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Yeast Diphosphopyridine Nucleotide Specific Isocitrate Dehydrogenase. Purification and Some Properties*

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ABSTRACT: Yeast diphosphopyridine nucleotide specific isocitrate dehydrogenase (EC 1.1.1.41) has been purified to homogeneity by the criteria of disc gel electrophoresis, sedimentation velocity, and sedimentation equilibrium. Purification of the enzyme is facilitated by preferentially eluting the enzyme from ion-exchange columns with citrate, a modifier of the enzyme. Citrate also stabilizes the enzyme during isolation and preserves the kinetic cooperativity with respect to isocitrate. The enzyme has an apparent molecular weight of 3×10^5 as determined by sedimentation equilibrium. A subunit molecular weight of 3.9×10^4 was estimated by polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate. Thus the enzyme probably contains eight subunits. The kinetic order of the reaction catalyzed by the purified enzyme, as determined by the slope of a Hill plot, is 3.5 with respect to isocitrate in the absence of AMP. Addition of AMP decreases this order, to a value as low as 2.6 in some preparations. The order of reaction with respect to DPN^+ is 1 at both high and low isocitrate concentrations, in contrast to the order of 2 observed for the partially purified enzyme.

The DPN-specific isocitrate dehydrogenase from yeast (*threo*-D_s-isocitrate + $DPN^+ \rightarrow$ α-ketoglutarate + CO_2 + DPNH, EC 1.1.1.41) is activated by AMP, and citrate may either activate or inhibit, depending upon the isocitrate and citrate concentrations (Hathaway and Atkinson, 1963). Kinetic studies on relatively crude preparations of the enzyme demonstrated a reaction order of 4 for isocitrate and of 2 for DPN^+, AMP, and Mg^{2+} (Atkinson *et al.*, 1965). Because of the linearity of Hill plots over a wide concentration range, it was suggested that their slopes might be an indication of the actual number of binding sites for each ligand.

The enzyme has been purified to apparent homogeneity as a prerequisite to a study of its physical properties and ligand-binding behavior. The purification and some physical properties are reported here, and the results of binding studies in the following paper (Kuehn *et al.*, 1971).

Materials and Methods

Materials. Sodium dodecyl sulfate from Fisher was further purified by recrystallization from ethanol according to Burgess (1969). Glyceraldehyde 3-phosphate dehydrogenase (rabbit muscle), hexokinase (yeast), and ribonuclease (beef pancreas) were from Boehringer Mannheim, fumarase (pig heart) from Calbiochem, glutamate dehydrogenase (beef liver) from Sigma, and bovine serum albumin from Pentex. DEAE-cellulose (Whatman DE-52 microgranular) was purchased from H. Reeve Angel Inc., phosphocellulose (standard grade) from Schleicher & Schuell, and Bio-Gel A-1.5m (100–200 mesh) from Bio-Rad Laboratories. Fresh, compressed baker's yeast (*Saccharomyces cerevisiae*) was purchased from Standard Brands, Inc. A Manton-Gaulin single-stage homogenizer was

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made available through the courtesy of Calbiochem of Los Angeles. Conductivity measurements were made with a Radiometer, Model CDM 2, equipped with a flow-type electrode. The Diaflo ultrafiltration unit was a product of Amicon.

Enzyme Assay. Isocitrate dehydrogenase activity was determined by following DPN^+ reduction at 340 nm with a Gilford Model 2000 spectrophotometer and accessory system. A typical assay mixture contained 100 mM Hepes[1]–KOH (pH 7.6), 4 mM $MgSO_4$, 0.34 mM DPN^+, 1 mM AMP, 3 mM dithiothreitol, 0.39 mM *dl*-isocitrate, and enzyme preparation. All components except the isocitrate were incubated simultaneously in a cuvet at 30° for 2 min before the reaction was initiated by addition of isocitrate. For the kinetic experiments, the substrate concentrations were determined enzymatically and the AMP concentration calculated from its absorbance at 259 nm. One unit of activity is the amount of enzyme which reduces 1 μmole of DPN^+/min under standard assay conditions. Specific activity is defined as the number of units per milligram of protein.

Protein Determination. A modification of procedure II described by Klungsøyr (1969) was employed to determine the protein concentration. The 10 mM 2-mercaptoethanol present in all buffers interferes with the Lowry method (Vallejo and Lagunas, 1970) and also with the Klungsøyr procedure. This interference in the latter method was prevented by adding 5 μl of 15% H_2O_2 to the protein-reagent incubation mixture. In the presence of H_2O_2, the standard curves for bovine serum albumin were identical in the presence and absence of 10 mM 2-mercaptoethanol. The presence of citrate in the protein samples had no effect on the standard curves. A bovine serum albumin standard curve was employed for the protein determinations during the initial purification. After purified isocitrate dehydrogenase was available, it was used as a protein standard in subsequent experiments.

Polyacrylamide Disc Gel Electrophoresis. The electrophoretic system of Davis (1964) was utilized to achieve a running pH of 9.5, and that of Richards *et al.* (1965), with minor modifications in buffer concentrations, for pH 8.2. The ratio of acrylamide to *N,N'*-methylenebisacrylamide was 39 to 1 in all gels. Electrophoresis was performed using equipment similar to that described by Davis (1964) and a Heathkit IP-32 power supply. Experiments were performed at 4° at 2 mA/gel until the marker dye was about 5 mm from the end of the gel. Gels were stained for protein (70–80 μg of protein was applied per gel) by immersion in 1% Amido-Schwarz in 4% acetic acid for 1 hr, and destained electrophoretically at 5 mA/tube in 7% acetic acid. When staining for isocitrate dehydrogenase activity (25 μg of protein was applied per gel), gels were incubated in 100 mM Tris-SO_4 (pH 8.5), 4 mM $MgSO_4$, 50 mM *dl*-isocitrate, 1.8 mM DPN^+, 0.52 mM nitroblue tetrazolium, and 0.24 mM phenazine methosulfate for 7 min at room temperature in a dark room. The gels were stored in 7% acetic acid.

Electrophoresis on polyacrylamide gels containing 0.1% sodium dodecyl sulfate was performed according to Weber and Osborn (1969). Prior to electrophoresis the proteins were incubated at 50° in 10 mM sodium phosphate (pH 7.0), containing 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol, for 2 hr to achieve dissociation. The standard proteins and the isocitrate dehydrogenase were run on separate gels. Gel parameters necessary for calculation of the mobility were measured with vernier calipers.

Analytical Ultracentrifugation. Prior to sedimentation studies, the enzyme was dialyzed for 48 hr against several changes of 0.10 M potassium phosphate (pH 7.0). The meniscus depletion technique of Yphantis (1964) was utilized for the sedimentation equilibrium experiments which were performed with a Spinco Model E ultracentrifuge. The buffer external to the dialysis tubing was employed as the solvent blank. The 12-mm, 2.5-deg double-sector cell employed was equipped with a charcoal-filled Epon centerpiece and sapphire windows. The rotor speed was 11,000 rpm and the rotor temperature was electronically maintained at 20°. Fringe displacements were observed with interference optics and were recorded on Kodak metallographic plates. Equilibrium was assumed when the fringe displacement was invariant with time after 35–45 hr. The displacement of the fringe as a function of the radial distance was measured with a Nikon Model 6C microcomparator at 50× magnification. Fringe displacements greater than 130 μm were considered significant.

Differential sedimentation velocity experiments were performed according to the method of Schumaker and Adams (1968) using schlieren optics and a rotor temperature of 20°. The average speed of the rotor (approximately 59,780 rpm) was calculated by timing the odometer with an electronic stopwatch. The single-sector reference cell contained 3.4 mg of isocitrate dehydrogenase/ml of 0.10 M potassium phosphate (pH 7.0) and was equipped with a 2-deg positive wedge window. The single-sector experimental cell contained a different concentration of enzyme for each run, and was equipped with a plane window. Sedimentation patterns were photographed at either 4- or 8-min intervals and were recorded on Kodak metallographic plates. Initial values of $\delta s^0_{20,s}$ for the individual runs were calculated assuming a value of k of 0.005 cm^3/mg. Subsequent computations of $\delta s^0_{20,s}$ $\delta s_{20,w}$, and $\delta s^{c_1c_2}_{20,w}$ gave a value of k of 0.00566 cm^3/mg. The value of $\delta s^c_{20,w}$ was calculated for each protein concentration using the computed value of k, and $\delta s^0_{20,w}$ was determined by extrapolation to infinite dilution in the usual manner.

Sedimentation experiments were also performed without a reference solution, and the sedimentation coefficients were calculated according to methods described by Schachman (1957).

The viscosity of 0.10 M potassium phosphate (pH 7.0) was measured with a Ubbelhode viscometer, and the density was measured pycnometrically.

Results and Discussion

Purification of Yeast DPN-Specific Isocitrate Dehydrogenase. All steps were performed at 4° except addition of protamine sulfate, which was carried out at room temperature. All additions of $(NH_4)_2SO_4$ were as the solid salt. Equilibration of chromatographic eluates and contents of dialysis sacs with their respective buffers was determined by measurement of the conductivity and pH at 4°. Baker's yeast (1.8 kg) was suspended in 900 ml of 0.10 M $NaHCO_3$, 10 mM sodium citrate, and 10 mM 2-mercaptoethanol solution (buffer A) using a Waring Blendor. The yeast was ruptured by two passes through a Manton-Gaulin homogenizer at about 550 atm. After dilution with 1500 ml of buffer A, the solution was stirred for 4 hr, centrifuged at 13,000*g* for 15 min, and the supernatant suspension was decanted through cheesecloth to remove lipid. The filtrate was diluted to 30 mg of protein/ml with buffer A, and brought to 50% saturation in $(NH_4)_2SO_4$.

[1] Abbreviations used are: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; $(S)_{0.5}$, the concentration of substrate required for half-maximal activity; $(M)_{0.5}$, the concentration of modifier required for half-maximal effect.

TABLE I: Purification of Yeast DPN-Specific Isocitrate Dehydrogenase.

Fraction	Vol (ml)	Protein (mg)	Total Act. (Units)	Sp Act. (Units/mg)	Yield (%)
Crude extract	2420	150,040	5420	0.036	(100)
$(NH_4)_2SO_4$ I	1150	53,245	4045	0.076	75
PS supernatant	2260	14,532	4552	0.31	84
$(NH_4)_2SO_4$ II	145	7,308	4329	0.59	80
DEAE-cellulose	1420	1,037	3586	3.46	66
Phosphocellulose	730	77	2224	30.2	41
Concentrated phosphocellulose	16.2		2151		40
Bio-Gel A-1.5m	381	40.6	1446	35.6	27
Concentrated Bio-Gel A-1.5m	4.3	39	1424	35.6 (26.5)[a]	26

[a] Specific activities are based on a bovine serum albumin protein standard except that the specific activity of 26.5 is based on the isocitrate dehydrogenase protein standard.

After equilibration, the solution was centrifuged at 13,000*g* for 15 min. The supernatant solution was decanted from the soft pellets, which were subsequently recentrifuged at 31,000 for 10 min. All supernatants were discarded, and the pellet was dissolved in buffer A. The protein concentration was adjusted to 30 mg/ml and $(NH_4)_2SO_4$ was added to yield a 20% saturated solution. After centrifugation at 13,000*g* for 15 min, the supernatant fluid was carefully decanted from the soft pellets which were recentrifuged at 31,000*g* for 10 min. The two supernatant fractions were combined and the pellet discarded. The solution was brought to 33% saturation in $(NH_4)_2SO_4$. Centrifugation and recentrifugation of the soft pellets were accomplished as noted previously. The pellets were suspended in 1 l. of 5 mM potassium phosphate (pH 7.6) and 10 mM 2-mercaptoethanol (buffer B) containing 0.5 mM sodium citrate. This solution was dialyzed against four 18-l. portions of the same buffer. This preparation is designated as $(NH_4)_2SO_4$ I in Table I.

The dialyzed ammonium sulfate fraction was diluted to 25 mg of protein/ml by addition of a buffer solution containing 10 mM potassium phosphate (pH 7.6), 1 mM sodium citrate, and 10 mM 2-mercaptoethanol. A 3% protamine sulfate solution (23°) was added dropwise into the stirred protein solution (4°) until a ratio of 0.25 mg of protamine sulfate/mg of protein was reached. After centrifugation at 13,000*g* for 10 min the pellet was discarded. To the clear, yellow supernatant solution (PS supernatant in Table I) was added 19 g of $NaHCO_3$ and 6.65 g of sodium citrate. The protamine sulfate fractionation always resulted in apparent activation of enzyme activity (10–40%).

The protamine sulfate supernatant solution was brought to 37% saturation in $(NH_4)_2SO_4$. A small pellet obtained by centrifugation at 13,000*g* for 15 min was discarded. $(NH_4)_2SO_4$ was added to the supernatant solution to bring the final salt concentration to 52%. The pellet obtained on centrifugation at 13,000*g* for 15 min was suspended in a minimal volume of buffer B containing 0.5 mM sodium citrate. This solution was dialyzed against three 4-l. portions of the same buffer. A small amount of material precipitated during dialysis and was removed by centrifugation prior to DEAE-cellulose chromatography. The supernatant solution is noted as $(NH_4)_2SO_4$ II in Table I.

The 37–52% $(NH_4)_2SO_4$ fraction was applied to a 32 × 6 cm column of DEAE-cellulose equilibrated with buffer B containing 0.5 mM sodium citrate. The column was washed with buffer B containing 5 mM sodium citrate at a flow rate of 240 ml/hr until the column effluent had an absorbance of 0.21 at 280 nm (about 15 hr). The isocitrate dehydrogenase was then eluted with buffer B containing 10 mM sodium citrate. The pooled fractions containing activity were dialyzed against three 18-l. portions of buffer B containing 0.5 mM sodium citrate.

The preparation was applied to a 21 cm × 4.2 cm column of phosphocellulose equilibrated with buffer B containing 0.5 mM sodium citrate. The column was washed with 1200 ml of buffer B containing 20 mM sodium citrate, after which the column effluent had an absorbance of 0.005 at 280 nm. The enzyme was eluted with buffer B containing 50 mM sodium citrate. The fractions containing activity were pooled and dialyzed against two 6-l. portions of buffer A. The dialyzed solution was concentrated to 16.2 ml under 0.7 atm of nitrogen in an Amicon Diaflo apparatus containing an XM-50 membrane.

The clear, colorless solution was applied to a 215 × 4.2 cm column of Bio-Gel A-1.5m previously equilibrated with the eluting solution, 0.10 M $NaHCO_3$, 0.5 mM sodium citrate, 10 mM 2-mercaptoethanol. Pooled fractions containing activity were concentrated to 4 ml in a Diaflo unit under 0.7 atm of nitrogen.

Debris were removed by centrifugation at about 1800*g* for 5 min. An equal volume of glycerol was added to the supernatant solution, and the purified enzyme was stored in 50% glycerol, 0.05 M $NaHCO_3$, 0.25 mM sodium citrate, 5 mM 2-mercaptoethanol at −20°. No loss of activity has been observed for enzyme stored in this manner for 6 months. The summary of a typical purification is given in Table I.

The specific activity of 26.5 units/mg noted here for assays performed in Hepes–KOH buffer is equivalent to the specific activity of 65 units/mg, obtained in Tris-HCl buffer, reported previously (Kuehn *et al.*, 1970). Although the apparent V_{max} is decreased in Hepes–KOH, the $(\text{isocitrate})_{0.5}$ is lowered by a factor of two.

Polyacrylamide Disc Gel Electrophoresis. Gel electrophoresis was performed at two values of pH and two gel concentrations to evaluate the degree of homogeneity of the purified enzyme (Hedrick and Smith, 1968). A single protein band was seen at pH 9.5 on 5 and 3% acrylamide gels and also at pH 8.2 on 5 and 3.5% gels. At each value of pH, the lower percentage gel was also stained for enzymic activity,

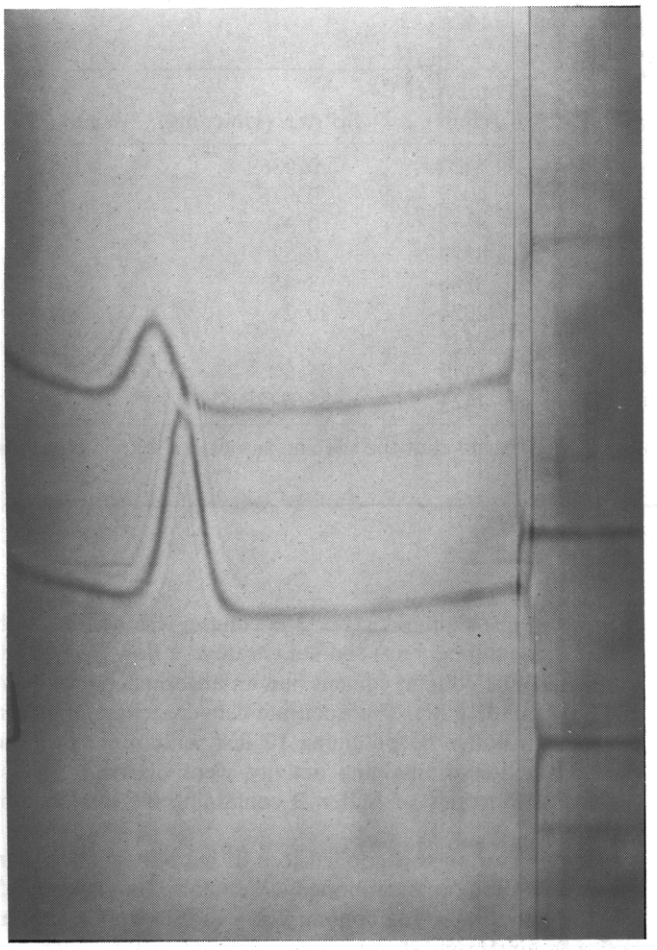

FIGURE 1: Sedimentation velocity pattern of yeast DPN isocitrate dehydrogenase. The upper pattern was obtained with 3.4 mg of protein/ml (reference cell with 2-deg positive wedge window) and the lower pattern with 6.3 mg of protein/ml (experimental cell with plane window). The sedimentation was performed in 0.10 M potassium phosphate (pH 7.0) at 29° and 59,780 rpm. The exposure was taken 45 min after reaching speed.

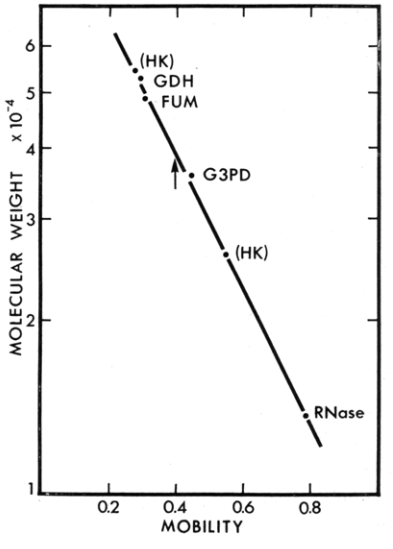

FIGURE 2: Electrophoretic mobility of yeast DPN isocitrate dehydrogenase subunit on polyacrylamide gels containing 0.1% sodium dodecyl sulfate. Abbreviations used are: GDH, glutamate dehydrogenase; FUM, fumarase; G3PD, glyceraldehyde 3-phosphate dehydrogenase; HK, hexokinase; and RNase, ribonuclease. The arrow corresponds to the mobility of the isocitrate subunit.

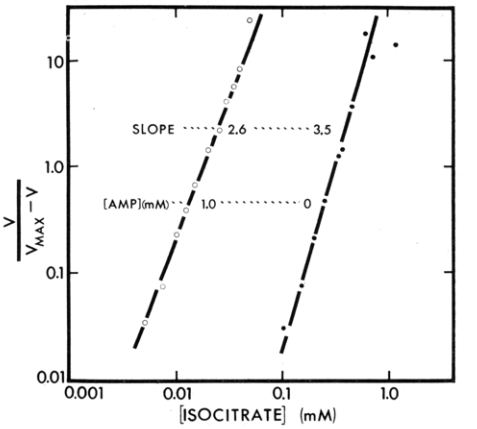

FIGURE 3: Hill plot of the dependence on isocitrate concentration of the velocity of the reaction catalyzed by yeast DPN isocitrate dehydrogenase. Reaction mixtures contained: 100 mM Hepes–KOH (pH 7.6), 4 mM $MgSO_4$, 3 mM dithiothreitol, 0.4 mM DPN^+, isocitrate and AMP at the concentrations indicated, and enzyme.

using the tetrazolium assay. Activity was found at the same position at which protein stained. A control gel, incubated in the absence of isocitrate, did not show any production of formazan.

Sedimentation Experiments. Sedimentation velocity experiments revealed a single boundary in 0.10 M potassium phosphate (pH 7.0) as shown in Figure 1. From measurements of the sedimentation rate at different protein concentrations, an $s^0_{20,w}$ value of 11.2×10^{-13} sec was obtained by extrapolation when the differential sedimentation technique was employed. A $s^0_{20,w}$ value of 11.0×10^{-13} sec was determined using conventional sedimentation velocity methods.

The data obtained in sedimentation equilibrium studies yielded a linear plot of the logarithm of the fringe displacement as a function of the square of the radial distance. The molecular weight was calculated from the slope of the graph in the usual manner. A partial specific volume for the enzyme of 0.74 cm^3/g was estimated from the amino acid composition (data not shown) as described by Cohn and Edsall (1943). The apparent molecular weight was determined from sedimentation equilibrium experiments employing 0.035, 0.054, and 0.070% protein solutions in 0.10 M potassium phosphate (pH 7.0). Values of 309,000, 295,000, and 289,000, respectively, were calculated.

Subunits. After the purified enzyme was dissociated in 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol, the molecular weight of its subunits was estimated by electrophoresis on polyacrylamide gels containing 0.1% sodium dodecyl sulfate. By comparing the mobility of the subunit with the mobilities of several protein standards, the molecular weight of the single protein band was estimated at 39,000 (Figure 2). Values for the subunit molecular weights of the standards were taken from Weber and Osborn (1969). Hexokinase subunit molecular weights are plotted both as 54,000 (Pringle, 1970) and as 26,000 (Klotz and Darnall, 1969) since two major bands were observed on electrophoresis of this enzyme.

Kinetics. The kinetic order[2] of the reaction with respect to isocitrate is approximately 3.5 in the absence of AMP, and 2.6 in the presence of this modifier (Figure 3). However, some

[2] In this paper, the terms "order" and "kinetic order" refer to the slopes of Hill plots.

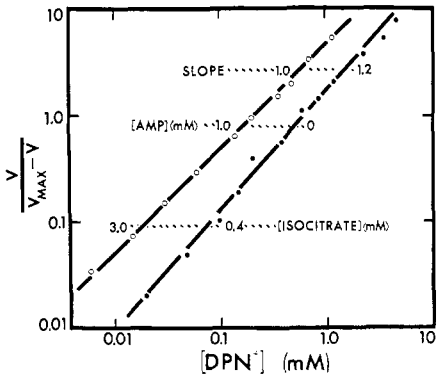

FIGURE 4: Hill plot of the dependence on DPN^+ concentration of the velocity of the reaction catalyzed by yeast DPN isocitrate dehydrogenase. Reaction mixtures contained: 100 mM Hepes–KOH (pH 7.6), 4 mM $MgSO_4$, 3 mM dithiothreitol, DPN^+, isocitrate, and AMP at the concentrations indicated, and enzyme.

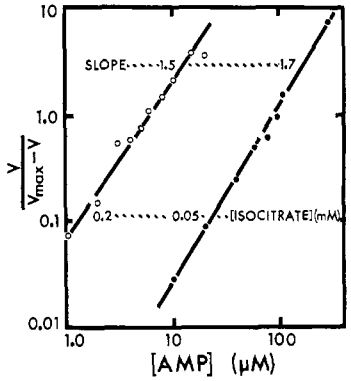

FIGURE 5: Hill plot of the dependence on AMP concentration of the velocity of the reaction catalyzed by yeast DPN isocitrate dehydrogenase. Reaction mixtures contained: 100 mM Hepes–KOH (pH 7.6), 4 mM $MgSO_4$, 3 mM dithiothreitol, 0.4 mM DPN^+, isocitrate and AMP at the concentrations indicated, and enzyme.

preparations have exhibited an order for isocitrate as low as 3 in the absence of AMP. In these cases the order remained unchanged when AMP was added.

The order with respect to DPN^+ is approximately 1 in the presence of either high or low concentration of isocitrate (Figure 4). The $(M)_{0.5}$ for AMP was strongly affected by the level of isocitrate, but the order of reaction with respect to AMP varied only from 1.5 at high isocitrate concentrations to 1.7 at low isocitrate concentrations (Figure 5). The purified enzyme shows no cooperativity with regard to Mn^{2+} (Figure 6), and yields a Hill slope for Mg^{2+} of 1.2 (L. D. Barnes, unpublished results). Results of kinetic experiments are summarized in Table II.

Modifications During Isolation. Citrate, a modifier of the reaction catalyzed by yeast DPN-specific isocitrate dehydrogenase, facilitates the isolation of the enzyme in terms of yield and purity. Isolation in the absence of citrate results in poor overall recovery of activity (<10%), and the preparation contains a minor contaminant. This contaminant is eliminated by preferential elution of isocitrate dehydrogenase with citrate from the DEAE-cellulose and phosphocellulose columns. The order with respect to isocitrate of a crude preparation is about 3.8 in the presence or absence of AMP (Atkinson *et al.*, 1965). If the enzyme is purified in the absence of citrate, the order of reaction with respect to isocitrate decreases to about 3 in the absence of AMP, and approximately 2 in the presence of AMP. In spite of this loss in cooperativity, the effect of AMP on affinity for isocitrate is as great with the purified enzyme as with the crude preparation. Purification in the presence of citrate yields a preparation with a degree of cooperativity intermediate between those of the crude preparation and of the enzyme purified without citrate. When the enzyme is only partially purified by ammonium sulfate fractionation in the presence of citrate, the order of reaction with respect to isocitrate is between 6 and 8 when assayed without AMP (Kuehn *et al.*, 1970). Such a high order for isocitrate is difficult to rationalize in view of the observation that the purified enzyme binds only four molecules of isocitrate (Kuehn *et al.*, 1971).

Even when purified in the presence of citrate, the enzyme loses its cooperative response to DPN^+ and Mg^{2+}, both of which exhibit second-order kinetics with crude preparations

TABLE II: Kinetic Parameters of Purified Yeast DPN-Specific Isocitrate Dehydrogenase.[a]

Varied Component	Other Assay Components (mM)	Kinetic Order[b]	$(X)_{0.5}$[c] (μM)
Isocitrate		3.5	320
Isocitrate	AMP (1.0)	2.6	18
DPN^+	Isocitrate (0.4)	1.2	620
DPN^+	Isocitrate (3.0)		
	AMP (1.0)	1.0	210
AMP	Isocitrate (0.05)	1.7	90
AMP	Isocitrate (0.2)	1.5	6
Mn^{2+}	Isocitrate (1.5)	1.0	5
Mn^{2+}	Isocitrate (4.0)		
	AMP (1.0)	1.0	0.95

[a] Reaction mixtures contained 100 mM Hepes–KOH (pH 7.6), 4 mM $MgSO_4$ (except when Mn^{2+} was the varied component), 3 mM dithiothreitol, 0.4 mM DPN^+ (except when DPN^+ was the varied component), and enzyme. [b] Slope of Hill plot. [c] Concentration of the varied component at which reaction velocity was half-maximal velocity.

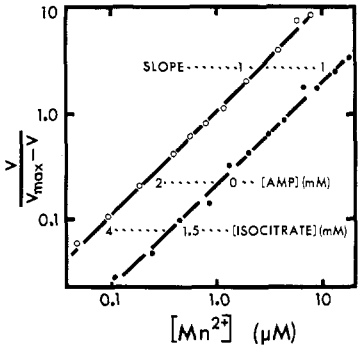

FIGURE 6: Hill plot of the dependence on Mn^{2+} concentration of the velocity of the reaction catalyzed by yeast DPN isocitrate dehydrogenase. Reaction mixtures contained: 100 mM Hepes–KOH (pH 7.6), 3 mM dithiothreitol, 0.4 mM DPN^+, isocitrate, AMP, and Mn^{2+} at the concentrations indicated, and enzyme. Concentrations of free Mn^{2+} were calculated from the total concentration of Mn^{2+} and isocitrate using a pK of 3.06 for dissociation of the Mn^{2+}–isocitrate complex (Grzybowski *et al.*, 1970).

(Atkinson *et al.*, 1965), but show little or no cooperativity with the purified enzyme (Figure 4 and unpublished results). The order with respect to the alternative cationic cofactor, Mn^{2+}, is also 1 (Figure 6).

Comparison with Other Enzymes. Yeast DPN isocitrate dehydrogenase resembles several other pyridine nucleotide specific dehydrogenases that can be dissociated into subunits of mol wt 30,000–40,000 (Sund, 1968). The native enzyme is probably composed of eight subunits of similar molecular weight. In a preliminary report (Kuehn *et al.*, 1970), we suggested that the enzyme contained two types of nonidentical subunits. Subsequent experiments have indicated that one of the bands on sodium dodecyl sulfate gel was probably due to a minor contaminant. The intensity of the contaminating band as observed visually decreased with increasing specific activity of the isocitrate dehydrogenase preparation. The purified enzyme reported here exhibited only one band on sodium dodecyl sulfate gels. Beef heart DPN-specific isocitrate dehydrogenase has been reported to have a molecular weight of 330,000 and to consist of eight subunits (Giorgio *et al.*, 1970). DPN-specific isocitrate dehydrogenases from other sources such as pea (Cox and Davies, 1969), *Blastocladiella emersonii* (LéJohn *et al.*, 1969), and *Neurospora crassa* (Sanwal and Stachow, 1965) exhibit molecular weights greater than 100,000, and in some cases high molecular weight aggregates are formed. Reported molecular weights of TPN-specific isocitrate dehydrogenases are lower: *Azotobacter vinelandii*, 80,000 (Chung and Franzen, 1969); pig heart, 58,000 (Colman, 1968); and *Bacillus stearothermophilus*, 92,500 (Howard and Becker, 1970).

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