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Alteration of the Conformative Response and Inhibition of Xanthosine 5'-Phosphate Aminase by Adenine Glycosides*

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ABSTRACT: Modification of the conformative response of xanthosine 5'-phosphate aminase to its substrate, xanthosine 5'-phosphate, by inorganic pyrophosphate has been found to be essential for its sensitivity to inhibition by adenine glycosides. The millimolar concentration of xanthosine 5'-phosphate which induces a half-maximal conformative response, the conformative response constant or K_{cr}, is 0.1. In the presence of inorganic pyrophosphate this value is reduced 33-fold. Such modification can be eliminated by chemical treatment or genetic alteration with the further consequences of loss or diminution of sensitivity to irreversible inhibition by the adenine glycoside antibiotics, psicofuranine and decoyinine, as well as diminution of sensitivity to reversible inhibition by adenosine. Catalytic activity however is not appreciably affected by elimination of the modifying action of inorganic pyrophosphate.

Previous studies (Udaka and Moyed, 1963; Fukuyama and Moyed, 1964) have shown that the adenine glycoside antibiotic psicofuranine (6-amino-9-D-psicofuranosylpurine) causes an irreversible inhibition of XMP aminase (xanthosine 5'-phosphate:ammonia ligase (AMP), EC 6.3.4.1). The inhibition was found to depend upon the presence of the substrates, XMP, and on one of the products, PP_i, of the catalytic reaction. A subsequent report (Zyk *et al.*, 1969) presented evidence that exposure of this enzyme to XMP results in a conformative response, a reversible change in the conformation of the enzyme associated with the binding of the substrate, which is greatly enhanced by PP_i and magnesium ions. Enhanced conformative response was found to be essential for the reversible interaction of the enzyme with adenosine. In the present communication we examine the role of the conformative response in the reversible and irreversible binding of adenine glycosides. The results to be presented indicate that the susceptibility of XMP aminase to inhibition by adenine glycosides is determined by the extent of response of the enzyme to the modifying effect of PP_i. Loss of responsiveness to PP_i resulting from environmental or genetic changes confers resistance to adenine glycosides.

Materials and Methods

Chemicals. Psicofuranine and decoyinine were gifts of the Upjohn Co. The other adenine glycosides, XMP, trypsin, and pronase were commercial preparations.

Purification of XMP Aminases. The previously published purification procedure was employed (Fukuyama and Moyed, 1964). There were modifications in the strains of bacteria and in the method of cultivation. The bacterial strains were *Escherichia coli* B-96, a purine-requiring mutant blocked in inosinicase, and its derivatives B-96-7, B-96-17, and B-96-24, which were selected by Mr. Kerry Donovan for ability to grow in the presence of bacteriostatic concentrations of psicofuranine. Derepressed synthesis of XMP aminase was achieved in all strains by growth for 8 hr in a mineral salts–glucose medium supplemented with 2 mg/ml of Bacto Difco Casamino Acids (vitamin free) and 40 μg/ml of AMP.

XMP Aminase Assay. The procedure was as previously described (Zyk *et al.*, 1969).

Heat Treatment, Pronase Treatment, and Trypsin Treatment. The procedures were as previously described (Zyk *et al.*, 1969).

Results

Interaction of XMP Aminase with Adenine Glycosides. In a previous report (Zyk *et al.*, 1969) we described the XMP-

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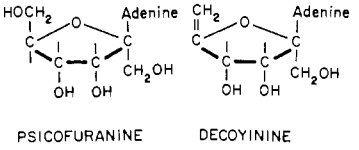

FIGURE 1: Structures of psicofuranine and decoyinine.

and PP_i-mediated interaction of XMP aminase with adenosine. This interaction is wholly reversible in contrast to the essentially irreversible interaction with psicofuranine in the presence of XMP and PP_i (Udaka and Moyed, 1963; Fukuyama and Moyed, 1964). Since adenosine and psicofuranine differ only in the structure of the sugar moieties, we examined the effect of other replacements for ribose on the interaction with XMP aminase. The adenine glycosides tested are listed in Table I. Except for psicofuranine and the closely related decoyinine all other compounds interacted reversibly with the enzyme. The reversible interactions were reflected in measurable inhibition of the catalytic activity which, however, was very low on a molar basis compared with that observed with adenosine. Similar results were obtained when the interaction was tested in terms of stabilization of the enzyme to proteolytic inactivation (Table I). Psicofuranine and decoyinine (Figure 1), which cause irreversible inhibition, differ from the other adenine glycosides tested having a hydroxyl group substituted in position 1 of the sugar moiety. It seems likely that such substitution is necessary for the irreversible binding of these inhibitors.

TABLE I: Effect of Adenine Glycosides on the Rate of Inactivation of XMP Aminase by Pronase.[a]

Adenine Glycoside	$1 - (V/V_0)$[b]
None	
Adenosine	0.93
2′-Deoxyadenosine	0.24
3′-Deoxyadenosine	0.12
9-D-Lyxosyladenine	0.20
9-D-Xylosyladenine	0.15
Psicofuranine[c]	*d*
Decoyinine[c]	*d*

[a] XMP aminase (6.5 μg of protein), 1.5 μmoles of Tris-HCl (pH 7.4), 2.5 μmoles of $MgCl_2$, 0.3 mμmole of XMP, 30 mμmoles of sodium pyrophosphate, 30 mμmoles of adenine glycoside as indicated above, and 2 μg of pronase in a volume of 0.30 ml were incubated at 37°. The treatment was terminated at 30-sec intervals by dilution with 2.2 ml of the assay reagents. The mixture was then transferred to cuvets for assay of the residual activity. [b] The rates of inactivation in the presence, V, and absence, V_0, of adenine glycoside were determined from the linear slopes obtained by plotting log of residual activity against duration of treatment. The extent of protection from inactivation by pronase is expressed as $1 - (V/V_0)$. [c] The structures of these compounds are shown in Figure 1. [d] The enzyme is irreversibly inhibited by these compounds.

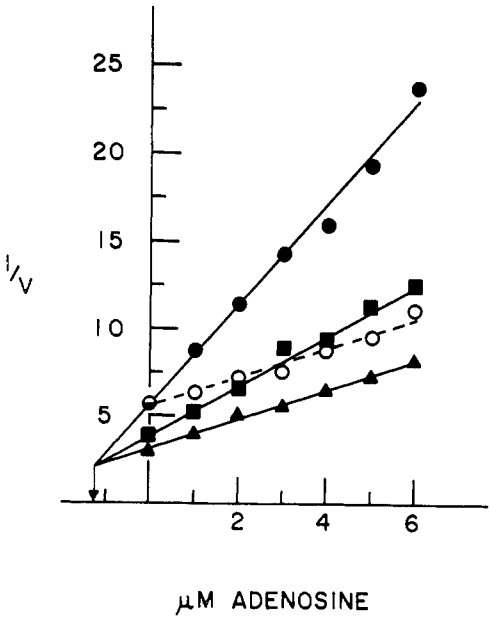

FIGURE 2: Competitive inhibition of XMP aminase by adenosine. Initial rates are measured by following the decrease in absorption at 290 mμ at pH 8.5 due to the conversion of XMP into GMP. The reagent mixture contained 180 μmoles of Tris-HCl (pH 8.5), 25 μmoles of $MgCl_2$, 0.25 μmole of ATP (●), 0.50 μmole of ATP (■), 1.0 μmole of ATP (▲), 0.3 μmole of XMP, 160 μmoles of $(NH_4)_2SO_4$, and 0.25 μmole of sodium pyrophosphate (pH 7.4). For the experiment in which PP_i was omitted (○), 0.25 μmole of ATP was employed. $V = -\Delta$ absorbancy at 290 mμ/min.

Competitive Inhibition of XMP Aminase by Adenosine. The inhibition of catalytic activity by adenosine is fully reversible under the experimental conditions of Figure 2. Adenosine acts as a competitive inhibitor with respect to ATP with a K_i of 1.2 μM as determined from a plot of the reciprocals of the observed velocities *vs.* inhibitor concentration (Figure 2). The K_i determination was performed in the presence of excess, 100 μM, PP_i. In the absence of added PP_i slight inhibition is observed (Figure 2) which may be attributable to PP_i produced in the course of the catalytic reaction. Thus, the effect of adenosine on aminase activity, like its stabilizing effect on the aminase described in a previous report (Zyk *et al.*, 1969), depends upon the presence of PP_i. Since both effects reflect the reversible binding of adenosine, we conclude that this binding depends upon the conformative response induced by XMP and modified by PP_i (Zyk *et al.*, 1969).

Irreversible Inhibition by Psicofuranine and Decoyinine. The inhibition of XMP aminase by psicofuranine and decoyinine is irreversible under the conditions described in Table II and Figure 3. Rates of inactivation appear to follow first-order kinetics as illustrated by the linear slopes obtained for psicofuranine in a semilogarithmic plot (Figure 3). The pseudo-first-order rate constants of inactivation, k', determined from these slopes are listed in Table II which also lists the k' values for decoyinine obtained in an analogous manner. Although the rate of inactivation by decoyinine is somewhat slower than that by psicofuranine, the two compounds are otherwise very similar in their effect on the enzyme; in both cases the effect is strictly dependent upon the presence of XMP, $MgCl_2$, and PP_i. Furthermore, the rates of inhibition

TABLE II: Requirements for Inactivation of XMP Aminase by Psicofuranine and Decoyinine.[a]

Additions			k'[b]	
			Psicofuranine	Decoyinine
None			0.010	0.010
XMP	PP_i	Mg^{2+}	0.255	0.208
XMP	–	Mg^{2+}	0.021	0.010
XMP	PP_i	–	0.021	0.018
–	PP_i	Mg^{2+}	0.010	0.010

[a] XMP aminase (6.5 μg of protein), 1.5 μmoles of Tris-HCl (pH 7.4), and, as indicated above, 0.30 mμmole of XMP, 0.30 mμmole of sodium pyrophosphate, 2.5 μmoles of $MgCl_2$, and 0.30 mμmole of psicofuranine or decoyinine were incubated in a volume of 0.30 ml at 37°. The treatment was terminated at 30-sec intervals and residual activity was measured as described in Table I. [b] Pseudo-first-order rate constants, k', for inactivation were determined by plotting the log of residual activity against duration of treatment.

by both compounds are reduced by an equimolar concentration of ATP and completely prevented by an excess, 100 μM, of ATP. This is illustrated for psicofuranine in Figure 3.

Relationship between Inhibition and Conformative Response. Since inactivation by psicofuranine or decoyinine depends upon the presence of XMP it is possible to vary the rate of inactivation by varying the concentration of XMP. The XMP-dependent variation in the rate constant of inactivation k', is illustrated in Figure 4. Half-maximal rates of inactivation by identical amounts of psicofuranine and decoyinine are achieved with an identical concentration of XMP, 2.0 μM, even though the observed rates of inactivation by the two compounds are not equal. The value of 2.0 μM is reasonably close to the conformative response constant, K_{cr}, obtained for XMP, 3.0 μM, in the presence of PP_i by the trypsin inactivation method (Zyk *et al.*, 1969). This is consistent with the proposal that the recognition of the two inhibitors depends upon a conformational transition induced by XMP and modified by PP_i. The slightly greater affinity of the aminase for XMP obtained by inactivation with the adenine glycosides than by the tryptic inactivation method in the presence of PP_i probably results from the mutual enhancing effects of binding previously observed for XMP, PP_i, and psicofuranine (Fukuyama and Moyed, 1964).

Elimination of the Modifying Effect of PP_i by Urea and Guanidine. Our previous observations on the PP_i-induced modification of the conformative response to XMP have indicated that this modification can be partly eliminated by raising the temperature. Thus, the PP_i-dependent decrease in K_{cr} for XMP determined from rates of thermal inactivation at 55° is only 3-fold as compared with the 33-fold decrease recorded by proteolytic inactivation at 37° with either trypsin or pronase. In contrast, the unmodified K_{cr} values for XMP in the absence of PP_i were identical by the three methods and thus not affected by the higher temperature (Zyk *et al.*, 1969). These observations suggested that the PP_i-induced modification of the conformative response

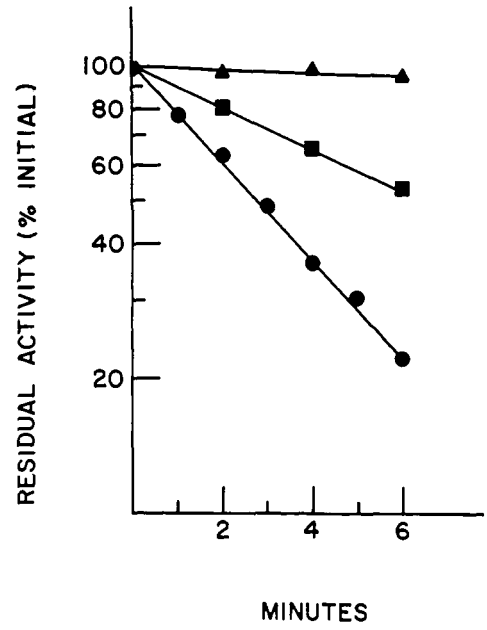

FIGURE 3: Effect of ATP on inactivation of XMP aminase by psicofuranine. XMP aminase (6.5 μg of protein), 1.5 μmoles of Tris-HCl (pH 7.4), 0.30 mμmole of XMP, 0.30 mμmole of sodium pyrophosphate (pH 7.4), and 0.30 mμmole of psicofuranine were incubated at 37° in a volume of 0.30 ml. ATP levels: (●) none, (■) 1.0 μM, and (▲) 100 μM. The treatment was terminated by dilution with 2.2 ml of the reagents for assay of XMP aminase. The mixture was then transferred to cuvets for assay of residual activity.

involved a superimposed conformational change which could be readily disrupted. Consequently, we tested the effect of a relatively mild treatment with other conformation-disrupting agents, urea and guanidine-HCl. Typical results are summarized in Table III. The rate constants of inactivation

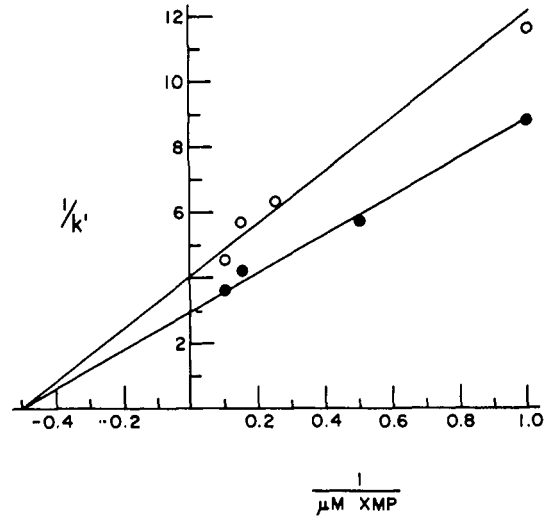

FIGURE 4: Effect of XMP on the inactivation of XMP aminase by psicofuranine and decoyinine. Rates of inactivation were determined as in Figure 3 with 0.30 mμmole of psicofuranine (●) or 0.30 mμmole of decoyinine (○) and amounts of XMP varied from 0.30 to 3.0 mμmoles. Pseudo-first-order rate constants were determined as in Table II.

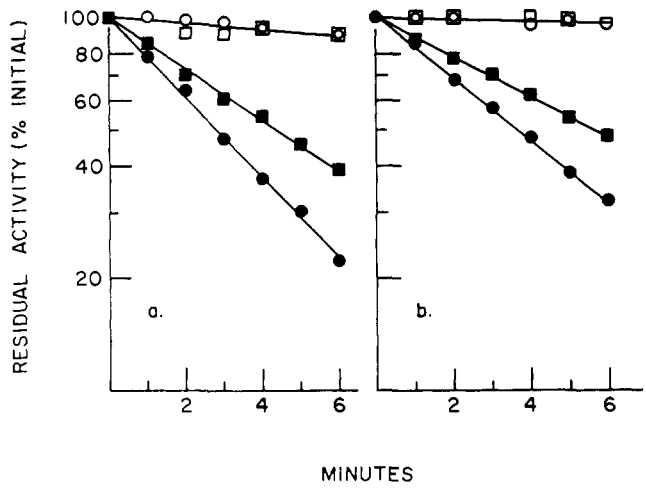

FIGURE 5: Inactivation of parental and mutant XMP aminases by psicofuranine and decoyinine. The rate of inhibition was determined as in Figure 3, but without added ATP and with either 0.30 mμmole of psicofuranine (a) or 0.30 mμmole of decoyinine (b). Purified aminases were obtained from strains B 96 (●), B 96-17 (■), B 96-7 (○), or B 96-24 (□).

in the presence of 0.1 mM XMP and 0.1 mM PP_i are compared with the corresponding constants in the presence of XMP alone. The difference between the rate constants is markedly decreased by 3.3 M urea and 0.33 M guanidine-HCl at 55°. Moreover, the effects of urea and of heat are additive as expected.

Elimination of Sensitivity to Adenine Glycosides by Urea and Guanidine-HCl. The correlation between the effect of PP_i on the conformative response (Zyk *et al.*, 1969) and on the XMP-dependent rates of inactivation (Table II) suggest that PP_i may be required for inhibition by the adenine glycosides because of its effect on the conformative response. If so the susceptibility of the enzyme to inactivation by the adenine glycosides should be eliminated by preventing the

TABLE III: Elimination of PP_i Effect by Urea or Guanidine.[a]

Treatment	k_0'[b]	k_P'	k_P'/k_0'
37°	0	0	
37°, 2 μg of trypsin	0.19	0.09	0.47
37°, 2 μg of Pronase	0.25	0.12	0.48
55°	0.29	0.17	0.59
37°, 3.3 M urea	0.20	0.15	0.75
55°, 1.67 M urea	0.56	0.50	0.89
55°, 0.33 M guanidine-HCl	0.66	0.67	1.02

[a] XMP aminase (6.5 μg of protein) was treated in the presence of 1.5 μmoles of Tris-HCl (pH 7.4), 30 mμmoles of XMP, 2.5 μmoles of $MgCl_2$, and as indicated above, 30 mμmoles of sodium pyrophosphate, in a volume of 0.30 ml. The treatments were terminated at 30-sec intervals and residual activity was measured as described in Table I. [b] Pseudo-first-order rate constants for inactivation in the absence, k_0', and in the presence, k_P', of PP_i were determined as in Table II.

TABLE IV: Effects of Urea and Guanidine-HCl on the Rates of Inactivation of Parental, B 96, and Mutant, B 96-17, Forms of XMP Aminase by Psicofuranine and Decoyinine.[a]

Inhibitor	Additions	k'[b] B 96	k'[b] B 96-17
None	None	0.00	0.00
None	2.5 M urea	0.081	0.083
None	0.33 M guanidine	0.034	0.036
Psicofuranine	None	0.255	0.187
Psicofuranine	2.5 M urea	0.128	0.083
Psicofuranine	0.33 M guanidine	0.098	0.034
Decoyinine	None	0.208	0.127
Decoyinine	2.5 M urea	0.083	0.058
Decoyinine	0.33 M guanidine	0.119	0.043

[a] XMP aminase (6.5 μg of protein), 1.5 μmoles of Tris-HCl (pH 7.4), 2.5 μmoles of $MgCl_2$, 30 mμmoles of XMP, 30 mμmoles of sodium pyrophosphate, and, as indicated, 0.30 mμmole of psicofuranine or decoyinine, and urea or guanidine-HCl were incubated in a volume of 0.30 ml at 37°. The treatments were terminated at 30-sec intervals. [b] Pseudo-first-order rate constants for inactivation were determined as in Table II.

PP_i modification of the conformation of the enzyme. In the previous section we have demonstrated conditions under which the effect of PP_i on the conformation is largely abolished (Table III). We have determined the effects of similar conditions on the rates of inactivation of the aminase by psicofuranine and decoyinine. The results, presented in Table IV, are consistent with the above interpretation; urea or guanidine-HCl, which cause a marked reduction in the PP_i effect on the conformation of the enzyme (Table III), also cause a reduction in the susceptibility of the enzyme to inactivation by psicofuranine and decoyinine (Table IV). Also included in Table IV are the results obtained with a mutant enzyme, B-96-17, which is less sensitive than the parental enzyme to inactivation by these adenine glycosides. The susceptibility of the latter enzyme is completely eliminated by 2.5 M urea or by 0.33 M guanidine-HCl. Additional properties of this and two other mutant enzymes are described in the following section.

Inhibition Patterns of Enzyme Preparations Derived from Psicofuranine-Resistant Mutants. XMP aminases from psicofuranine-resistant strains (B-96-7, B-96-17, and B-96-24) derived from *E. coli* B-96 have altered susceptibility to inhibition by adenine glycosides. Two of the mutant aminases, B-96-7 and B-96-24, are completely resistant to irreversible inhibition by psicofuranine and decoyinine; the third mutant, B-96-17, retains sensitivity to irreversible inhibition by these antibiotics, but its rate of inactivation is lower than that of the parental aminase (Figure 5). A tenfold increase in the concentration of XMP under otherwise unchanged conditions had no effect on the rates of inhibition of any of the aminases. A similar increase in the concentration of psicofuranine or decoyinine did not result in inactivation of the B-96-7 and B-96-24 aminases, but the rates of inhibition of the

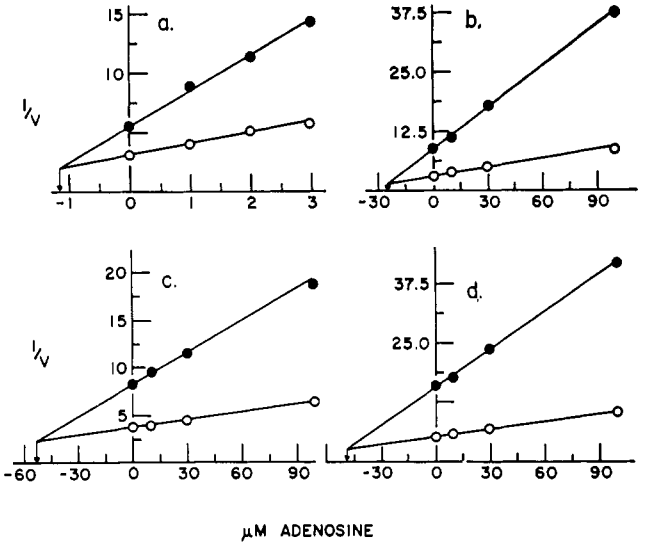

FIGURE 6: Competitive inhibition of parental and mutant XMP aminases by adenosine. The effects of the indicated concentrations of adenosine were measured as in Figure 2 in the presence of 30 mμmoles (●) or 120 mμmoles of ATP (○). Purified aminases were obtained from strains B 96 (a), B 96-17 (b), B 96-7 (c), or B 96-24 (d). $V = -\Delta$ absorbancy at 290 mμ/min.

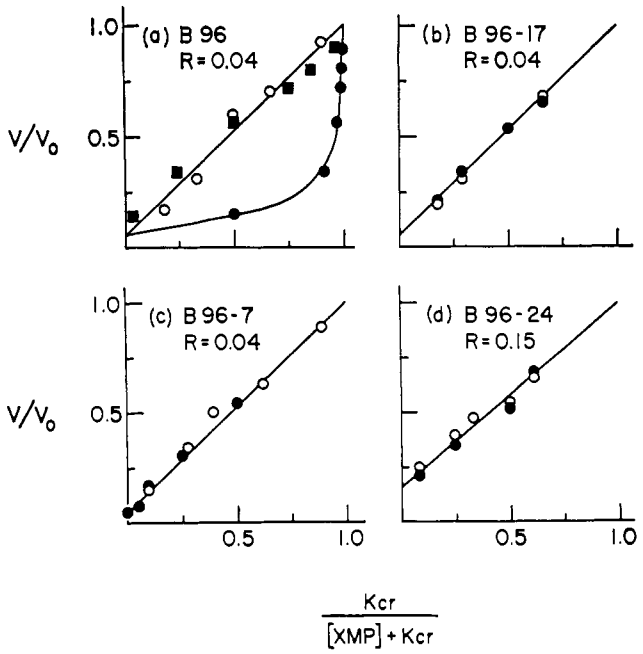

FIGURE 7: Effect of PP_i on the conformative responses to XMP of mutant and parental XMP aminases. XMP aminase (6.5 μg of protein), 1.5 μmoles of Tris-Hcl (pH 7.4), varying amounts of XMP, 2.49 μmoles of $MgCl_2$, and 2 μg of trypsin were incubated with (●) or without (○) 30 mμmoles of sodium pyrophosphate (pH 7.4) in a volume of 0.30 ml. The treatments were terminated at 30-sec intervals, residual activity was determined and plotted as described in Table I for calculation of the rates of inactivation in the presence, V, or absence, V_0, of XMP. The values for R were obtained by extrapolation to infinite XMP concentration in plots of V/V_0 *vs.* 1/(XMP). K_{cr} = conformative response constant, the concentration of XMP at which 50% of the enzyme has undergone the conformative response. The fraction of the enzyme in conformative response, F_{cr}, = $(1 - (V/V_0))/(1 - R)$. K_{cr} values are determined from plots of $1/F_{cr}$ *vs.* (XMP). Details for the derivation and calculation of the constants have been published (Zyk *et al.*, 1969). A K_{cr} value of 100 μM was assumed in all cases, and, in addition, a K_{cr} value of 3 μM XMP in the presence of PP_i (■) was assumed in one case (Figure 7a).

parental, B-96, and the intermediate, B-96-17, were accelerated as expected.

The two mutant aminases, B-96-7 and B-96-24, which are resistant to *irreversible* inhibition by psicofuranine and decoyinine are nevertheless susceptible to *reversible* inhibition by these antibiotics. Furthermore, all three of the mutant aminases, like the parental enzyme, are reversibly inhibited by adenosine; the inhibition is competitive with respect to ATP (Figure 6). However, the K_i values are elevated for the mutant aminases, ranging from 25.0 μM adenosine for B-96-17 to 51.0 and 56.0 μM adenosine for B-96-24 and B-96-7, respectively. The K_i value for the parental enzyme is 1.2 μM adenosine.

Conformative Response of Mutant Aminases. In view of the role of the PP_i effect in the sensitivity of the parental aminase to adenine glycosides, we examined the possibility that a genetic loss of sensitivity could be associated with a loss of conformative responsiveness to PP_i. This proved to be the case as shown in Figure 7 which compares the conformative response of the parental and mutant enzymes with XMP in the presence and absence of PP_i. Results are plotted by a method which permits a comparison of widely divergent K_{cr} values on a single set of coordinates (Zyk *et al.*, 1969). The relative rates of inactivation, V/V_0, obtained as described in the legend to Figure 7, are plotted against $K_{cr}/(XMP) + K_{cr}$. The theoretical straight line in this plot joins the point for (XMP) = 0 where $V = V_0$ and the point for (XMP) approaching infinity. The value of the vertical intercept, $R = V_s/V_0$ (where V_s is the rate of inactivation with saturating XMP), depends upon the method used for inactivation (Zyk *et al.*, 1969); in this case with tryptic digestion the R values are 0.15 for one of the mutant aminases, B-96-24, and 0.04 for the other mutant and parental aminases.

The experimental values, expressed as V/V_0 follow the theoretical slope when plotted as a function of $K_{cr}/(XMP) + K_{cr}$ if the value substituted for K_{cr} is correct (Zyk *et al.*, 1969). This relationship is illustrated in Figure 7a where the previously established values, $R = 0.04$ and $K_{cr} = 100$ μM, for the parental aminase, B-96 have been substituted. We have also determined and substituted the unmodified K_{cr} and R values for the three mutant enzymes (Figure 7b–d). The significance of the higher R value for B-96-24 is not clear, but it seems unrelated to the level of resistance.

The unmodified K_{cr} values for all three mutant enzymes as well as for the parental enzymes were 100 μM (Figure 7a–d). In contrast, there was a striking difference between the parental and mutant enzymes in their responses to PP_i. PP_i causes a 33-fold decrease in the K_{cr} value for XMP with the parental enzyme (Zyk *et al.*, 1969). There was, however, no detectable change in the K_{cr} values for XMP of mutant enzymes in response to PP_i. This is illustrated in Figure 7a–d where the value of 100 μM is shown to fit all data obtained in the absence of PP_i and all but one set of data obtained in the presence of PP_i. The one exception is the response of the parental enzyme to PP_i as reflected in the deviation from the linear slope shown in Figure 7a. This deviation is corrected

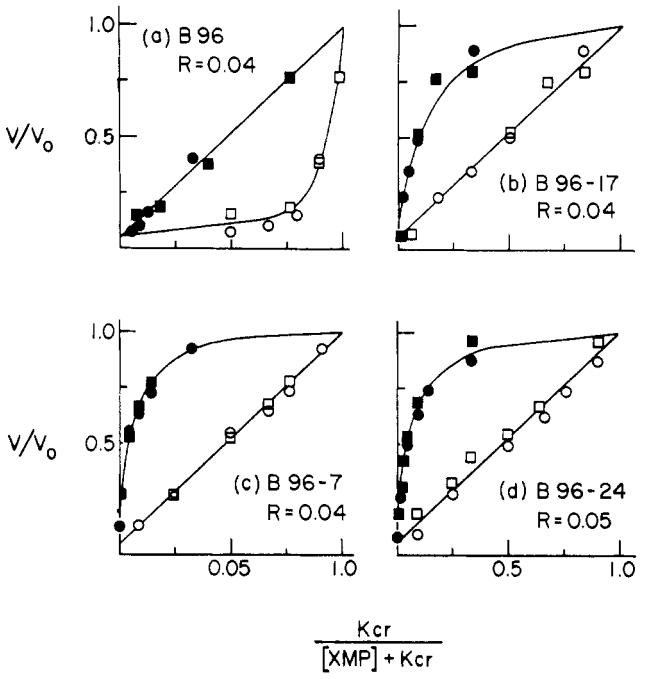

FIGURE 8: Effect of adenosine and ATP on the conformative response of parental and mutant aminases. The procedures were as described in Figure 7 except that either 30 mμmoles of sodium pyrophosphate (pH 7.4) plus 30 mμmoles of adenosine (●, ○) or 30 mμmoles of ATP (■, □) were included in the treatments. The V/V_0 values are plotted twice, with high (○, □) or low (●, ■) K_{cr} values substituted in the abscissa. The following K_{cr} values were employed: (a) B 96, K_{cr} = 0.5 or 0.05 μM XMP; (b) B 96-17, K_{cr} = 0.5 or 0.05 μM XMP; (c) B 96–7, K_{cr} = 1.0 or 0.05 μM XMP; and (d) B 96-24, K_{cr} = 1.0 or 0.05 μM XMP.

when the correct K_{cr} value for the parental aminase, 3.0 μM XMP, is substituted in the abscissa.

A further comparison of the responsiveness of the mutant and parental aminases is presented in Figure 8. A previous detailed study of the parental aminase has shown that 100 μM ATP causes a decrease of more than 2000-fold in the K_{cr} value for XMP; this effect of ATP can be duplicated by equimolar concentrations of adenosine and PP_i (Zyk *et al.*, 1969). This is evident in Figure 8a where the experimental points are seen to follow the theoretical slope in the presence of either ATP or adenosine + PP_i when the correct K_{cr} value of 0.05 μM XMP is used in the abscissa. By contrast, the K_{cr} values which give the best fits for the mutant enzymes are 1.0 μM XMP for B-96-7 and B-96-24 and 0.5 μM XMP for B-96-17, K_{cr} values 10–20 times higher than for the parental aminase. For closer comparison V/V_0 values have been plotted in each case substituting the low, 0.05 μM and the high, 0.5 or 1.0 μM, K_{cr} values. Although the mutant enzymes respond to ATP or to the adenosine–PP_i combination, the response is poor compared with that of the parental enzyme. It may be significant that the response of the partially resistant aminase, B-96-17 is somewhat better, K_{cr} = 0.5 μM XMP, than that of the highly resistant mutants, B-96-7 and B-96-24, K_{cr} = 1.0 μM XMP.

Discussion

The inhibition of XMP aminase by psicofuranine and other adenine glycosides has been of particular interest because of the unique specificity of the inhibition and its unusual dependence on the participation of a substrate and a product of the catalytic reaction. Both the reversible inhibition by adenosine and the irreversible inhibition by psicofuranine or decoyinine appear to involve the ATP binding site as shown by the competitive relationships with the substrate. Yet, other similar ATP-driven reactions in the bacterial cell are not similarly affected by these inhibitors; they are either insensitive or several 1000-fold less sensitive than the aminase (Bloch and Nichol, 1964; Spencer and Preiss, 1967). The requirement for one of the substrates, XMP, and one of the products, PP_i, for inhibition of the aminase by the adenine glycoside was equally puzzling.

A possible explanation of these observations was provided by a detailed study of the interaction of XMP aminase with XMP, PP_i, and adenine glycosides (Zyk *et al.*, 1969). XMP was found to induce a marked change in the conformation of XMP aminase, which affects the stability and the binding characteristics of the enzyme. The conformative response to XMP facilitates the binding of PP_i which in turn modifies the conformative response. It was shown, furthermore, that modification of the conformative response to XMP is a prerequisite for a still further modification by adenosine. The experiments described here establish that both PP_i and XMP are required for competitive inhibition by adenosine as well as for interaction with other adenine glycosides. These observations confirm earlier direct binding studies with psicofuranine (Fukuyama and Moyed, 1964) and similar binding measurements with adenosine (N. Zyk, N. Citri, and H. S. Moyed, 1968, unpublished observations).

These findings focus attention on the role of XMP and PP_i in the inhibition of XMP aminase by adenine glycosides. We believe that the recognition of the inhibitor depends upon a specific conformational state of the aminase molecule and that transition to that state is induced by XMP and PP_i. The correlation between the concentration-dependent effect of XMP on inhibition (Figure 4) and on the conformative response (Zyk *et al.*, 1969) provide evidence for the postulated role of XMP. The participation of the PP_i-induced modification of the conformation response in inhibition by adenine glycosides has been inferred from two independent experimental approaches. The rationale in both cases is that since the response to PP_i, unlike the response to XMP, is not an integral component of the catalytic reaction, it could be conceivably eliminated without loss of enzymatic activity. According to this view, elimination or reduction of the effect of PP_i on the conformative response should have a corresponding effect on sensitivity to adenine glycosides.

This proposition was examined by both chemical and genetic modification of XMP aminase. Previous observations (Zyk *et al.*, 1969) have indicated that the PP_i-dependent transition in the conformation of XMP aminase is partially reversed at higher temperatures. We have now shown that the PP_i effect is even more readily eliminated by relatively mild treatment with urea or guanidine-HCl (Table III). Significantly, these reagents eliminate susceptibility to irreversible inhibition by psicofuranine and decoyinine but have little effect on the survival of enzymatic activity.

Similarly, loss of sensitivity to adenine glycosides by mutation could be correlated with the loss of responsiveness to PP_i. The mutants examined in this report were isolated on the basis of their ability to grow on psicofuranine. The resistant XMP

aminases have significant differences in K_i values for adenosine (Figure 5) which is consistent with resistance resulting from independent mutational events in each strain. Thus, it appears that structural changes which eliminate the effect of PP_i on conformative response of the aminase of *E. coli* permit the growth of this organism in the presence of psicofuranine.

The resistance thus acquired is not simply due to loss of recognition of the antibiotic. Although the affinity of the mutant aminases for adenine glycosides is reduced by a large factor (Figure 5), the more drastic change is the *complete* loss of the ability of PP_i to modify the response to XMP. The only mechanism, so far observed, which confers resistance to adenine glycosides *via* modification of the aminase depends upon breaking the sequence of conformational transition which is required for recognition of the inhibitors. This appears to be accomplished principally by disrupting the PP_i-induced conformation with either urea or guanidine-HCl or by mutational events, presumably appropriate replacements of one of several amino acid residues, which impair the flexibility of the enzyme.

The only other situation in which the conformative response was studied and related to drug resistance led to a similar conclusion (Zyk and Citri, 1968). That study included observations on the enzymatic inactivation of methicillin. Normally, the conformative response of the relevant enzyme, penicillin β-lactamase, is modified by the side chain of methicillin in a way which is unfavorable for catalysis. Elimination of this side-chain-induced modification results in an effective destruction of the antibiotic (Citri and Kalkstein, 1967; Zyk and Citri, 1968). Thus, in the case of penicillin β-lactamase, as in the present case, constraint of the conformative response in the relevant enzyme appears to provide a mechanism of drug resistance involving either the level of binding or the rate of drug destruction. Since the two cases which have been studied are totally unrelated, it is probable that similar mechanisms based on a modification of conformative response may be frequently responsible for drug resistance.

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