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GUANOSINE MONOPHOSPHATE SYNTHETASE FROM EHRLICH ASCITES CELLS

MULTIPLE INHIBITION BY PYROPHOSPHATE AND NUCLEOSIDES

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Summary

GMP synthetase (xanthosine-5′-phosphate : ammonia ligase (AMP-forming), EC 6.3.4.1) from Ehrlich ascites cells was found to be subject to multiple inhibition by its reaction product, PP_i, and some analogs of adenosine. PP_i and the nucleoside (N) inhibitors were also capable of individually inhibiting this enzyme. Under no conditions did the inhibition appear to be irreversible or "pseudoinactivating" in nature. The individual inhibition by PP_i was competitive with respect to ATP (K_I = 0.42 mM). Conversely, in the absence of PP_i, the binding of N was noncompetitive with ATP, but shifted to a competitive pattern when PP_i was present. Furthermore, with the inhibitors in concert, there was an apparent lowering of the K_I values for both inhibitors. This data was consistent with either PP_i functioning to tighten the binding of N at a non-catalytic site (positive cooperativity) or with PP_i actually opening a second binding site for N in addition to the non-catalytic site. Although this study did not distinguish which of these events was occurring, it did reveal that the intensity of the effect of PP_i appeared to be constant. That is, for various N inhibitors with a range of independently determined K_I values from 26 to 1650 μM, the ratio of their K_I values determined in the absence of PP_i to the values determined in the presence of PP_i was always 38 ± 1.

In 1960, Slechta determined that the nucleosides, psicofuranine and decoyenine, exert their antibiotic activities by inhibiting the enzyme GMP synthetase (xanthosine-5′-phosphate : ammonia ligase (AMP-forming), EC 6.3.4.1) *

* GMP synthetase catalyses the following reaction:

$$XMP + ATP + [(Gln + H_2O) \text{ or } (NH_3)] \overset{Mg^{2+}}{\longleftrightarrow} GMP + AMP + PP_i + (Glu)$$

[1]. Subsequent investigations have unraveled the intricate mechanism of inhibition [2—5] and the nucleoside binding specificity [4—5] of the purified enzyme from *Escherichia coli*. Since it has been established that GMP synthetase from mammalian and bacterial sources have many similar catalytic properties [6], it was of interest to determine whether any differences exist either with respect to the mechanism of inhibition or the binding specificity.

In the study reported here, GMP synthetase purified from Ehrlich ascites cells was investigated and found to be subject to inhibition by nucleosides. The mechanism of inhibition and nucleoside binding specificity were determined and compared with the corresponding aspects of the bacterial enzyme.

Experimental Procedure

Materials

Biochemicals. GMP synthetase was isolated from Ehrlich ascites cells according to the method described earlier [6]. The sources for the nucleosides are listed in Table II. The elemental analysis of the nucleosides synthesized in these laboratories agreed within 0.5% of the theoretical values. The sources of the other reagents used in these studies are reported elsewhere [7].

Methods

GMP synthetase assay. The radiochemical assay previously described [6,7] was used in these studies. The amount of [^{14}C]GMP derived from [^{14}C]xanthosine 5'-phosphate (XMP) was determined following acid hydrolysis and base analysis. The standard reaction mixture contained 0.25 mM [^{14}C]XMP (approx. 1.2—2.4 μCi/μmol), 2.0 mM glutamine, 10 mM $MgSO_4$, and 75 mM Tris · HCl, pH 7.6. The concentrations of ATP and other reagents are indicated in the table and figure legends. An 'ATP-regenerating system' was used in order to ensure that all the adenine nucleotides were in the triphosphate form and to maintain a constant concentration of ATP throughout the reaction. The final concentration of the compounds of this regenerating system were: myokinase, 3.6 international units/ml; pyruvate kinase, 2.0 international units/ml; phospho*enol*pyruvate, 0.4 mM. Unless specified otherwise, the reactions were initiated with GMP synthetase, performed at 37° C in a total volume of 60 μl, and stopped with acid as previously described [7]. Velocities are expressed as nmol of GMP produced/min per ml enzyme and represent the initial, linear portion of the reaction rates. The data were analyzed by direct fit to the Michaelis-Menten equation according to the method of Wilkinson [8].

Results

Mechanism of inhibition

Inhibition by nucleosides. Initial studies with GMP synthetase from Ehrlich ascites cells clearly indicated that this enzyme was subject to inhibition by many of the same nucleosides that were reported to inhibit the enzyme from *E. coli* [1,4,5]. However, during the present experiments, it was observed that these nucleosides were capable of exerting their inhibition in the absence of

PP_i *. This is in sharp contrast to the observations with the bacterial GMP synthetase where the binding of the nucleoside inhibitors was strictly dependent upon the preceding binding of the reaction product, PP_i [2—5].

A detailed investigation of the nature of this inhibition was performed with 5′-deoxyadenosine. The results of initial velocity experiments with ATP as the variable substrate are summarized in Table I. It can be seen that 5′-dAdo was a noncompetitive inhibitor with respect to ATP. Its inhibition constant (K_N) was 0.38 mM. The K_m for ATP was 0.28 mM and is identical to the earlier determination [6]. Similar experiments where the concentration of XMP was varied while that of ATP was fixed at 2.0 mM clearly indicated that the inhibition by 5′-dAdo was not competitive with respect to XMP. However, the data did not permit the distinction between noncompetitive and uncompetitive inhibition (data not shown).

Inhibition by PP_i. The enzymatic animation of XMP occurs with the concomitant hydrolysis of ATP to yield AMP and PP_i [9,10]. The reaction product, PP_i, which was previously shown to inhibit GMP synthetase from *E. coli* [5] and calf thymus [10], was also found to inhibit the enzyme from Ehrlich ascites cells. KF was included in all reactions with PP_i present in order to inhibit the endogenous inorganic pyrophosphatase and thereby protect the PP_i. At the concentration used (6.9 mM), KF inhibits the pyrophosphatase by more than 95% and the GMP synthetase by 15%. The data shown in Table II indicate that PP_i is a competitive inhibitor with respect to ATP. The inhibition constant, K_{PP_i}, was determined to be 0.35 mM while the K_m for ATP was 0.25 mM. The average K_{PP_i} determined from two separate experiments was 0.42 mM. The inhibition of the bacterial enzyme by PP_i was also competitive with respect to ATP with K_{PP_i} and K_m values of 0.48 and 0.33 mM, respectively [5].

Inhibition by nucleosides and PP_i in concert. When the inhibition by nucleosides was studied in the presence of PP_i, a marked enhancement of the nucleoside's inhibitory potency was noted. The data from initial velocity studies of the inhibition by 5′-deoxyadenosine in the presence of 0.10 mM PP_i (K_{PP_i} = 0.42 mM) is shown in Table I. It should be noted the PP_i not only greatly decreased the apparent dissociation constant (i.e. 10-fold) for 5′-deoxyadenosine, but also shifted the pattern of the inhibition from noncompetitive to competitive with respect to ATP.

The initial velocity studies with the enzyme from *E. coli* [4,5] indicated that in the presence of PP_i, these nucleosides also behaved as competitive inhibitors with respect to ATP. However, the double-inhibitor complex formed with the bacterial enzyme was shown to slowly isomerize into a non-dissociating species that existed in equilibrium with the initial complex [4,5]. This isomerization was manifested in a time-dependent (progressive) increase in inhibition that occurred either during the reaction (PP_i present) or during the preincubation of the nucleoside with the enzyme in the presence of PP_i, XMP, and Mg^{2+} [2]. It was, therefore, of interest to examine the enzyme from Ehrlich ascites cells in order to ascertain whether any non-dissociating species were formed. In contrast

* The high levels of inorganic pyrophosphatase present in this enzyme preparation [6] assured the immediate hydrolysis of all endogenously produced PP_i. Furthermore, the phosphate produced from this hydrolysis was found not to inhibit GMP synthetase (tested at a range of 0.1—0.3 mM).

TABLE I

INHIBITION OF GMP SYNTHETASE BY 5′-DEOXYADENOSINE (5′-dAdo)

The reaction vials contained the 'standard reaction mixture', the 'ATP generating system', enzyme, and PP_i and 5′-dAdo as indicated. 6.9 mM KF was included in the vials which contained PP_i. The concentration of ATP varied between 0.1 and 0.4 mM. Each determination consists of 6 or 7 data points. Other details are described in Methods and Results.

Apparent kinetic constants for ATP

PP_i absent (noncompetitive inhibition)				0.1 mM PP_i present (apparent competitive inhibition)			
[5′-dAdo] (mM)	K'_m ± S.E. (mM)	V' ± S.E. (nmol/min per ml enzyme)		[5′-dAdo] (mM)	K'_m ± S.E. (mM)	V' ± S.E. (nmol/min per ml enzyme)	
0.0	0.28 ± 0.02	2.68 ± 0.09		0.0	0.53 ± 0.12	3.22 ± 0.51	
0.20	0.22 ± 0.01	1.31 ± 0.04		0.020	0.79 ± 0.16	3.27 ± 0.49	
0.40	0.27 ± 0.05	1.15 ± 0.11		0.050	0.94 ± 0.25	2.62 ± 0.25	
0.68	0.22 ± 0.05	0.86 ± 0.09					
Inhibition constant (K_N) (mM):			0.37 ± 0.04 (slopes) 0.39 ± 0.04 (intercepts)	Apparent inhibition constant (K_N(app)) (mM):			0.035 ± 0.004

TABLE II

INHIBITION OF GMP SYNTHETASE BY PP_i

The reaction conditions were identical to those of Table I.

Apparent kinetic constants for ATP

5′-dAdo absent (competitive inhibition)			0.1 mM 5′-dAdo present (competitive inhibition)		
$[PP_i]$ (mM)	K'_m ± S.E. (mM)	V' ± S.E. (nmol/min per ml enzyme)	$[PP_i]$ (mM)	K'_m ± S.E. (mM)	V' ± S.E. (nmol/min per ml enzyme)
0.0	0.25 ± 0.01	1.83 ± 0.05	0.0	0.25 ± 0.02	1.68 ± 0.08
0.20	0.30 ± 0.04	1.74 ± 0.13	0.020	0.35 ± 0.08	1.72 ± 0.23
0.40	0.74 ± 0.26	2.40 ± 0.61	0.050	0.45 ± 0.08	1.60 ± 0.18
0.70	1.50 ± 0.42	2.98 ± 0.70			
Inhibition constant (K_{PP_i}) (mM):		0.35 ± 0.04	Apparent inhibition constant (K_{PP_i} (app)) (mM):		0.048 ± 0.003

to the bacterial enzyme, no evidence for the formation of any secondary species was detected with this enzyme. Reactions in the presence of nucleoside inhibitors and PP_i were always linear within the normal zero-order portion of the substrate depletion curve and preincubation of the inhibitors with the enzyme in the presence of PP_i, XMP and Mg^{2+} did not increase the inhibition. This inability of the nucleoside · PP_i double inhibitor complex with this enzyme to isomerize into a non-dissociating species constitutes the second major difference in the mechanisms of inhibition of this and the *E. coli* enzyme.

In Table II, it can be seen that the inhibition by PP_i was also markedly enhanced by 5′-deoxyadenosine. While the nature of the inhibition remained competitive, the K_{PPi} decreased from 0.42 mM to an apparent value of 0.048 mM.

Kinetic analysis. In order to simplify the analysis of the inhibition mechanism, all substrates except ATP were fixed at saturating concentrations relative to their K_m concentrations [6], and the reactions were analyzed with respect to ATP as the variable substrate. The above data for the inhibition by 5′-deoxyadenosine is consistent * with either a "one binding site" or a "two-binding site"

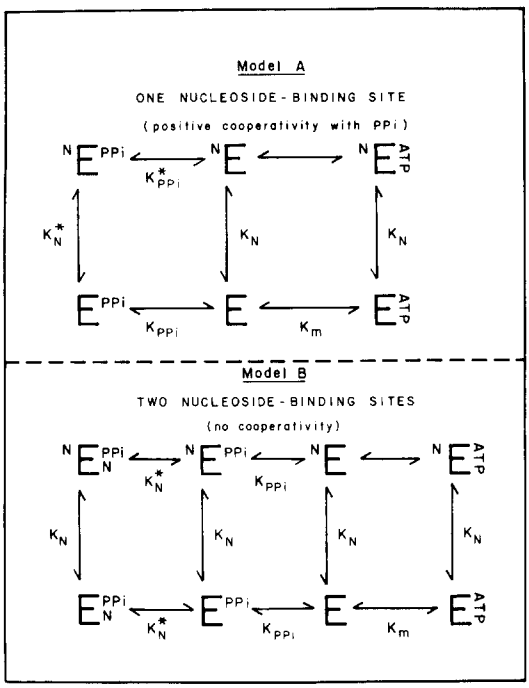

Fig. 1. Proposed mechanisms for the multiple inhibition of GMP synthetase by PP_i and nucleosides. The symbols used are: E, GMP synthetase from Ehrlich ascites cells (the right-hand side indicates the ATP-binding site); N, nucleoside inhibitor; K_m, Michaelis-Menten constant for ATP; K_N, K_N^*, dissociation constants for the reactions shown in the direction of the additions of N; K_{PP_i}, $K_{PP_i}^*$, dissociation constants for reactions shown in the direction of addition of PP_i. The lack of progressive inhibition of this enzyme renders it difficult to establish whether E represents free enzyme or enzyme complexed with XMP. The binding of nucleosides to the enzyme from *E. coli* required the preceding formation of the enzyme · XMP complex [2,3] as well as the binding of PP_i [2—5]. Model A represents random binding of inhibitors with positive cooperativity. Model B represents ordered sequential binding. Details are discussed within the text.

* A comprehensive elucidation of the reaction mechanism of this enzyme may possibly reveal alternative inhibition mechanisms.

mechanism. In both mechanistic models (see Fig. 1) the nucleoside N can bind to a site distinct from the site that ATP or PP_i can occupy. Thus the inhibition by N in the absence of PP_i will be noncompetitive in both models with an inhibition constant of K_N.

A second feature common to both models is that the binding of PP_i in some way decreases the dissociation constant of N from K_N to K_N^*. Since the binding of N with dissociation constant K_N^* is absolutely dependent upon prior binding of PPi and PPi is competitive with respect to ATP, inhibition by N with constant K_N^* will also be competitive. Furthermore, since K_N^* is much less than K_N, under the experimental conditions where K_N^* is being determined (i.e. very low ratios of $[N]/K_N$) the noncompetitive component, K_N, will be undetectable and the inhibition by N in the presence of PP_i will appear competitive.

The "one site" model (Figure 1A) ascribes the effect of PP_i on the binding of N to positive cooperativity, i.e. binding of either N or PP_i decreases the dissociation constant for the second ligand at its original binding site. The rate equation for the initial velocity of this type of inhibited reaction is presented below in its double reciprocal form. Rate equations Eqns. 1 and 2 were derived from the models of Fig. 1 by conventional procedures [12]. All reactions involving the binding of inhibitors were assumed to be at equilibrium. Those involving ATP were assumed to be at a steady-state. Thus, the binding of ATP to the N · E complex could be ignored for the derivations.

$$\frac{1}{v} = \frac{K_m}{V}\left[1 + \frac{[N]}{K_N} + \frac{[PP_i]}{K_{PP_i}}\left(1 + \frac{[N]}{K_N^*}\right)\right]\frac{1}{[ATP]} + \frac{1}{V}\left(1 + \frac{[N]}{K_N}\right) \tag{1}$$

The "two site" model (Fig. 1B) assumes no cooperativity between the inhibitors at their original binding sites, but proposes a second site for the binding of N. The accessibility of this site to N is absolutely dependent upon the formation of the enzyme · PP_i complex. The rate equation for this mechanism is presented below.

$$\frac{1}{v} = \left(1 + \frac{[N]}{K_N}\right)\frac{K_m}{V}\left[1 + \frac{[PP_i]}{K_{PP_i}}\left(1 + \frac{[N]}{K_N^*}\right)\right]\frac{1}{[ATP]} + \frac{1}{V}\left(1 + \frac{[N]}{K_N}\right) \tag{2}$$

Two of the above assertions can be confirmed with the inspection of Eqns. 1 and 2. First, since K_N^* appears only in the expression for the slopes of both equations, inhibition due to N with dissociation constant K_N^* will be competitive; and second, under the conditions where K_N^* is being determined, $[N]/K_N$ approaches zero. Therefore the expression $(1 + [N]/K_N)$ approaches unity and thus inhibition by N in the presence of PP_i will appear purely competitive. Unfortunately, if $(1 + [N]/K_N)$ simplifies to units, Eqns. 1 and 2 become identical. Therefore, the two models cannot be distinguished by initial velocity experiments of this nature. Thus, for 5′-deoxyadenosine, the average value of K_N^* was calculated to be 9.0 μM from the data of Table I (PP_i present) using Eqn. 1 and 10.0 μM using Eqn. 2.

In order to test the internal consistency of the data and the proposed mechanisms, K_{PP_i} was determined from the data of Table II (5′-dAdo present) using the above determined values of K_N^*. The average values for K_{PP_i} were 0.48 mM

and 0.60 mM when determined from Eqns. 1 and 2 respectively. These values were neither significantly different from each other nor from the value determined in the absence of 5′-deoxyadenosine (0.42 mM).

Inhibitor specificity

Initial velocity studies similar to those of Table I were performed with decoyenine and 2-fluoroadenosine (data not shown). The patterns of inhibition for these inhibitors were identical to those for 5′-deoxyadenosine. That is, the inhibition was noncompetitive with respect to ATP in the absence of PP_i and competitive in the presence of PP_i. The inhibition constants are summarized in Table III. If it is assumed that all adenosine analog inhibitors inhibit this enzyme by the same mechanism, then, K_N and K_N^* values could be more easily obtained from Dixon plots [13] of $1/v$ vs. [N] performed in the absence and in the presence of PP_i at a constant concentration of ATP. The negative value of the x-axis intercept of the line determined in the absence of PP_i is equal to K_N [13] while the negative value of the x-axis intercept of the line with PP_i present is equal to:

$$K_N^* \left[\frac{K_{PP_i}}{[PP_i]} \left(1 + \frac{[ATP]}{K_m} \right) + 1 \right] \tag{3}$$

Although Eqns. 1 and 2 gave statistically identical K_N^* values when they were transformed to fit a plot of $1/v$ vs. [N], the preferable transformation was from Eqn. 2 because when it was solved for the expression of the (negative) x-axis intercept (Eqn. 3), the K_N term was absent.

The K_N and K_N^* values determined from this type of Dixon plot for decoyenine and 2-fluoroadenosine were identical to the values obtained from the double reciprocal plots described above. The inhibition constants for inhibitors for which both K_N and K_N^* were determined are presented in Table III. It is of interest that while these inhibitors had K_N values ranging from 26 to 1650 μM, their K_N/K_N^* ratios were 38 ± 1.

The K_N^* values for all the nucleosides studied are presented in Table IV. Apart from those listed in Table III, the K_N^* values were determined from one-line Dixon plots as described above. The respective concentrations of ATP and PP_i were fixed at 0.30 and 0.20 mM for these experiments.

TABLE III

RELATIONSHIP BETWEEN THE INDEPENDENT AND PP_i-FACILITATED BINDING OF NUCLEOSIDES TO GMP SYNTHETASE

Nucleoside	Binding constants (μM)		K_N
	PP_i absent (K_N)	PP_i present (K_N^*)	K_N^*
2-Fluoroadenosine	26	0.66	39
Decoyenine	26	0.68	38
Psicofuranine	51	1.3	39
5′-Deoxyadenosine	380	9.5	40
Tubercidin	1650	49	34

TABLE IV

INHIBITORS OF GMP SYNTHETASE FROM EHRLICH ASCITES CELLS

Source †	Compound	K^*_N(μM)
BW	2-Fluoroadenosine	0.7
UC	Decoyenine	0.7
UC	Psicofuranine	1.3
S	2-Chloroadenosine	5
S	Adenosine **	10
PL	5′-Deoxyadenosine	10
BW	N^6-Allyladenosine	26
BW	8-Azaadenosine **	41
S	N^6-Methyladenosine	44
C	Tubercidin	49
S	6-Hydroxylamino-purine ribonucleoside **	61
BW	*Erythro*-9-(2-hydroxy-3-nonyl)adenine	150
S	Purine ribonucloside	>400
S	Kinetin ribonucleoside	>400
C	Zeatin ribonucleoside	>400
S	N^6,N^6-Dimethyladenosine	
BW	3-(N^6-Adenosyl)-*N*-butylpropionamide	
BW	N^6-(5-Carboxypentyl)-adenosine	
S	N^6-Isopentenyladenosine	
C	N^6-Benzyladenosine	
TM	6-Chloropurine ribonucleoside **	
S	6-Methylpurine ribonucleoside	
S	6-Methoxypurine ribonucleoside	
S	6-Methylmercaptopurine ribonucleoside	
BW	2-Amino-6-methylthiopurine ribonucleoside	
BW	2-Aminoadenosine **	
BW	2-Amino-2′-deoxyadenosine **	>400
BW	2-Methyladenosine	
BW	2-Methylthioadenosine	
TM	8-Bromoadenosine	
S	1-Methyladenosine	
NB	Xanthosine	
PL	Guanosine	
BW	4-Amino-1-(β-D-ribofuranosyl)pyrazolo-[3,4-d]pyrimidine **	
BW	4-Amino-1-(β-D-2′-deoxyribofuranosyl)pyrazolo[3,4-d]pyrimidine **	
U	Formycin A **	
C	Formycin B	
TM	α-Adenosine	
TM	9-α-D-Lyxosyladenine	
BW	9-D-Xylosyladenine **	
BW	9-D-Glucofuranosyladenine	
Pf	9-D-Arabinosyladenine **	
PL	2′-Deoxyadenosine **	
S	3′-Deoxyadenosine **	
BW	5′-Chloro-5′-deoxyadenosine	
BW	5′-Amino-5′-deoxyadenosine	
PL	Cytidine	
S	Thymidine	
PL	*Uridine*	
S	1-D-Arabinosylcytosine	
MS	*Mycophenolic acid*	

† *Source abbreviations used*: BW, *synthesized at these Wellcome Laboratories*; C, Calbiochem; MS, gift from Dr. M.J. Sweeny (Eli Lily Co.); NB, Nutritional Biochemicals Co.; PF, Pfanstiehl; PL, P.L. Biochemicals; S, Sigma; TM, Terra-Marine Bioresearch; U, gift from Dr. H. Umezawa (Inst. Microbiol. Chem., Tokyo) via Dr. S.H. Chu (Brown Univ.); UC, gift from Dr. G. Fonken (Upjohn Co.).

** Since this preparation of GMP synthetase contained traces of adenosine deaminase (6), the deaminase inhibitor [11], *erythro*-9-(hydroxy-3-nonyl)adenine, was included in the assays because the nucleoside was found to be deaminated by calf intestinal adenosine deaminase at a rate ≥5% the rate of adenosine. At the concentration employed, 7 μM, this compound was found to inhibit Ehrlich ascites adenosine deaminase by 100% and GMP synthetase by less than 1%

Discussion

The above results demonstrate that while the GMP synthetases from both this tumor source and *E. coli* [1,4,5] were inhibited by certain nucleosides, only the tumor enzyme was subject to inhibition in the absence of PP_i. The observation that the nucleosides bind to the tumor enzyme (in the absence of PP_i) at a site that is distinct from the catalytic sites of the nucleotide substrates

is of particular interest. Earlier reports that suggested that the nucleosides bind to a noncatalytic or regulatory site on the bacterial enzyme [1,14—16] became less credible with the discovery that the nucleosides appeared to compete with ATP in the presence of PP_i [4,5]. However, the present finding that the apparent competitive binding of the nucleosides in the presence of PP_i is consistent with the existence of a regulatory binding site may renew the possibility that such a site does exist on the bacterial enzyme as well as the tumor enzyme.

The nature of the role of the reaction product, PPi, in the facilitated binding of the nucleosides to the Ehrlich ascites enzyme is worthy of consideration. Unfortunately, the data do not permit the distinction as to whether PP_i serves to tighten the binding of the nucleosides at a non-catalytic site (Model A) or actually opens a second site (possibly the ATP site) to allow binding at that locus (Model B). In either case, it is apparent from the common K_N/K_N^* ratios that the intensity of the effect of PP_i is constant for all of the nucleoside inhibitors.

Inspection of relative nucleoside-binding specificities of GMP synthetase from Ehrlich ascites cells and *E. coli* B-96 [5] (K_N^* values of this study should be compared with K_B values of the earlier study) reveals that they were rather similar, * with the major exception that the tumor enzyme had a greatly diminished capacity to bind compounds with substituents on the N^6-position. Although the specificities of the two enzymes were similar, with a few exceptions, the PP_i-facilitated dissociation constants for the nucleosides were approximately an order of magnitude lower with the bacterial enzyme. The exceptions were decoyenine, 5′-deoxyadenosine and tubercidin which had similar inhibition constants for both enzymes and 2-fluoroadenosine and *erythro*-9-(2-hydroxy-3-nonyl)adenine which had lower inhibition constants with the tumor enzyme.

The comparison of the inhibition of GMP synthetase from these two sources is complicated by factors other than the nucleoside dissociation constants. The high levels of PP_i in *E. coli* [17] coupled with the ability of the bacterial enzyme to isomerize into non-dissociating complexes with nucleosides and PP_i [4,5] suggest that the bacterial enzyme may be more susceptible to inhibition under physiological conditions. However, the possibility that this tumor enzyme has lost its ability to isomerize and be "pseudoirreversibly" inhibited as a result of the purification procedure cannot be ignored. Evidence has been presented for chemically or genetically altered enzyme [2,4,14] and heterogeneous populations of enzyme [5] from *E. coli* with diminished capacities to be inhibited in this manner. It is conceivable that differences in the molecular weights of the bacterial enzyme (126 000 [18]) and this enzyme (85 000 [6]) may represent either a physical or genetic loss of a peptide that was crucial to the isomerization process.

* The analogy is continued by the finding that inosine and 6-mercaptopurine riboside were weak inhibitors of both this and the bacterial enzyme [5]. However, the data for these compounds were excluded from Table II because, for unknown reasons, the Dixon plots with inosine began to parallel the x-axis above 0.1 mM inosine while those for 6-mercaptopurine riboside were curvilinear upwards above this concentration.

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References

1 Slechta, L. (1960) Biochem. Biophys. Res. Commun. 3, 596—598
2 Vdaka, S. and Moyed, H.S. (1963) J. Biol. Chem. 238, 2797—2803
3 Zyk, N., Citri, N. and Moyed, H.S. (1969) Biochemistry 8, 2787—2794
4 Zyk, N., Citri, N. and Moyed, H.S. (1970) Biochemistry 9, 677—683
5 Spector, T. and Beacham, III, L.M. (1975) J. Biol. Chem. 250, 3101—3107
6 Spector, T. (1975) J. Biol. Chem. 250, 7372—7376
7 Spector, T., Miller, R.L., Fyfe, J.A. and Krenitsky, T.A. (1974) Biochem. Biophys. Acta 370, 585—591
8 Wilkinson, G.N. (1961) Biochem. J. 80, 324—332
9 Moyed, H.S. and Magasanik, B. (1957) J. Biol. Chem. 226, 351—363
10 Abrams, R. and Bentley, M. (1959) Arch. Biochem. Biophys. 79, 91—110
11 Schaeffer, H.J. and Schwender, C.F. (1974) J. Med. Chem. 17, 6—8
12 Webb, J.L. (1963) Enzyme and Metabolic Inhibitors, Vol. 1, p. 32, Academic Press, New York
13 Dixon, M. (1953) Biochem. J. 55, 170—171
14 Fukuyama, T.T. and Moyed, H.S. (1964) Biochemistry 3, 1488—1492
15 Kuramitsu, H. and Moyed, H.S. (1966) J. Biol. Chem. 241, 1596—1601
16 Donovan, K.L., Rowe, J.A. and Moyed, H.S. (1967) Antimicrob. Agents Chemother. 289—296
17 Heinonen, J. (1974) Anal. Biochem. 59, 366—374
18 Sakamoto, N., Hatfield, G.W. and Moyed, H.S. (1972) J. Biol. Chem. 247, 5880—5887