Inosinic Acid Dehydrogenase of Sarcoma 180 Cells*

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SUMMARY
Inosinic acid dehydrogenase was partially purified from sarcoma 180 cells. The concentrations of each reactant, inosine 5'-phosphate, nicotinamide adenine dinucleotide, and K⁺, altered both the apparent Kₐ and Vₘₐₓ values for each of the other reactants. Enzymatic activity was inhibited by both products of the reaction, xanthosine 5'-phosphate and reduced nicotinamide adenine dinucleotide; the inhibition produced by XMP was competitive with respect to IMP, whereas the inhibition caused by NADH was not competitive with respect to either IMP, NAD⁺, or K⁺. The data are consistent with an enzymatic mechanism involving ordered sequential addition of IMP, NAD⁺, and K⁺ to the enzyme to form active enzyme-substrate complexes. Substrate inhibition occurred at NAD⁺ concentrations several-fold higher than the optimal concentration of NAD⁺; the kinetics suggests that formation of an abortive ternary complex between the enzyme, XMP, and NAD⁺ is involved in producing this substrate inhibition.

Inosinic acid dehydrogenase (IMP dehydrogenase; IMP:NAD⁺ oxidoreductase EC 1.2.1.14) catalyzes the following reaction:

\[ \text{IMP} + \text{NAD}^+ + \text{H}_2\text{O} \rightarrow \text{XMP} + \text{NADH} + \text{H}^+ \]

This enzyme was first described by Magasanik, Moyed, and Gehring (1) with partially purified extracts of *Aerobacter aerogenes*; similar enzymatic activity has subsequently been shown in extracts of other bacteria (2, 3), higher plants (4, 5), and animals (5-7).

The reaction catalyzed by IMP dehydrogenase occurs subsequent to a branch point in the pathway involved in the biosynthesis of purine nucleotides de novo; therefore, IMP dehydrogenase is the first enzyme uniquely involved in the biosynthesis of guanine nucleotides. Accordingly, the activity of this enzyme is subject to repression in bacterial cells (8) and to negative feedback control by several guanine nucleotides; the inhibition produced by GMP is kinetically competitive with IMP (2, 3, 9-12). IMP dehydrogenase is also subject to inhibition by thyroxin (5), sulfhydryl reagents (4, 11, 13), and several purine nucleotide analogues such as the ribonucleotides of 6-chloropurine, 2-amino-6-chloropurine, 6-thiopurine, and 2-amino-6-thiopurine (7, 10-12, 14-16). The relationship of the inhibition in *vitro* of IMP dehydrogenase activity to the inhibition *in vivo* of the growth of cells and the biosynthesis of guanine nucleotides by these purine analogues is under investigation (see, for example, Reference 17).

The data in this report describe a partial purification and some kinetic properties of the IMP dehydrogenase activity of sarcoma 180 ascites tumor cells; a preliminary report of some of these results appeared previously in abstract form (11, 18).

**EXPERIMENTAL PROCEDURE**

**Materials**—Adenosine diphosphoribose, AMP, ATP, IMP, α-NAD⁺, 3-pyridinealdehyde AD, and XMP were obtained from P-L Biochemicals. NAD⁺ was also purchased from Sigma. GSH, NADH, NADP⁺, and NADPH were obtained from Nutritional Biochemicals. Nicotinic acid and nicotinamide were purchased from Eastman; DEAE-cellulose was from Carl Schleicher and Schuell Company; and crystalline yeast alcohol dehydrogenase was from Worthington. Tris and KCl were purchased from Fisher. Enzyme grade ammonium sulfate was supplied by Mann.

**Preparation of IMP Dehydrogenase**—Six-day growths of sarcoma 180 ascites tumor cells were obtained from female CD-1 Swiss mice of approximately 9 to 11 weeks of age. The cells were separated from peritoneal fluid by centrifugation and washed with distilled water and 0.9% NaCl. All subsequent steps were performed at 4°C. Washed cells were suspended in 2 volumes of 0.1 M Tris·Cl, pH 8, and 50-ml aliquots were subjected to sonic disruption for 30 sec with a Branson sonifier set at 6 d.c. amps. Unbroken cells, nuclei, and debris were removed by centrifugation at 10,000 × g for 10 min. Particulate matter was then precipitated by centrifugation for 2 hours at 105,000 × g. The lipid was removed with a pipette and the remaining 1 The abbreviations used are: α-NAD⁺, 3-carbamoyl-1-α-D-ribofuranosylpyridine 5'-ester with adenosine 5'-pyrophosphate; 3-pyridinealdehyde-AD, α-D-ribofuranosyl-3-pyridinal 5'-ester with adenosine 5'-pyrophosphate.
supernatant solution was fractionated by the addition of a saturated solution of ammonium sulfate. The precipitate that formed between 20 and 40% saturation with ammonium sulfate contained most of the IMP dehydrogenase activity; this precipitate was dissolved in 5 ml of 0.1 M Tris-Cl, pH 8, and dialyzed for 24 hours against 2,000 ml of 0.01 M Tris-Cl, pH 8. About 15 ml of dialyzed enzyme preparation, containing about 35 mg of protein per ml, were obtained from the ascites cells from 50 mice. The enzyme preparations were usually stored for about 2 weeks at -17° before use; a small amount of material that precipitated during dialysis was removed by centrifugation at 10,000 X g for 10 min after thawing the frozen preparations. Freezing did not appear to alter the properties of the enzyme, but preparations which had been frozen for more than 3 weeks generally had lower activity. The actual degree of purification cannot be stated with accuracy because IMP dehydrogenase activity could not be measured by either spectrophotometric or isotopic techniques with nonfractionated preparations; the fraction used for enzymatic measurements contained 4% of the total cellular protein; assuming negligible losses of enzyme in the fractionation procedure it can be calculated that less than a 25-fold purification of enzyme was attained.

The enzymatic activity could be absorbed to and eluted from DEAE-cellulose, DEAE-sephadex, hydroxylapatite, and calcium phosphate gel; with each of these procedures the total recovery of enzyme activity was never greater than 30% and no significant increase in the degree of purification was achieved. Attempts to stabilize the enzymatic activity during chromatography on DEAE-cellulose by the presence of either EDTA, GSH, NAD+, IMP or a mixture of NAD+, IMP, and GSH were unsuccessful. The enzyme activity was totally excluded from Sephadex G-100, and had a Vm/Vo of about 1.1 with Sephadex G-200; a single symmetrical peak of enzymatic activity was observed in each of these experiments.

Assay—IMP dehydrogenase activity was determined by the method of Magasanik et al. (1), which involves monitoring the change in absorbance at 340 mµ with time, a measure of the net production of NADH. Assays were routinely conducted in the following manner: 100 µmoles of Tris-Cl (pH 8), 100 µmoles of KCl, 0.28 µmole of NAD+ (K+ salt), 0.1 ml of enzyme preparation (generally 1 to 3 mg of protein), and water to give a volume of 0.9 ml were mixed by inversion in a 1-ml quartz cuvette. The cuvettes were allowed to equilibrate to 37°; the temperature of the cell chamber was maintained at 37° ± 1° with a Tafmond thermostated circulating water bath. After 5 min of previous incubation, 2.3 µmoles of IMP (Na+ salt) in a volume of 0.1 ml, prewarmed to 37°, were added to initiate the reaction. The absorbance at 340 mµ was monitored with a Gilford model 2000 multiple sample absorbance recorder. The change in the molar extinction coefficient for the conversion of NAD+ to NADH is 6.22 X 10³ (19). One unit of enzymatic activity was defined as the formation of 1 µmole of NADH per min at 37°.

The apparent Kₚ and Vₘₐₓ values were calculated with an IBM computer with the program of Cleland (20).

The NAD+ and NADH contents of sarcoma 180 cells were determined by enzymatic method (21).

**RESULTS**

The activity of IMP dehydrogenase was measured by the change in absorbance at 340 mµ; under the conditions used the reaction velocity was constant for about 30 min and was a linear function of the amount of enzyme used in the assay. Enzymatic activity was similar with either 0.1 M Tris-Cl or 0.1 M KCl as buffers; optimal activity occurred at approximately pH 8. The activity of the enzyme was dependent upon the presence of both IMP and NAD+ and was markedly stimulated by the presence of inorganic salts. The concentration of inorganic cations had a marked effect on the extrapolated maximal velocity for the enzymatically catalyzed reaction; however, the absolute requirement for an inorganic cation in the reaction was difficult to establish. About 10 to 20% of the maximum activity was obtained when an inorganic salt was not specifically added to the assay mixture except as counterions with the substrates and enzyme. When the IMP dehydrogenase preparation was passed through a column of Sephadex G-25 with 0.01 M Tris-Cl and assayed with IMP and NAD+ which had Tris as their counterions, the preparation exhibited 8% of the activity of a comparable reaction mixture containing 0.1 M K+.

Thus, although a total dependence of enzymatic activity on inorganic cations was not shown, it is apparent that activity is markedly dependent upon the salt concentration. Maximum activity occurred at a K+ concentration of 0.1 M (Fig. 1), nonivalent cations other than K+ were less effective, Na+ being more stimulatory than NH₄+. Li+ and Mg²⁺ at relatively high concentrations inhibited in a competitive manner the stimulatory effects of K+.

In the presence of 0.1 M K+ relatively high concentrations of NH₄+ (0.1 M NH₄Cl) and Ca²⁺ (0.25 M CaCl₂) were also inhibitory (34 and 29%, inhibition, respectively), but high concentrations of Na⁺ (0.4 M NaCl) did not inhibit enzymatic activity. The relative effectiveness of several anions was found to depend upon the cation used (Table I); thus, while the stimulatory activity of potassium acetate was similar in magnitude to that of KCl, sodium acetate and ammonium acetate were distinctly more active than NaCl and NH₄Cl, respectively. The stimulatory activity of sodium formate was similar in magnitude...
**TABLE I**

*Effects of several salts on activity of IMP dehydrogenase*

Assays were conducted with 0.1 M Tris-Cl, pH 8, as described in the text. The nature and the concentration of the added salt was varied as shown in the table; the enzymatic activity with 0.1 M KCl was set equal to 1.00, and the other enzymatic activities were expressed relative to this value.

<table>
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<th>Salt</th>
<th>Concentration</th>
<th>Relative enzymatic activity</th>
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</thead>
<tbody>
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</tr>
<tr>
<td>Potassium acetate</td>
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</tr>
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<td>Sodium acetate</td>
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</tr>
<tr>
<td>Ammonium chloride</td>
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<td>0.66</td>
</tr>
<tr>
<td>Ammonium acetate</td>
<td>0.5</td>
<td>1.05</td>
</tr>
<tr>
<td>Sodium formate</td>
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<td>1.35</td>
</tr>
<tr>
<td>Potassium EDTA</td>
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<td>1.00</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.64</td>
</tr>
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</table>

It was noticed that, whereas potassium EDTA at a concentration of 0.01 M induced stimulation of enzymatic activity comparable to that produced by 0.1 M KCl.

*Apparent Irreversibility of Reaction*—Incubation of the enzyme at pH 8 with XMP (2.3×10^{-3} M), NADH (2×10^{-4} M), and 0.1 M K^+ did not result in a decrease in the absorbance at 340 nm. Such a decrease would have resulted from net conversion of NADH and XMP to NAD^+ and IMP, respectively. Thus, the reaction equilibrium strongly favors formation of XMP and NADH.

*Initial Velocity Kinetics*—Several experiments were performed to validate the performance of kinetic studies with this enzyme preparation. First, the enzyme preparation was incubated with NADH; barely detectable decreases in the absorbance at 340 nm occurred at pH values 7, 8, and 9.5; these results were demonstrative of the absence of significant oxidation of NADH by the enzyme preparation, assuring that NADH production could be determined by absorbance measurements. Second, the enzyme was previously incubated with K^+ and a rate-limiting concentration of either IMP or NAD^+ for various durations prior to the addition of the other reaction component. In each case the reaction velocity that was subsequently attained was identical, indicating the effective absence of nucleotidase activities from

![Graphs](http://www.jbc.org/)

**Fig. 2.** Double reciprocal plots of the initial velocity of the IMP dehydrogenase reaction as functions of the concentrations of IMP, NAD^+, and K^+. V is the initial velocity in millimicro moles per min. **A**, effect of NAD^+ on the substrate-velocity relationship for IMP. The concentration of K^+ was 0.1 M. a, 4.2×10^{-4} M NAD^+; b, 5.6×10^{-4} M NAD^+; c, 7.0×10^{-4} M NAD^+; d, 9.3×10^{-4} M NAD^+; e, 1.4×10^{-3} M NAD^+; f, 2.8×10^{-3} M NAD^+. **B**, effect of K^+ on the substrate-velocity relationship for IMP. The concentration of NAD^+ was 2.8×10^{-4} M. a, 4×10^{-4} M K^+; b, 5×10^{-4} M K^+; c, 1×10^{-3} M K^+; d, 2×10^{-3} M K^+; e, 1×10^{-2} M K^+. **C**, effect of IMP on the substrate-velocity relationship for NAD^+. The concentration of K^+ was 0.1 M. a, 4.6×10^{-4} M IMP; b, 9.3×10^{-4} M IMP; c, 2.3×10^{-3} M IMP; d, 2.3×10^{-2} M IMP. **D**, effect of K^+ on the substrate-velocity relationship for NAD^+. The concentration of IMP was 2.3×10^{-4} M. a, 4×10^{-4} M K^+; b, 5×10^{-4} M K^+; c, 1×10^{-3} M K^+; d, 2×10^{-3} M K^+; e, 1×10^{-2} M K^+. **E**, effect of K^+ on the activator-velocity relationship for IMP. The concentration of NAD^+ was 2.8×10^{-4} M. a, 2.3×10^{-4} M IMP; b, 3.5×10^{-4} M IMP; c, 7.0×10^{-4} M IMP; d, 2.3×10^{-3} M IMP; e, 2.3×10^{-2} M IMP. **F**, effect of NAD^+ on the activator-velocity relationship for K^+. The concentration of IMP was 2.3×10^{-3} M. a, 2.8×10^{-3} M NAD^+; b, 4.2×10^{-3} M NAD^+; c, 7.0×10^{-4} M NAD^+; d, 1.4×10^{-3} M NAD^+; e, 2.8×10^{-4} M NAD^+. 

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the preparation, thereby assuring that velocity measurements at relatively low concentrations of substrates would not be influenced by destruction of the substrates by contaminating enzymes.

The initial velocity of the enzymatically catalyzed reaction was determined at several concentrations of IMP, NAD\(^+\), and K\(^+\). The reciprocal of the initial velocity was graphed as a function of the reciprocal of the concentration of IMP, NAD\(^+\), or K\(^+\) at several fixed concentrations of one of the other reaction components (Fig. 2, A to F). Each line in Fig. 2A, for example, represents determinations of the initial velocity of the reaction as a function of the concentration of IMP at one of several particular concentrations of NAD\(^+\); all of the data in Fig. 2A were determined with 0.1 M K\(^+\) in the assay mixture. Most of the data in Fig. 2, A to F, appear to fit straight lines; significant deviations from linearity occurred, however, at high concentrations of NAD\(^+\); this inhibition is discussed subsequently. Use of concentrations of IMP greater than 10\(^{-2}\) M or of K\(^+\) greater than 10\(^{-3}\) M also produced decreases in the reaction velocity; the mechanisms of the inhibitory effects produced by the latter two compounds were not investigated since they involved such high concentrations of reaction components.

The lines in Fig. 2, A to F, intersect in the upper left-hand quadrants. The slopes and intercepts of these lines appeared to be linear functions of the reciprocal of the concentration of the changing fixed reactant\(^2\) for each experiment. The extrapolated \(V_{max}\) was essentially the same, 1.00 to 1.18 (average 1.08) relative to the control activity, in each of these six experiments. The lowest apparent \(K_m\) values obtainable for IMP, NAD\(^+\), and K\(^+\) were 1.6 \times 10^{-5} M, 4.6 \times 10^{-5} M, and 2 \times 10^{-3} M, respectively.

**Product Inhibition Patterns**—Inhibition of IMP dehydrogenase by NADH was noncompetitive with respect to NAD\(^+\) (Fig. 3A). The effects of NADH on the slopes and intercepts of Fig. 3A were linear functions of the concentration of the changing reaction component; this inhibition is discussed subsequently. Use of concentrations of IMP greater than 10\(^{-2}\) M or of K\(^+\) greater than 10\(^{-3}\) M also produced decreases in the reaction velocity; the mechanisms of the inhibitory effects produced by the latter two compounds were not investigated since they involved such high concentrations of reaction components.

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after 10 min; thus, product inhibition by NADH was not usually encountered in assaying the initial activity of IMP dehydrogenase. The inhibition of IMP dehydrogenase activity by NADH was not competitive with respect to IMP (Fig. 3B); the degree of variation in the data was too large to allow a distinction between noncompetitive and uncompetitive inhibition. The effect of NADH on the intercept of this plot appeared to be directly proportional to the concentration of NADH. The inhibition produced by NADH was noncompetitive with respect to K+ (Fig. 3C). The effects of NADH on the slope and intercept of this plot appeared to be linear.

XMP, the other product of the reaction, also inhibited IMP dehydrogenase activity. Kinetically the inhibition by XMP was competitive with respect to IMP (Fig. 3D). The effect of XMP on the slopes of the lines of the double reciprocal plot was a linear function of the concentration of XMP. Inhibition by XMP was noncompetitive with respect to both NAD+ (Fig. 3E) and K+ (Fig. 3F).

Inhibition by NAD+—A plot of the initial velocity of the enzymatically catalyzed reaction against the logarithm of the concentration of NAD+ described a bell-shaped curve (Fig. 4); a relatively narrow range of concentrations of NAD+ (3 to 4 × 10⁻⁴ M) was required for maximal enzymatic activity. The inhibition at the high concentrations of NAD+ was immediate in onset and did not increase in magnitude as the reaction continued, indicating that inhibition was not mediated through the products of the enzymatic reaction. Chromatographic purification of NAD+, with a procedure that removed adenosine diphosphoribose (25), did not affect the inhibition of IMP dehydrogenase by high concentrations of NAD+; furthermore, a concentration of 1.8 × 10⁻⁵ M adenosine diphosphoribose caused only 8% inhibition of IMP dehydrogenase activity under the standard assay conditions. The α isomer of NAD+ is not removed by the method of chromatographic purification that was used; a concentration of 3 × 10⁻³ M of α-NAD+ caused only 7% of this isomer (26), the degree of inhibition (38%) produced by 1.5 × 10⁻³ M NAD+ cannot be attributed to contaminating α-NAD+.

Several compounds that structurally resemble part of the NAD+ molecule were also examined both as substrates and inhibitors of IMP dehydrogenase activity (Table II). AMP at a relatively high concentration produced 20% inhibition, whereas BTP and adenosine diphosphoribose were essentially noninhibitory at higher concentrations, nicotinamide mononucleotide was only a weak inhibitor of IMP dehydrogenase activity, inducing 25% inhibition at a concentration of 1 × 10⁻³ M. NADP+, which appeared to replace NAD+ partially as a substrate, caused no inhibition at a concentration of 1.1 × 10⁻³ M. In contrast, NADPH was a potent inhibitor of enzymic activity, producing 57% inhibition at a concentration of 1.1 ×
Activity of analogues of NAD⁺ as inhibitors of IMP dehydrogenase

Inhibition of the activity of IMP dehydrogenase was determined in the presence of 0.1 M K⁺, 2.3 × 10⁻³ M IMP, and 2.8 × 10⁻⁴ M NAD⁺ in 0.1 M Tris-Cl, pH 8.

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<th>Compound</th>
<th>Concentration</th>
<th>Inhibition</th>
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<td>NAD⁺</td>
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</tr>
<tr>
<td>AMP</td>
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10⁻⁴ M. 3-Pyridinealdehyde-AD was capable of replacing NAD⁺ as a substrate, the Kₘ value (6.6 × 10⁻⁴ M) being 14-fold higher than the value for NAD⁺; furthermore, 3-pyridinealdehyde-AD produced substrate inhibition at concentrations above 3 × 10⁻⁴ M.

Pyridine Nucleotide Content of Sarcoma 180 Cells—Enzymatic determination of the NAD⁺ and NADH contents of sarcoma 180 cells revealed 96 ± 16 μg of NAD⁺ and less than 1.5 μg of NADH per g, wet weight, of cells. These findings are slightly lower than those reported for NAD⁺ for other ascites tumor cells (21, 27); a similar low content of NADH has been reported for Ehrlich ascites cells (21), although other neoplastic cell lines contain considerably more NADH (21, 27). If NAD⁺ were uniformly distributed throughout the cell, the concentration of NAD⁺ would be about 2 × 10⁻⁴ M, a concentration which is close to the optimum concentration for IMP dehydrogenase activity and well out of the range of NAD⁺ concentrations that produce significant substrate inhibition of the enzyme. Since strong inhibition of enzymatic activity is produced by NADH, the low level of NADH in these cells also would appear to constitute a favorable environment for IMP dehydrogenase activity.

**DISCUSSION**

Although the IMP dehydrogenase of sarcoma 180 cells is similar to the IMP dehydrogenases obtained from other sources with respect to pH optimum, apparent irreversibility of the reaction, and inhibition by sulfhydryl reagents (11), the enzyme obtained from sarcoma 180 cells differs from the other enzymes in several parameters. Thus, while the apparent Kₘ value for IMP is similar (1.4 to 2.6 × 10⁻⁵ M) for the IMP dehydrogenases of A. aerogenes (1, 12), pea seedlings (4), Ehrlich ascites tumor cells (7), and sarcoma 180 cells, the apparent Kₘ value obtained for NAD⁺ is considerably lower with the IMP dehydrogenase of sarcoma 180 cells (4.6 × 10⁻⁵ M) than with the enzyme from pea seedlings (2.5 × 10⁻⁴ M) (4) or from A. aerogenes (1.1 to 3.8 × 10⁻⁴ M) (1, 12). The activator constant for K⁺ also is lower with the sarcoma 180 enzyme (about 2 × 10⁻⁴ M) than with the IMP dehydrogenase of A. aerogenes (1.6 × 10⁻⁴ M) (1). Furthermore, Na⁺ could not replace K⁺ as an activator of the IMP dehydrogenase of A. aerogenes (1, 12), whereas Na⁺ is a fairly potent activator of the sarcoma 180 enzyme. Thus, compared with the enzyme from bacteria, the IMP dehydrogenase of sarcoma 180 cells has apparent K⁺ values which are one or two orders of magnitude lower for two of its three reactants.

Reactivity Sequence—For the discussion which follows it can be assumed that K⁺ stimulates IMP dehydrogenase activity by direct interaction with the enzyme. It is conceivable, however, that some of the effects of ions such as K⁺ are mediated through nonspecific mechanisms such as dissociation of the enzyme from contaminating proteins.

The findings that the double reciprocal plots of the initial velocities of IMP dehydrogenase activity are linear (below the range of significant substrate inhibition) and that the lines intersect (Fig. 2, A to F) indicate that the enzymatic reaction mechanism involves either ordered addition or rapid equilibrium random addition of the substrates to the enzyme to form active enzyme-substrate complexes. The results of the product inhibition experiments are inconsistent with a rapid equilibrium random addition mechanism because with such a mechanism each product would be expected to compete with each substrate for binding to the free enzyme (22); the data in Fig. 3A show noncompetitive inhibition by NADH. Ordered addition reaction mechanisms are, on the other hand, adequate to explain the experimental findings if one assumes that IMP is the first reactant to bind to the enzyme and that XMP is the last product to be released. Mechanisms in which K⁺ binds to the free enzyme to produce activation are consistent with the initial velocity studies but are inconsistent with the product inhibition study which shows noncompetitive inhibition by XMP with K⁺ as the variable reactant (Fig. 3F). Thus the data are consistent with mechanisms in which IMP binds to the free enzyme and K⁺ and NAD⁺ are bound subsequently; analyses of the initial velocity and product inhibition kinetics do not allow a definite assignment of the order of addition of K⁺ and NAD⁺.

With regard to inhibition of the enzyme by its substrates, NAD⁺ has not been reported to be an inhibitor of IMP dehydrogenase from other sources, although a slight inhibition by a relatively high concentration of NAD⁺ is detectable in the data obtained with the IMP dehydrogenase of pigeon liver (6). With the enzyme from sarcoma 180, the substrate inhibition produced by high concentrations of NAD⁺ appears to be relatively specific since at similar concentrations several other nucleotides did not produce inhibition. Thus, only dinucleotides were found to be significantly inhibitory at concentrations below 10⁻⁴ M; molecules which resembled portions of the NAD⁺ molecule were inhibitory only at much greater concentrations; it would appear, therefore, that the intact NAD⁺ molecule is required for the formation of an inhibitory complex with the enzyme. Formation of an abortive complex between the enzyme and NAD⁺ should produce inhibition which is competitive with IMP as the variable reactant (28); the data in Fig. 5A show, however, noncompetitive inhibition. With the method of King and Altman (29) to derive the appropriate equations, it was determined that formation of abortive complexes between the enzyme, NAD⁺, and XMP can result in noncompetitive inhibition with IMP as variable substrate. Thus, the findings are consistent with the possibility that the inhibition produced by high concentrations of NAD⁺ involves formation of an enzyme-XMP-NAD⁺ complex.

Studies of inhibition of the activity of IMP dehydrogenase of sarcoma 180 ascites tumor cells by purine nucleotide analogues...
show that IMP and its analogues can, at low concentrations, freely interact with a catalytically important site on IMP dehydrogenase in the absence of NAD+, and that the presence of NAD+ does not appear to have a significant effect on this interaction (18). Hampton and Nomura (12) have recently described similar results with the IMP dehydrogenase of A. aerogenes. Both of these studies corroborate the suggestion that IMP is bound to the enzyme to form an enzyme-substrate complex prior to the binding of NAD+.

An ordered addition mechanism with IMP as the first ligand is consistent with all of the available data from investigations of the kinetics of inhibition by products, substrate, and purine nucleotide analogues. A more complicated mechanism may eventually be required for consideration of the kinetic role of the source of the hydroxyl group and the mechanisms of the inhibition produced by high concentrations of IMP and K+. IMP dehydrogenase, however, appears to differ from several other dehydrogenases which have been shown to bind the pyridine nucleotide prior to the attachment of other substrates (30).

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