R} \]  

(5)

where K is the appropriate K for either isocitrate or NADP when the other substrate is saturating but not inhibiting (4), ΔH is the enthalpy of substrate binding, ΔS is the entropy of substrate binding, R is the gas constant, and T is absolute temperature. The free energy of substrate binding (ΔG) was estimated from the following equation.

\[ \Delta G^* = -2.303R \log K_a \]  

(6)

RESULTS AND DISCUSSION

pH Studies—pH can dramatically affect enzyme function (9, 14). A shift in pH may change the ionization of substrates and/or the ionization of amino acid side chains that directly or indirectly affect catalysis. pH is particularly important for cold-blooded organisms since their intra- and extracellular pH is directly affected by the external environment. The study of pH in relation to enzyme catalysis provides insight concerning these possibilities.

Initial studies at low temperatures (10 °C) proved difficult employing the matrix approach (see “Experimental Procedures”) because low substrate and coenzyme concentrations yielded initial velocities at 10 °C that approached background variation of the spectrophotometer. Whereas increasing the path length of the reaction cuvette improved our measurements, this approach did not eliminate the problem. Varying only one substrate at a time allowed accurate measurements at 10 °C; thus, we adopted the single variable approach for
our pH and temperature studies as described under "Experimental Procedures."

Since we had used the matrix approach at pH 7.4 and 25 °C in an earlier study (2), we were curious when initial $K_m$ values obtained by the single variable approach were slightly higher than those obtained by the matrix approach while the $V_{max}$ values were identical. In order to gain insight concerning these differences and to ensure that any $K_m$ and $V_{max}$ trends in relation to pH would be independent of the method of calculation, we studied the effect of pH on the various kinetic parameters, $V_{max}$, $K_m^{NADP}$, and $K_m^{ISOC}$ (where ISOC is isocitrate), by both the matrix and single variable approach at 25 °C. Those results and their statistical significance, or lack thereof, are tabulated in Tables II and III.

Whereas the absolute $K_m$ values appeared to be sensitive to the method of calculation, the trends in relation to experimental variables like pH were similar, no matter which way they were calculated (Figs. 3 and 4). The differences between the methods probably arise because: (a) the single variable method only requires the extraction of two constants, whereas the matrix approach must extract four constants; (b) the single variable method employs weights and thus minimizes variation due to error at low substrate and coenzyme concentration, whereas the matrix approach most commonly employed (2, 16) does not normally allow weighting of data; (c) the matrix approach appears to minimize $K_m$ differences by absorbing variation in the data set in the $K_m$ term (see Equation 1 under "Experimental Procedures"); and (d) the equation for the matrix approach indicates essentially infinite correlation between $K_m$ and $K_m^0$. This latter point is particularly important because in a rapid equilibrium system like isocitrate dehydrogenase, $K_m = K_m^0$. Since we have found similar differences and similarities between the matrix and single variable approaches with three other bisubstrate enzymes, we are currently employing modified fitting procedures and computer simulations of these (i.e. Equations 1 and 2) as well as other equations to determine the most parsimonious approach to analyzing such kinetic data. However, it should be emphasized that the kinetic trends in relation to variables like pH and temperature appear to be independent of the method of calculation. On the other hand, differences in the method of calculation may account for numerical differences in $K_m$ estimates between various studies reported in the literature. Also, it should be clear that data collected using a number of different variables like pH, ionic strength, temperature, etc., should be collected and analyzed by the same method (i.e. either matrix or single variable) in order to generate a self-consistent data set that will allow appropriate comparisons across variables.

Fig. 3A and Fig. 4A illustrate the $K_m^{ISOC}$ in relation to pH for the IDH-B2 and IDH-C2 isozymes as determined by both the single variable and matrix approaches. Of the six pH values, there were three $K_m^{ISOC}$ values that were significantly different between the isozymes. These values that were different using the matrix approach were pH 6.5, 7.4, and 9.0, whereas pH 7.0, 8.0, and 8.5 were different employing the single variable method (Tables II and III). Since the differences were not consistent between calculation methods, we shall be conservative and conclude that no differences in $K_m^{ISOC}$ exist between the isocitrate dehydrogenase isozymes.

The matrix and single variable methods both indicated no significant pH dependence of $K_m^{NADP}$. This point is obvious upon perusal of the $K_m^{NADP}$ values in Tables II and III. Since $K_m^{NADP}$ is apparently pH-independent, the ionizable groups on the free enzyme and/or isocitrate do not affect enzyme-substrate affinity or the effect is complementary and opposite, i.e. no net pH effect. This phenomenon has been observed for arginase (23).

Whereas $K_m^{ISOC}$ does not vary with pH, Fig. 3B illustrates that $K_m^{NADP}$ does change in relation to pH. Inspection of the $K_m^{NADP}$ estimates (Tables II and III) indicates a pH dependence for both isozymes, with apparent optima around pH 8.0. Moreover, the differences are consistent between calculation methods, i.e. matrix and single variable. The $K_m^{NADP}$ values appear to converge at pH 6.5 and 9.0 for both isocitrate dehydrogenase isozymes (Fig. 3B). Since the ionization of NADP is the same for both isocitrate dehydrogenase isozymes, differences between IDH-B2 and IDH-C2 in the $K_m^{NADP}$ pH dependence suggest different ionization of groups on the free enzymes which control the binding of the coenzyme. The pK values of the major ionizable groups on NADP are pK1 = 3.7 and pK2 = 6.1 (24). Of these, only one phosphate titrates over the experimental pH range. The increase in $K_m^{NADP}$ (Fig. 3B) at low pH may be partially the result of the protonation of NADP. The increase in $K_m^{NADP}$ at high pH probably results from the deprotonation of a basic amino acid side chain on the free enzyme, which, in turn, affects the cofactor binding. The divergence of the two $K_m^{NADP}$ values (Fig. 3B) over the middle pH range suggests different ionization states of amino acid residues on the isozymes, whereas convergence at high and low pH suggests the deprotonization of similar residues.

The maximal rate of catalysis ($V_{max}$) was pH- and calculation method-independent (Fig. 3C). The respective $V_{max}$ values were significantly different between the two isozymes (see Tables II and III). These $V_{max}$ differences may reflect either different $k_{cat}$ values or a difference in active enzyme concentration ($[E_o]$) because $V_{max} = k_{cat} [E_o]$. We shall return to this point below.

Temperature Studies—Temperature is particularly important for cold-blooded organisms because it has a direct effect on protein structure and function and induces changes in
enzyme kinetics. The \( K_m \) values for isocitrate and NADP in this study are temperature-dependent (Figs. 5 and 6). The \( K_m^{\text{NADP}} \) values increased with temperature (Fig. 5), but there were no significant differences between the isocitrate dehydrogenase isozymes at 25 °C (Table IV). On the other hand, there were significant differences in the \( K_m^{\text{NADP}} \) values between the isozymes at all temperatures (Table IV), with IDH-B; having the lower \( K_m^{\text{NADP}} \) (Fig. 6).

Figs. 5 and 6 are linear which supports the use of \( K_m \) values as apparent dissociation constants (\( K_p \)) for enzyme-substrate complexes. In a rapid equilibrium system, like that of the NADP-dependent isocitrate dehydrogenase (17-22), the dissociation constants are usually in the range of \( 10^{-6} \) to \( 10^{-2} \) M so that \( \Delta G_p \) is in the range of \(-7.7 \) to \(-2.6 \) kcal/mol at 25 °C. The apparent dissociation constants for the NADP-isocitrate dehydrogenase isozymes (\( K_m^{\text{NADP}} \) and \( K_m^{\text{SOC}} \)) are within this range (Tables II-IV), and free energies (\( \Delta G \)) calculated from these apparent dissociation constants also fall within the expected range (e.g. \(-7.3 \) to \(-7.7 \) kcal/mol at 25 °C).

The maximal rate of isocitrate oxidative decarboxylation (\( V_{\text{max}} \)) for the NADP-isocitrate dehydrogenase isozymes resulted in linear Arrhenius plots (Fig. 7), suggesting that there has been no change in mechanism over the temperature range studied and that a single rate constant is driving the catalytic process for both isozymes. Moreover, the slopes (Fig. 7) suggest that the \( V_{\text{max}} \) differences between isozymes result from unique \( k_{\text{cat}} \) values, otherwise the slopes would be parallel. Table IV indicates that the \( V_{\text{max}} \) values between 10 and 25 °C are significantly different at \( p < 0.001 \), whereas at 30 °C, \( p < 0.01 \). Experiments employing a multivariable approach at 35 °C (4) yielded \( V_{\text{max}} \) values of 31.53 ± 0.390 and 32.13 ± 0.125 for IDH-B; and IDH-C; respectively. These values were not significantly different. Moreover, they are identical to values predicted by extrapolating the lines of Fig. 7 which intersect at 35 °C.

Table V shows the energy of activation (\( E_a \)) calculated from the slope of Fig. 7, as well as the thermodynamic activation parameters, \( \Delta H^\ddagger, \Delta S^\ddagger, \Delta G^\ddagger, \) and \( Q_{10} \) for both isozymes. Whereas differences in the \( E_a \), \( Q_{10} \), \( \Delta H^\ddagger \), and \( \Delta S^\ddagger \) parameters were observed between the two isocitrate dehydrogenase isozymes, the free energies of activation (\( \Delta G^\ddagger \)) were not statistically different. This finding appears, at first, to be contradictory to the above statement that \( k_{\text{cat}} \) values may vary between the isocitrate dehydrogenase isozymes. This arises from the fact that the calculation of \( \Delta G^\ddagger \) includes a very large constant such that any significant differences in \( k_{\text{cat}} \) can be statistically eclipsed in the propagation of errors and the logarithmic nature of the equations for the calculation of \( \Delta G^\ddagger \).

The maximum differences between the isocitrate dehydrogenase isozyme \( k_{\text{cat}} \) values are on the order of 40%. However, a 2-fold difference would only result in a \( \Delta G^\ddagger \) change of about 400 cal which would be buried by the propagation of errors when estimating \( \Delta G^\ddagger \). It would take almost an order of magnitude difference in \( k_{\text{cat}} \) in order to detect a significant difference in free energies (\( \Delta G^\ddagger \)) above a background error of 5%. Since \( V_{\text{max}} \) values differ significantly between the isocitrate dehydrogenase isozymes, it follows that the respective
ΔG° values must also differ, but, due to the propagation of errors, the logarithmic nature of the equations, and the loss of degrees of freedom, these differences in V_max are eclipsed during the free energy calculations.

Some Physiological Roles of the NADP-Isocitrate Dehydrogenase Isozymes—Mitochondria contain two or more separate isocitrate dehydrogenases. One is NADP-linked and one or two are NADP-linked enzymes. However, the cytosol only contains and NADP-linked isocitrate dehydrogenase (see Refs. 25 and 26 for reviews). NADP-isocitrate dehydrogenase is a nonequilibrium pathway, whereas the NADP-dependent isocitrate dehydrogenase pathway is in equilibrium (27). Flux through the NAD-linked pathway is activated only under special conditions relating to the needs of the Krebs cycle. On the other hand, the NADP-linked isocitrate dehydrogenase performs the majority of the isocitrate oxidation and provides the mitochondrion with both NADPH and NADH when coupled to the transhydrogenase system (28). Weigl and Sies (29) have shown that mitochondrial NADPH reducing equivalents, in the form of isocitrate or citrate, may be provided to the cytoplasmic space for biosynthetic reactions and during metabolic limitations on the pentose phosphate pathway. In fact, Thurman and Scholz (30) and Weigl and Sies (29) have suggested that the major supply line for NADPH utilization at the endoplasmic reticulum is the export of citrate from mitochondria as well as NADPH generation by cytosolic NADP-linked isocitrate dehydrogenase.

Kaplan (3) suggested that the cytoplasmic NADP-isocitrate dehydrogenase is kinetically favored over the mitochondrial form for isocitrate oxidation and thus favors NADPH shuttling into the cytoplasm. However, there are several important factors that must be considered, such as: (a) the kinetic parameters for the reverse direction (i.e. K_Co, K_NADPH, K_[O], and V_max, where K_O indicates K_oxoglutarate), (b) the substrate and cofactor inhibition constants, (c) the concentration of substrates and products in the cytoplasm and mitochondria of various tissues, (d) the role of NADP-isocitrate dehydrogenase in various tissues, (e) the metabolic linkages to other enzymes involved in the shuttling of reducing equivalents across the mitochondrial membrane (e.g. the malate dehydrogenase isozymes, the aspartate aminotransferase isozymes, the malic enzyme, etc.,) (f) the differential transport of substrates across the mitochondrial membrane and the temperature and pH sensitivity of that transport, and (g) the relative concentration of the various NADP-isocitrate dehydrogenase isozymes in different tissues.

We have presented kinetic data in the forward direction previously (2) for the IDH-A2 and IDH-B2 isozymes that are consistent with Kaplan's (3) concept for liver tissue. The fractional distributions of these isozymes (Table VI) are also consistent with the greater catalytic efficiency for the IDH-B2 isozyme in liver. However, Table VI illustrates that the IDH-C2 isozyme is the major NADP-isocitrate dehydrogenase in heart and brain and the only detectable NADP-isocitrate dehydrogenase form in white muscle. Whereas the V_max / K_NADPH values for IDH-B2 and IDH-C2 differ by a factor of 2, in red muscle and brain, these catalytic differences would be offset by the greater concentration of the IDH-C2 isozyme. Moreover, in white muscle, the only detectable NADP-isocitrate dehydrogenase is the IDH-C2 isozyme. Since IDH-C2 is not present in liver, its role in the alleged NADPH shuttle must be very limited. These data are consistent with those of the literature. For example, Hogeboom and Schneider (31) and others (32, 33) have found that tissue such as liver, with high cytosolic demand, has 80% of its NADP-isocitrate dehydrogenase in the cytoplasmic form; whereas red muscle and heart, with low cytosolic NADPH demand, have 90% mitochondrial NADP-isocitrate dehydrogenase. Thus, the major role of IDH-C2 must be to provide reducing equivalents in the form of NADPH or NADH when coupled into the transhydrogenase system to the electron transport chain.

This point is emphasized by the results illustrated in Table VII, wherein it is clear that NADP-isocitrate dehydrogenase makes up only 6-7% of the total isocitrate dehydrogenase activity in any given tissue. Since white muscle isocitrate dehydrogenase activity is 93% IDH-C2 (see Tables VI and VII), it must supply the major oxidation of isocitrate and thus reducing equivalents for energy production. These results are consistent with those of Alp et al. (34), who surveyed tissues of numerous vertebrates and found that the vast majority of isocitrate dehydrogenase activity resided in the NADP-dependent isocitrate dehydrogenase enzyme.

These data, together with studies presented here and elsewhere (2, 3), lead us to conclude that the IDH-C2 isozyme is significantly involved in energy production, whereas IDH-B2 is involved in supplying cytosolic NADPH reducing equivalents for biosynthetic reactions.

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**Table 1:**

| Buffer | pH | pMg | pNa | pCa | pDextran | Total |
|-------|----|-----|-----|-----|----------|-------|
| Isotonic Glucose | 0.1 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 |
| Magnesium chloride | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Tris-HCl | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| MOPS | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |

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**Table 2:**

| Buffer | pH | pMg | pNa | pCa | pDextran | Total |
|-------|----|-----|-----|-----|----------|-------|
| Isotonic Glucose | 0.1 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 |
| Magnesium chloride | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Tris-HCl | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| MOPS | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |

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**Figure 1:** Effect of NADP/UDP ratio on the activity of NADP-Isocitrate Dehydrogenase. The activity of NADP-Isocitrate Dehydrogenase was assayed under the following conditions: pH 7.0, 5 mM MgCl₂, 100 mM MgCl₂, and 200 mM MgCl₂. The results are expressed as micromoles of NADP reduced per minute per milligram of protein.

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**Figure 2:** Effect of substrate concentration on the activity of NADP-Isocitrate Dehydrogenase. The activity of NADP-Isocitrate Dehydrogenase was assayed under the following conditions: pH 7.0, 5 mM MgCl₂, 100 mM MgCl₂, and 200 mM MgCl₂. The results are expressed as micromoles of NADP reduced per minute per milligram of protein.

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**Table 3:** Comparison of Kinetic Parameters Obtained from the Matrix Operation (Least Squares Procedure), equation 1 for NADP, and equation 2 for UDP, as a function of pH at 30°C. Error Ellipses Plotted at ± Standard Error of the Mean.
NADP-Isocitrate Dehydrogenase Isozymes

TABLE I: Comparison of kinetic parameters for NADH and NADPH as a function of temperature at pH 7.5. Error Estimates are Presented as Standard Error of the Mean. Significance of Tests are Presented as Probability as Determined by a t-test where Probabilities Above 0.05 are Considered Not Significant.

| pH | kcat (µmol/min/mg) | kcat/km (µmol/min/mg) | kcat/km, vs. NADH | kcat/km, vs. NADPH |
|----|-------------------|----------------------|-------------------|-------------------|
| 25 | 1.17 ± 0.03       | 2.52 ± 0.125         | 0.68 ± 0.056     | 0.56 ± 0.105      |
| 30 | 1.94 ± 0.110      | 2.50 ± 0.082         | 1.10 ± 0.020     | 0.85 ± 0.113      |
| 35 | 2.25 ± 0.356      | 0.76 ± 0.053         | 1.08 ± 0.040     | 0.57 ± 0.052      |
| 40 | 2.50 ± 0.390      | 0.38 ± 0.037         | 1.32 ± 0.101     | 0.57 ± 0.065      |
| 45 | 3.53 ± 0.175      | 0.72 ± 0.035         | 1.81 ± 0.035     | 0.64 ± 0.044      |

Fig. 4: Specificity of NADPH in isozymes on a function of pH at 25°C and PH 7.0. A, NADPH activity was measured by the Warburg apparatus and measured as an initial rate of oxygen consumption. B, NADPH activity was measured by the Warburg apparatus and measured as an initial rate of oxygen consumption. C, NADPH activity was measured by the Warburg apparatus and measured as an initial rate of oxygen consumption.

Table II: Measurement of NADPH in different fractions of tissue and brain.

| Tissue | NADPH Activity | NADPH Activity | NADPH Activity |
|--------|----------------|----------------|----------------|
| Brain  | 1.25           | 0.35           | 0.95           |
| Liver  | 1.30           | 0.45           | 0.90           |
| Muscle | 1.40           | 0.55           | 0.95           |

Note: All activities are expressed as a percentage of the total activity.