EFFECT OF ADENINE NUCLEOTIDES ON REACTIONS INVOLVING TRIPHOSPHOPYRIDINE NUCLEOTIDE*

by

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TPN has been found to contain a monoester phosphate grouping in the 2′-position of the adenylic acid ribose[1]. Some information relating to the significance of the monoester group in TPN has been obtained from studies on the pyridine nucleotide transhydrogenase from *Pseudomonas fluorescens*[2]. These studies suggested that 2′-adenylic acid by competing with TPN could activate certain electron transfer reactions catalyzed by the *Pseudomonas* enzyme. In the present paper, data will be presented showing that 2′ adenylic acid can act as a competitive inhibitor of TPN linked systems, whereas this nucleotide has no effect on DPN specific dehydrogenases.

MATERIALS AND METHOD

The TPN-*iso*citrate dehydrogenase was obtained from pig heart by the method of GRAFFLIN AND OCHOA[3]. The phosphogluconic acid dehydrogenase was the yeast preparation of HORECKER AND SMYRNIOTIS[4]. Glucose-6-phosphate dehydrogenase (Zwischenferment) was prepared by the procedure of KORNBERG[5]. The TPN-glutathione reductase was the enzyme of MAPSON AND GODDARD[6] and was prepared from peas as described previously[7]. The cytochrome *c* reductase was the liver enzyme of HORECKER[8]***. The yeast extract used in these experiments was prepared from brewers yeast by the method of KORNBERG AND PRICER[9]; the source of the "malic enzyme" was an extract from an acetone powder of pigeon liver[10]. Yeast alcohol dehydrogenase and heart lactic dehydrogenase were obtained by the procedure of RACKER[11] and STRAUB respectively[12].

The TPN and DPN preparations were obtained from the Sigma Chemical Co. and were approximately 80% pure. TPNH, DPNH and desamino TPN were prepared as outlined previously[13,14,7]. 5′-adenylic acid was obtained from the Ernst Bischoff Co., Inc. The 2′ and 3′-adenylic acids were preparations supplied by the Schwarz Laboratories. Cyclic 2-3-adenylic acid was prepared by the method of BROWN AND TODD[15]. The various inosinic isomers were prepared by the procedure described by SHUSTER AND KAPLAN[16].

2′-Phospho-adenosinediphosphoribose (P-ADPR) was prepared by cleaving TPN at the nicotinamide-ribose bond with *Neurospora* DPNase[17]. After the reaction was complete, the mixture was made acid to congo red with dilute nitric acid and the P-ADPR precipitated with five volumes of cold acetone. The product, after washing with acetone and ether, gave a molar ratio of phosphate to ribose to adenine of 3:2:1. 22 mg of P-ADPR were obtained by this method from 50 mg of TPN.

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The following abbreviations will be used: TPN, triphosphopyridine nucleotide; DPN, diphosphopyridine nucleotide; TPNH and DPNH, the corresponding reduced nucleotides; AMP, adenylic acid; IMP, inosinic acid; ADPR, adenosine diphosphate ribose; P-ADPR, phospho adenosinediphosphate ribose; and OAA, oxaloacetate.

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RESULTS

Studies with the TPN cytochrome c reductase

In the course of experiments on the reductase, it was observed that the oxidized form of TPN would inhibit the reduction of cytochrome *c* by TPNH. This inhibition is illustrated in Table I; the inhibition was found to be competitive as determined by the LINEWEAVER-BURK plot[18] in Fig. 1.

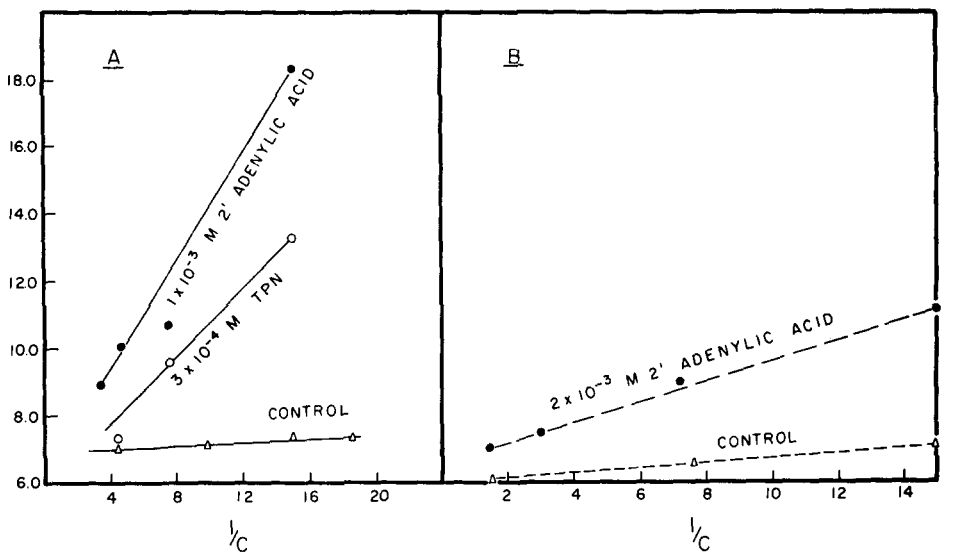

Fig. 1. Competitive inhibition of cytochrome *c* reductase and TPN *iso*citric dehydrogenase by nucleotides. (A) Lineweaver-Burk plot of TPN and 2′ AMP on liver cytochrome *c* reductase, (C = TPNH molar concentration × 1000) (V = velocity from 15 seconds to 180 seconds after start of reaction). Conditions same as in Table I. (B) $1/V \times 1/C$ plot of 2′ AMP inhibition of TPN *iso*citric dehydrogenase. (C = TPN concentration molar × 1000), V = velocity from 30 seconds to 120 after initiation of reaction). Conditions as in Table IV.

TABLE I

EFFECT OF VARIOUS NUCLEOTIDES ON INHIBITION OF TPN-CYTOCHROME *c* REDUCTASE

Reaction mixtures contained 0.05 ml of a 5% cytochrome *c* solution, 0.08 *M* K_2HPO_4, 0.04 micromoles TPNH. Total volume 3 ml. Reaction started by addition of 200 γ cytochrome *c* reductase. Rate of reduction of cytochrome *c* measured by change at 550 mμ between 15 and 180 seconds after addition of enzyme.

Nucleotide added	*Final concentration*	*Per cent inhibition*
TPN	$2.5 \cdot 10^{-5}$	36
TPN	$7 \cdot 10^{-5}$	46
TPN	$1.4 \cdot 10^{-4}$	81
P-ADPR	$1.4 \cdot 10^{-4}$	71
DPN	$1.4 \cdot 10^{-3}$	0
A DPR	$1.4 \cdot 10^{-3}$	0
desamino TPN	$1.0 \cdot 10^{-4}$	13
2′ AMP	$1.2 \cdot 10^{-3}$	73
3′ AMP	$1.2 \cdot 10^{-3}$	0
5′ AMP	$1.2 \cdot 10^{-3}$	8
2′ IMP	$1.0 \cdot 10^{-3}$	12
3′ IMP	$1.5 \cdot 10^{-3}$	7

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Table I lists the effect of other nucleotides on the reductase. DPN and ADPR, in concentrations fifty times higher than that found effective with TPN, do not inhibit this reaction. However, P-ADPR (TPN with the nicotinamide removed) is almost as potent an inhibitor as TPN. Desamino TPN has little inhibitory action on the cytochrome *c* reductase. This finding might be expected since the reduced form of the deaminated nucleotide reacts much more slowly than TPNH in this reaction.

2″ AMP also acts as a competitive inhibitor of the reaction (see Table I and Fig. 1). 5′ AMP and 3′ AMP have little or no effect on the reduction of cytochrome *c*. The inosinic acid isomers also do not compete with TPNH in the reaction.

Inhibition of Zwischenferment by adenylic acid isomers

Table II shows that 2″ adenylic acid is a much more effective inhibitor of the Zwischenferment of yeast than is either of the other isomers of adenylic acid. The inhibition by the 2′ adenylic was found to be competitive.

TABLE II

INHIBITION OF ZWISCHENFERMENT BY VARIOUS ADENYLIC ACID ISOMERS

Reaction mixtures contained 0.01 *M* glycylglycine buffer, pH 6.56, 10 micromoles $MgCl_2$, 0.1 ml of a 1/10 dilution of the Zwischenferment preparation[5], 0.25 micromoles TPN, in a total volume of 3 ml. Reaction started by addition of 10 micromoles glucose-6-phosphate. Rate measured by change in optical density at 340 mμ between 15 and 60 seconds after addition of substrate.

Nucleotide	*Final concentration*	*Per cent inhibition*
2′ AMP	$3 \cdot 10^{-3}$	72
2′ AMP	$6 \cdot 10^{-3}$	91
3′ AMP	$3 \cdot 10^{-3}$	15
3′ AMP	$6 \cdot 10^{-3}$	24
5′ AMP	$6 \cdot 10^{-3}$	24

Studies with phosphogluconic acid dehydrogenase

Phosphogluconic acid dehydrogenase is generally regarded as a TPN specific enzyme. However, it was found that when high concentrations of DPN are used some reaction between DPN and the dehydrogenase system occurs. This reaction of DPN with the phosphogluconate system is stimulated by cysteine. This stimulation takes place both with the purified and the crude enzyme. Stimulation is illustrated in Fig. 2. Activation with cysteine when TPN is the pyridine nucleotide has also been observed.

2′ AMP inhibits the reduction of both TPN and DPN which is promoted by the phosphogluconic acid dehydrogenase. The inhibition is much more pronounced when DPN is the oxidizing agent as demonstrated in Table III. This occurs when the level of DPN is 20 times higher than TPN. The data suggest that the affinity of the TPN for the enzyme is considerably greater than that of DPN, and because of this difference in affinity the 2′ AMP is more inhibitory when DPN is the coenzyme. However, the inhibition produced by 2′ AMP suggests that the reaction with DPN is catalyzed by the same protein that reacts with TPN.

Effect of adenylic acids on isocitric dehydrogenases

Table IV presents the effect of nucleotides on the TPN *iso*citric dehydrogenase of pig heart. 2′ AMP is a potent inhibitor of the reaction; the inhibition is competitive as

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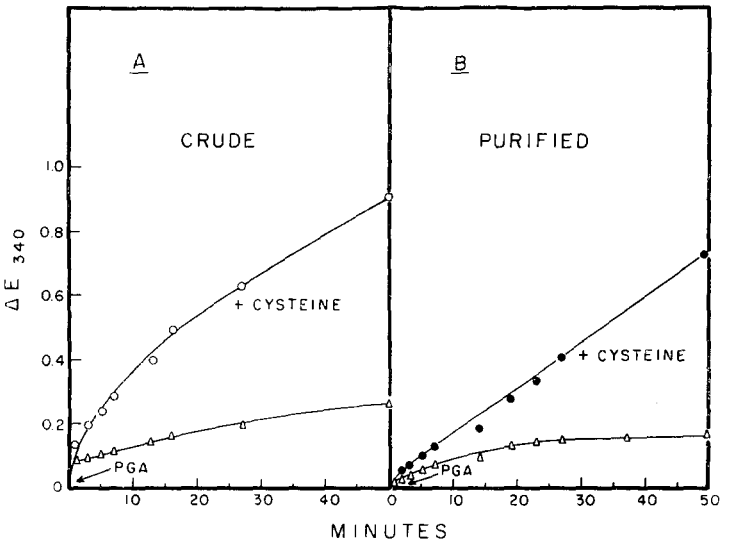

Fig. 2. Cysteine activation of phosphogluconic acid dehydrogenase. Reaction mixture contained 3.5 mg DPN, 5 μmoles phosphogluconic acid, 10 micromoles cysteine, 0.08 M phosphate pH 7.5, total volume 3 ml. 0.1 ml crude extract, and 0.1 ml of a 1/20 dilution of the purified enzyme was used respectively. Reaction started by addition of phosphogluconate.

TABLE III

EFFECT OF 2′-ADENYLIC ACID ON PHOSPHOGLUCONIC ACID DEHYDROGENASE

All samples contained 0.05 M phosphate (pH 7.5), 10 micromoles cysteine and 0.2 ml of a 1 to 20 dilution of enzyme. Reaction initiated with 10 micromoles phosphogluconate. Rate equals optical density change × 1000 at 340 mμ from 15 to 120 seconds after start of reaction.

Pyridine nucleotide added	*AMP* $2 \cdot 10^{-3}$ *M*	*Rate*	*Per cent inhibition*
TPN $6 \cdot 10^{-5}$	–	192	–
TPN $6 \cdot 10^{-5}$	+	104	46
DPN $1.3 \cdot 10^{-3}$	–	32	–
DPN $1.3 \cdot 10^{-3}$	+	4	87

TABLE IV

EFFECT OF VARIOUS NUCLEOTIDES ON THE TPN *iso*CITRIC DEHYDROGENASE OF PIG HEART

Reaction mixture contained 0.1 M phosphate (pH 7.5), 0.25 micromoles TPN, 0.1 ml of a 1/20 dilution of enzyme[5]. Total volume 3 ml. Reaction started with 10 micromoles *iso*citrate. Rate represents change in optical density × 1000 at 340 mμ between 1 and 3 minutes after start of reaction.

Nucleotide added $2 \cdot 10^{-3}$ *M*	*Rate*	*Per cent inhibition*
0	94	–
2′ AMP	15	84
3′ AMP	62	34
5′ AMP	85	9
Cyclic AMP	89	6
2′ IMP	94	0

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illustrated in Fig. 1B. Some inhibition is also observed with the 3′ AMP, whereas 5′ AMP has practically no effect. Although both the 2′ and 3′ adenylates inhibit the reaction, the 2–3 cyclic AMP is without effect. It appears from these studies that the monoester group is essential for the inhibition and that the 2′ AMP is a much better inhibitor than either the 3′ or 5′ isomers. 2′ IMP does not inhibit the reaction. The fact that this nucleotide is not active may be correlated with the finding that desamino TPN is completely inactive in the *iso*citrate reaction.

Magnesium ion has only a slight stimulatory effect on the rate of reduction of TPN by *iso*citrate. However, as shown in Table V, the presence of magnesium considerably decreases the inhibition exerted by the adenylic acids. This is particularly significant with 2′ AMP, where the rate is increased six fold by the presence of magnesium.

TABLE V

EFFECT OF MAGNESIUM ION ON INHIBITION OF *iso*CITRIC ACID DEHYDROGENASE BY ADENYLIC ACID ISOMERS

Conditions as in Table IV

Nucleotide added $6 \cdot 10^{-3}$ *M*	*Mg ion* $3 \cdot 10^{-2}$ *M*	*Rate*	*Per cent control rate*
0	0	109	—
2′ AMP	0	12	11
3′ AMP	0	39	36
5′ AMP	0	75	70
0	+	122	111
2′ AMP	+	73	68
3′ AMP	+	90	83
5′ AMP	+	108	100

KORNBERG AND PRICER[8] have reported two *iso*citric dehydrogenases in yeast. One is specific for TPN whereas the second reacts only with DPN. The purified TPN *iso*citric dehydrogenase from yeast was found to be inhibited by 2′ AMP. However, the DPN enzyme was not affected by the mononucleotide. An indication that two distinct *iso*citrate enzymes exist in yeast can be obtained from the effect of 2′ AMP on crude extracts. As can be seen from Fig. 3, 2′ AMP inhibits only the reduction of TPN and not of DPN in such extracts. This can be contrasted to the finding with the phosphogluconate dehydrogenase where the reaction with either TPN or DPN is inhibited by the 2′ AMP.

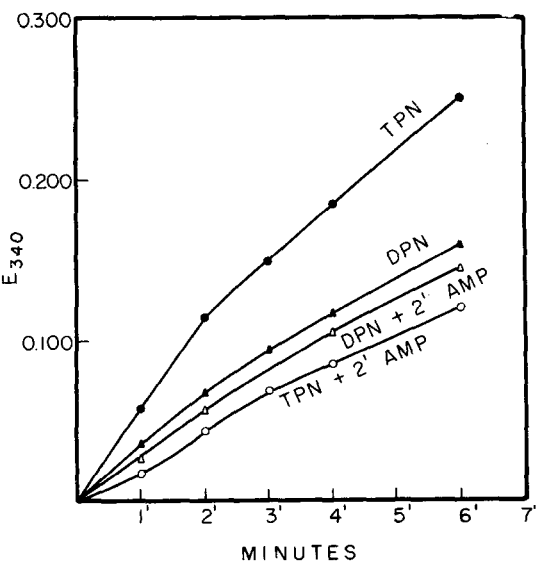

Fig. 3. Effect of 2′ AMP on TPN and DPN *iso*citric dehydrogenases of yeast. All reaction mixtures contained crude extract 0.4 ml, 0.1 *M* phosphate, pH 7.0, 2.1 ml, DPN, or TPN 0.3 micromoles, $MgCl_2$ 20 micromoles; 2 micromoles 5′ AMP, and 10 micromoles 2′ AMP when added. Total volume 3 ml. Reaction initiated with 10 micromoles of *iso*citrate.

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During the course of the above experiments, it was noted that some of our crude pig heart extracts could promote the reduction of DPN by *iso*citrate. This reduction took place at a much slower rate than with TPN. It was noted as in the case of the yeast extract that 2′ AMP would inhibit the TPN reduction but was without effect on the course of the DPN reaction. This suggested that two distinct *iso*citrate oxidizing enzymes were also present in pig heart*, and the following observation supports this view. It was found by ammonium sulfate fractionation, that a preparation could be obtained which would only catalyse DPN reduction by *iso*citrate. 2′ AMP had no effect on this partially purified fraction. Fig. 4 illustrated the difference in behavior of the DPN-linked *iso*citric and phosphogluconic dehydrogenase systems. With the former, which is DPN-specific, 2′ AMP does not inhibit the reaction appreciably, whereas with the latter, which is non-specific and primarily a TPN enzyme, 2′ AMP strongly inhibits the reduction of DPN.

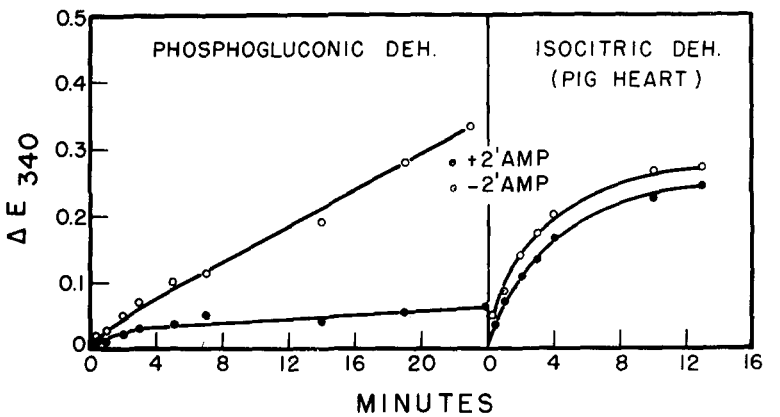

Fig. 4. Comparison of 2′ AMP action on phosphogluconic acid, dehydrogenase from yeast with DPN *iso*citric dehydrogenase from pig heart. For phosphogluconic dehydrogenase test, see Table III. The DPN *iso*citrate test contained 0.5 micromoles DPN, 5 micromoles 5′ AMP, phosphate pH 7.0 *M*/10 2.0 ml, 10 micromoles $MgCl_2$, 0.2 ml pig heart extract. Total volume 3 ml. Reaction initiated with 10 micromoles *iso*citrate. 10 micromoles of 2′ AMP added in each case.

Studies with the "malic enzyme" of pigeon liver

The TPN specific "malic enzyme" is also inhibited by 2′ AMP (Table VI). This inhibition was found to be competitive with TPN. Magnesium ion, as was found with the *iso*citrate system, exerted considerable protection against the effect of the 2′ nucleotide. Manganese also was active in decreasing the inhibition.

The effect of 2′ and 3′ adenylic acids on oxaloacetate carboxylase of pigeon liver

TPN has been reported to stimulate the decarboxylation of OAA by pigeon liver extracts[19,20]. It was therefore thought of interest to test the activity of the adenylic isomers on this decarboxylation. Table VII summarizes the results of such studies, and also confirms the marked TPN stimulation of the reaction. Neither 2′ nor 3′ adenylic acid affects the decarboxylation of OAA in the absence of TPN or metal (Expt. 1 A). However, in the presence of TPN and metal, inhibition by 2′ AMP results; no inhibition

* Since the completion of this work, a much more detailed and complete report on occurrence of DPN *iso*citric dehydrogenase in animal tissues has been published by PLAUT AND SUNG[19]. Our results confirm the existence of this enzyme in pig heart. It is of interest to note that addition of cyanide ion at time increased the activity of the pig heart enzyme.

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TABLE VI

EFFECT OF METAL IONS ON THE INHIBITION OF THE "MALIC ENZYME" BY 2′ AMP

Reaction mixtures all contained $M/10$ phosphate (pH 7.5), 0.2 μmoles TPN and 0.05 ml of crude pigeon liver extract. Reaction initiated by addition of 20 micromoles of d l malate. Other additions as given in Table. Total volume 3 ml. Rate equals optical density change × 1000 at 340 mμ, 15 seconds to 120 seconds after addition of the malate.

Metal added µmoles	*2′ AMP µmoles added*	*Rate*	*Inhibition per cent*
0	0	144	—
0	10	63	45
0	20	12	89
10 ($MgCl_2$)	0	159	—
10 ($MgCl_2$)	10	138	13
10 ($MgCl_2$)	20	96	30
0.3 ($MnCl_2$)	0	131	—
0.3 ($MnCl_2$)	20	98	25

TABLE VII

EFFECT OF NUCLEOTIDES ON DECARBOXYLATION OF OAA

Main compartment of Warburg vessel contained 30 micromoles of OAA, plus additions shown in Table, in 0.1 M acetate buffer pH 0.1 M NaAc. Final total volume 3 ml. After 3′ equilibration, 0.3 ml of pigeon liver extract was tipped in from sidearm. Temperature 30°. CO_2 production measured from 2 to 15 minutes after addition of enzyme. All values corrected for non-enzymic decarboxylation.

TPN added µmoles	*$MnCl_2$ added µmoles*	*2′ AMP added µmoles*	*3′ AMP added µmoles*	*CO_2 produced µl*	*Inhibition per cent*
Expt. 1 A					
0	0	0	0	46	—
0	0	0	0	46	—
0	0	40	0	50	0
0	0	0	40	43	7
Expt. 1 B					
0.2	3	0	0	150	—
0.2	3	40	0	88	42
0.2	3	0	40	146	3
Expt. 2 A					
0	3	0	0	67	—
0	3	60	0	28	60
0	3	0	60	36	48
Expt. 2 B					
0.2	3	0	0	135	—
0.2	3	60	0	69	49
0.2	3	0	60	127	5

under the same conditions takes place with 3′ AMP (Expt. 1 B). In the presence of Mn but in the absence of TPN, both mononucleotides inhibit (Expt. 2 A). However, addition of TPN abolishes the inhibition produced by the 3′ AMP (Expt. 1 B and 2 B). 5′ AMP has no effect under any conditions on the CO_2 release from OAA. The significance of the above experiments will be presented in the discussion.

Inhibition of TPNH glutathione reductase of peas by various nucleotides

It would appear from the data presented above that 2′ AMP can act as an inhibitor in all TPN specific systems. The inhibition, however, does not occur with the glutathione

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TABLE VIII

INHIBITION OF TPNH-GLUTATHIONE REDUCTASE BY VARIOUS NUCLEOTIDES

Reaction mixtures contained 0.3 micromoles TPNH, $M/10$ phosphate (pH 7.5), 0.1 ml enzyme. Reaction started with 10 micromoles oxidized glutathione. Rate measured by change in optical density at 340 mμ between 15 and 120 seconds after beginning of reaction. One micromole nucleotide added in each case.

Nucleotide	*Per cent inhibition*
TPN	69.0
DPN	52.0
2′ AMP	10.5
P-ADPR	4.9
ADPR	0
3′ AMP	6.0
5′ AMP	11.0
desamino TPN	5.0
NMN	17.0
NMN + 2′ AMP	34.6

reductase (Table VIII). Both TPN and DPN will act as inhibitors of this reductase. P-ADPR, which is almost as potent an inhibitor of the cytochrome reductase as TPN, (see Table I) has very little effect on the glutathione system. The fact that DPN is active and P-ADPR is not suggests that the nicotinamide moiety of the TPNH may be of more significance in the mechanism of action of the pea enzyme.

Effect of adenylic acids on crystalline yeast alcohol dehydrogenase

All three adenylic acid isomers at a concentration of 0.01 M were found to be without influence on the rate of the DPN-specific yeast alcohol dehydrogenase reaction.

Effect of 2′ AMP on lactic acid dehydrogenase

Lactic acid dehydrogenase reacts with TPN at approximately 1/500 the rate of DPN. However, the oxidation of TPNH by pyruvate is not affected by 0.01 M 2′ AMP. This further indicates that the inhibition produced by this mononucleotide is restricted to TPN specific enzymes.

DISCUSSION

WILLIAMS[22] has reported the inhibition of some DPN dehydrogenases by adenine, adenosine, and ATP. The concentrations required for inhibition were much higher than that obtained in this paper with 2′ AMP on the TPN systems. Furthermore, the effect on the TPN enzymes seems to be more specific since the inhibition occurs largely with 2′ AMP and to a much lesser degree with the other isomers of adenylic acid.

The data presented in this paper strongly suggest that the monoester phosphate group of TPN plays an important role in the binding of the coenzyme to the apoenzyme. This is illustrated in the experiments with the TPNH cytochrome *c* reductase in which TPN and P-ADPR are competitive inhibitors of TPNH whereas DPN and ADPR are without effect on the reaction of the reductase. The inhibition of the cytochrome reduction by 2′ AMP and the inactivity of the other adenylic acid isomers indicate that the 2′ position is intimately concerned with the linkage of TPN to the enzyme. The importance of the 2′ phosphate grouping is further shown by the inactivity of the synthetic 3′ TPNH to function as an electron donor[23].

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Consideration of the activities of the various mononucleotides on the TPN isocitric dehydrogenase are of interest in attempting to interpret the function of the 2′ phosphate grouping of TPN. The 2′ AMP inhibits the reaction to a much greater degree than the 3′ or 5′ isomers. The inhibition by the 3′ nucleotide is larger than that found with the 5′ compound. The fact that the cyclic 2′–3′ AMP has no inhibitory action indicates that the additional phosphate of TPN must be in the monoester form for activity. It is of significance that the 2′ IMP is inactive as an inhibitor of the *iso*citrate dehydrogenase, since desamino TPN is inactive in the reaction. This relationship strengthens the approach used in this paper in gaining information as to the enzyme-binding function of the various groupings in the TPN molecule.

The possibility exists that the monoester group in the 2′ position is involved in a bond to the amino group of the purine. Indirect evidence for such a binding comes from unpublished experiments with the takadiastase deaminase which has previously been found to deaminate 3′ and 5′ adenylic acids but not the 2′ isomer[24]. It has now been found that 2′, 3′ cyclic AMP is deaminated by the enzyme. This suggests that the 2′ phosphate may interact somehow with the amino group, and that this interaction may prevent the action of the deaminase. There is no evidence available as yet as to how either the amino group or the 2′ phosphate group of TPN interact chemically with the various enzymes. However, the evidence reported in this paper indicates that the monoester phosphate of TPN is intimately concerned with the linkage of the coenzyme to the enzyme.

The addition of Mg^{++} produces an interesting effect in lowering the inhibition by the adenylic nucleotides of the *iso*citric dehydrogenase. This is particularly so since the metal has little influence on increasing the rate of the reaction. One possibility for the protection by Mg may be that it increases the binding of TPN to the protein and that the enzyme-metallo-adenylate complex is much more dissociable than the corresponding TPN complex. From the results in Table VI, it is evident that both Mg and Mn cause similar effects on lowering the inhibition of the "malic enzyme" induced by 2′ AMP.

A relationship between the metal and nucleotide also appears to exist as indicated by the studies with OAA decarboxylase. Our results confirm the findings of others[20,21] on the TPN stimulated OAA decarboxylation of pigeon liver. It is of interest to note that metal appears to be essential for the inhibition by the 2′ or 3′ AMP on the OAA decarboxylase. The presence of TPN seems to completely overcome the inhibition produced by the 3′ AMP, but only partly prevents the inhibition of 2′ AMP (see Table VII, Expts. 1 B and 2 A). In the absence of TPN, but in the presence of metal, the 3′ AMP inhibits even though the compound appears to have a low affinity for the decarboxylase. It should be emphasized that TPN must have a considerable greater affinity for the enzyme than does 2′ AMP, for relatively large levels of the mononucleotide are required to produce inhibition. Because crude pigeon liver extracts were used in our experiments, it is possible that more than one decarboxylase was involved. One of these enzymes may be specifically TPN activated.

The results of this study suggest that when both DPN and TPN are active in a reaction, it may be possible to ascertain by the inhibitory action of 2′ AMP whether one or two enzymes are involved. This is illustrated by the observation that 2′ AMP does not inhibit DPN *iso*citric dehydrogenases, but will inhibit the reduction of DPN catalysed by the phosphogluconic acid dehydrogenase which is primarily a TPN enzyme. On the other hand, 2′ AMP does not inhibit the oxidation of TPNH by lactic dehydrogenase, which

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can be considered a DPN enzyme, since TPN reacts in this system at approximately 1/500 the rate of DPN.

SUMMARY

Oxidized TPN, and P-ADPR have been found to be competitive inhibitors of the TPNH-cytochrome *c* reductase of horse liver. 2′ AMP also competitively inhibits, at somewhat higher concentrations. DPN, ADPR, desamino TPN, 3′ AMP, 5′ AMP, 2′ IMP, and 3′ IMP produce very slight or no inhibition.

Glucose-6-phosphate dehydrogenase (Zwischenferment) is inhibited by a concentration of $3 \cdot 10^{-3}$ *M* 2′ AMP, whereas little inhibition results with either 3′ or 5′ AMP.

It has been found that DPN when present at high concentrations will react with the yeast phosphogluconic acid dehydrogenase system. Cysteine has been found to activate this enzyme particularly when DPN is used as coenzyme. 2′ AMP inhibits the reduction of both DPN and TPN; the inhibition is much greater when DPN is the electron acceptor.

2′ AMP competitively inhibits the TPN *iso*citric dehydrogenase from pig heart. 3′ AMP also inhibits but to a lesser degree. The cyclic 2′ 3′ AMP, 5′ AMP, and 2′ IMP do not inhibit. 2′ AMP has been found to inhibit the TPN specific *iso*citrate dehydrogenases from pig heart and brewers yeast, but is without effect on the activity of the DPN specific *iso*citric dehydrogenases from these sources.

Magnesium ions cause a considerable decrease in the inhibition produced by 2′ AMP on the TPN *iso*citrate system. A similar effect of Mg as well as Mn is observed with the "malic enzyme" from pigeon liver.

Both 2′ and 3′ AMP inhibits OAA decarboxylation in pigeon liver; this inhibition requires the addition of metal. TPN stimulation of OAA decarboxylation has been confirmed. TPN reverses the inhibition produced by 3′ AMP, but is less effective against 2′ AMP.

None of the adenylic acid isomers at a concentration of 0.01 *M* influence the yeast alcohol dehydrogenase reaction. 2′ AMP also does not inhibit the oxidation of TPNH by lactic dehydrogenase. The only TPN specific enzyme effect which has been found not to be inhibited by 2′ AMP is the glutathione reductase from peas.

The results are discussed with respect to the significance of the monoester phosphate group of TPN in the reactions of this coenzyme.

RÉSUMÉ

Le TPN oxydé et le P-ADPR sont des inhibiteurs compétitifs de la TPNH-cytochrome *c* réductase du foie de cheval. Le 2′ AMP inhibe aussi compétitivement, à des concentrations un peu plus élevées. Le DPN, l'ADPR, le désamino-TPN, le 3′ AMP, le 5′ AMP, le 2′ IMP et le 3′ IMP inhibent très peu ou pas du tout.

La glucose-6-phosphate deshydrogénase (Zwischenferment) est inhibée par une concentration de $3 \cdot 10^{-3}$ *M* en 2′ AMP, tandis que le 3′ ou le 5′ AMP produisent une inhibition très faible.

Si le DPN est présent à concentration élevée, il réagit avec le système phosphogluconique déshydrogénase de la levure. La cystéine active cet enzyme particulièrement quand le DPN est employé comme coenzyme. Le 2′ AMP inhibe la réduction à la fois du DPN et du TPN; l'inhibition est beaucoup plus grande quand le DPN est l'accepteur d'électrons. Le 2′ AMP inhibe compétitivement la TPN-*iso*citrique deshydrogénase du coeur du porc. Le 3′ AMP l'inhibe également, mais à un degré moindre. Le 2′-3′ AMP cyclique, le 5′ AMP et le 2′ IMP ne l'inhibent pas. Le 2′ AMP inhibe les *iso*citrate déshydrogénases liées au TPN du coeur de porc et de la levure de boulangerie, mais est sans effet sur l'activité des *iso*citrique deshydrogénases de même origine liées au DPN.

Les ions magnésium diminuent considérablement l'inhibition produite par le 2′ AMP sur le système TPN-*iso*citrate. Une action analogue du Mg, ainsi que du Mn, s'observe avec l'"enzyme malique" du foie de pigeon.

Le 2′ et le 3′ AMP inhibent tous les deux la décarboxylation de l'OAA par le foie du pigeon; cette inhibition n'a lieu qu'après addition d'un métal. La stimulation par le TPN de la décarboxylation de l'OAA a été confirmée. Le TPN supprime l'inhibition provoquée par le 3′ AMP, mais est moins efficace contre l'inhibition par le 2′ AMP.

Aucun des isomères de l'acide adénylique, à la concentration 0.01 *M*, n'a d'influence sur l'activité de l'alcool-deshydrogénase de la levure. Le 2′ AMP n'inhibe pas non plus l'oxydation du TPNH par la lactique déshydrogénase. Le seul enzyme lié au TPN qui n'est pas inhibé par le 2′ AMP est la glutathion-réductase des pois.

L'importance du groupe phosphate monoester du TPN dans les réactions de ce coenzyme est discutée à la lumière de ces résultats.

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ZUSAMMENFASSUNG

Es wurde festgestellt, dass oxydiertes TPN und P-ADPR kompetitive Hemmungsfaktoren von TPNH-Cytochrom *c*-Reduktase aus Pferdeleber darstellen. 2′ AMP ist bei einigermassen erhöhten Konzentrationen gleichfalls ein kompetitiver Hemmungsfaktor. DPN, ADPR, Desamino TPN, 3′ AMP, 5′ AMP, 2′ IMP und 3′ IMP verursachen nur sehr wenig oder gar keine Hemmung.

Glukose-6-Phosphat-Dehydrogenase (Zwischenferment) wird durch eine Konzentration von $3 \cdot 10^{-3}$ *M* 2′ AMP gehemmt, während die hemmende Wirkung von 3′- oder 5′ AMP nur sehr gering ist.

Es wird bewiesen, dass DPN in hohen Konzentrationen mit dem Phospho-Glukonsäuredehydrogenasesystem der Hefe reagiert. Es erwies sich, dass dieses Enzym besonders durch Cystein aktiviert wird, wenn man DPN als Coenzym benützt. 2′ AMP hemmt die Reduktion von sowohl DPN als TPN; die Hemmung ist viel bedeutender, wenn DPN den Elektronenannehmer darstellt.

2′ AMP wirkt kompetitiv hemmend auf TPN-*Iso*zitronensäuredehydrogenase aus Schweinsherz. Auch 3′ AMP wirkt hemmend, jedoch in geringerem Masse. Zyklische 2′–3′ AMP, 5′ AMP und 2′ IMP wirken nicht hemmend. Es erwies sich, dass 2′ AMP auf TPN-spezifische *Iso*zitronensäuredehydrogenase aus Schweinsherzen und Brauereihefe hemmend wirkt, jedoch keine Wirkung auf die Aktivität der aus denselben Quellen stammenden DPN-spezifischen *Iso*zitronensäuredehydrogenasen ausübt.

Magnesiumionen verursachen ein bedeutendes Herabsinken der Hemmung des TPN-*Iso*-zitronensäuresystems durch 2′ AMP. Durch Mg und Mn wird eine ähnliche Wirkung auf das "Apfelsäure-Enzym" (malic enzyme) aus Taubenleber ausgeübt.

Sowohl 2′- als 3′ AMP hemmen die Dekarboxylation von OAA in Taubenleber; diese Hemmung benötigt die Hinzufügung von Metall. Die durch TPN verursachte Steigerung der Dekarboxylation von OAA wurde bekräftigt. TPN macht die durch 3′ AMP verursachte Hemmung rückgängig, ist jedoch gegen 2′ AMP weniger wirksam.

Bei einer Konzentration von 0.01 *M* beeinflussen keine der Adenylsäurenisomere die Hefen-Alkoholdehydrogenasenreaktion. 2′ AMP wirkt nicht hemmend auf die Oxydation von TPNH durch Milchsäurendehydrogenase. Glutathionreduktase aus Erbsen ist das einzige TPN-spezifische Enzym, welches durch 2′ AMP nicht gehemmt wird.

Die Ergebnisse werden erörtert, indem die Bedeutung der Monoesterphosphatgruppe von TPN in den Reaktionen dieses Coenzyms in Betracht gezogen wird.

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