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PRELIMINARY COMMUNICATIONS

POTENT INHIBITION OF MUSCLE 5'-AMP DEAMINASE BY THE NUCLEOSIDE ANTIBIOTICS COFORMYCIN AND DEOXYCOFORMYCIN

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Recently, there has been a surge of interest in the identification of potent inhibitors of adenosine deaminase (ADA, adenosine aminohydrolase, EC 3.5.4.4), because this enzyme plays a key role in the inactivation of many adenosine analogs of chemotherapeutic interest, e.g. arabinosyl adenine and formycin A (1,2). Several potent ADA inhibitors are currently under intensive study and include the antibiotics coformycin and deoxycoformycin (3,4), and the synthetic compounds, erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) and 1,6-dihydro-6-hydroxymethyl purine ribonucleoside (DHMPR)(5,6). Inhibition constants (K_i) determined with human erythrocytic ADA are: deoxycoformycin, 2.5×10^{-12} M; coformycin, $1\text{-}10 \times 10^{-11}$ M; EHNA, 1.6×10^{-9} M; and DHMPR, 1.3×10^{-6} M (7,8). Marked potentiation of the antitumor action of arabinosyl adenine was observed when the analog was administered with deoxycoformycin or EHNA (9-12). Also, ADA inhibitors may impair the maturation (13) or mitogen-stimulated blastogenesis (14) of lymphocytes, suggesting the use of compounds of this class as immunosuppressive agents. In view of the therapeutic potential of potent ADA inhibitors, questions have arisen about possible interactions with other enzymes of purine nucleoside or nucleotide metabolism. Of special interest is 5'-AMP deaminase (5'-AMP aminohydrolase, EC 3.5.4.6), which, in vital tissues such as muscle and brain (15,16), is subject to strong allosteric control and may play a key role, not only in the regulation of intracellular purine nucleotide metabolism but also in amino acid deaminations (17). Since interference with the normal functions of 5'-AMP deaminase might cause toxicity, the interactions of several ADA inhibitors with purified rabbit muscle 5'-AMP deaminase were examined.

Coformycin, deoxycoformycin, EHNA, DHMPR and 2-fluoroadenosine were gifts as described previously (7, 8). When 66% glycerol suspensions of purified rabbit muscle 5'-AMP deaminase (Sigma, Sp. Act. 30 units per mg) were diluted for enzymatic assay, they became highly unstable. The enzyme could be stabilized, however, by dilution into a buffer mixture that contained: 2-(N-morpholino) ethane sulfonic acid buffer (MES-Tris, 50 mM with respect to

MES), pH 6.3; KCl, 0.5 M; β-mercaptoethanol, 1.0 mM; glycogen, 0.5 mg/ml; and bovine plasma albumin, 0.5 mg/ml. The concentrated enzyme preparation could be diluted approximately 50-fold in this mixture without loss of activity for several hrs at 0°. Omission of glycogen causes significant activity loss within 10 to 15 min. The above buffer mixture was employed both for dilution of the enzyme and for enzymatic assays. The enzyme was preincubated (15 min, 30°) in buffer mixture containing 0.5 mM ATP, unless stated otherwise, in the presence or absence of the inhibitor. The reaction was started by addition of AMP (2.0 mM, final concentration in routine assays). Increases in absorbance at 285 nm at 30° were recorded on a Beckman spectrophotometer equipped with Gilford recorder. The difference in molar absorbancy of AMP and IMP at 285 nm was assumed to be 300 (15).

As shown in Table I, ADA inhibitors EHNA, DHMPR and 2-fluoroadenosine did not inhibit 5'-AMP deaminase activity when used at concentrations of 1.1×10^{-4} M, 2.0×10^{-4} M and 1.0×10^{-3} M respectively. On the other hand, deoxycoformycin, a tight-binding inhibitor of ADA, inhibited the 5'-AMP deaminase 45% and 66% at the concentrations of 1.3×10^{-6} M and 2.8×10^{-6} M respectively. Preincubation of the enzyme with coformycin caused marked increases in inhibition (67-99.5% at concentrations of 5.5×10^{-8} M and 1.3×10^{-6} M), whereas preincubation in the presence of deoxycoformycin had little or no effect. Therefore, in the following experiments 15 min preincubation of the enzyme with the inhibitor was employed.

Figure 1A presents double reciprocal plots of 5'-AMP deaminase activity in the presence of several concentrations of coformycin. Patterns of non-competitive inhibition were observed. From the replots of intercepts (Fig. 1B) and slopes (Fig. 1C), the values of K_{ii} (K_i, intercept) and K_{is} (K_i, slope) for coformycin were estimated at 4.9×10^{-8} M and 5.2×10^{-8} M respectively. Deoxycoformycin also produced a pattern typical of non-competitive inhibition (data not shown), with the K_{ii} and K_{is} values of 3.6×10^{-6} M and 1.4×10^{-6} M respectively.

An observation of both interest and potential importance is that, although deoxycoformycin is at least four times more potent as an inhibitor of ADA than the related ribonucleoside, coformycin, the opposite situation exists with 5'-AMP deaminase. Here, the ribonucleoside, coformycin, is about 30- to 70-fold more potent as an inhibitor than deoxycoformycin. These findings are consistent with the markedly superior substrate activity of AMP over 2'-deoxy AMP, with 5'-AMP deaminase (18). Also significant is the observation that the K_i value of coformycin is about 2×10^{-8} M, whereas the K_m of 5'-AMP with 5'-AMP deaminase has been reported in the range of 0.7 to 2.0×10^{-3} M, i.e. a difference of about 1×10^5 fold. Perhaps when the 5'-monophosphate nucleotide of coformycin becomes available, it will prove still more potent as an inhibitor of 5'-AMP deaminase. One may also speculate that, as with ADA, both coformycin and deoxycoformycin represent transition-state inhibitors of 5'-AMP

Table I. Percent Inhibition of Rabbit Muscle 5'-AMP Deaminase by Various Inhibitors of Adenosine Deaminase*

Inhibitors		Preincubation (+)	Preincubation (-)
None		0	0
Coformycin	5.5×10^{-8} M	67	30
	1.3×10^{-6} M	> 99.5	83
Deoxycoformycin	1.3×10^{-6} M	45	42
	2.8×10^{-6} M	66	59
EHNA	1.1×10^{-4} M	0	0
DHMPR	2.0×10^{-4} M	0	0
2-Fluoroadenosine	1.0×10^{-3} M	0	-

*The enzyme was preincubated with (+) or without (-) the inhibitor in the buffer mixture containing 0.5 mM ATP for 15 min at 30°. The reaction was started by addition of AMP to (+) samples and AMP plus the inhibitor to (-) samples.

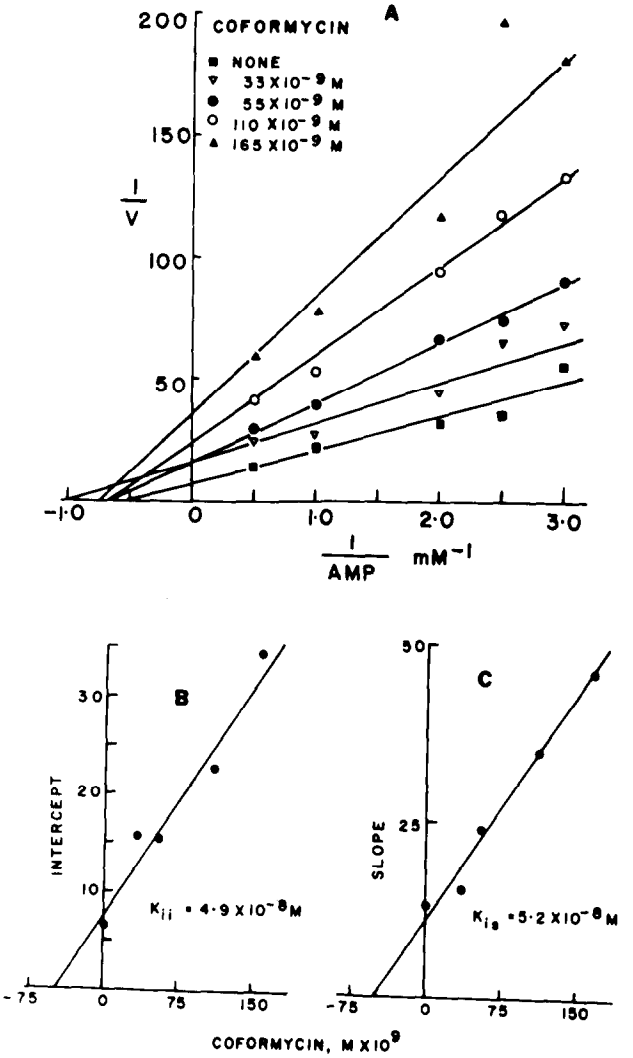

Fig. 1. (A) Double reciprocal plots of 5'-AMP deaminase reaction in the presence of various concentrations of coformycin. (B) Replots of 1/v-intercepts vs coformycin concentrations and (C) replot of slopes from A vs coformycin concentrations.

deaminase (7,8). Also of interest are the findings that EHNA, DHMPR, and 2-fluoroadenosine, all of which display significant inhibitory activity with ADA, did not inhibit muscle 5'-AMP deaminase, even at relatively high concentrations (see Table I).

The above observations may have relevance to the possible clinical applications of ADA inhibitors. Although the physiological role of 5'-AMP deaminase is not fully understood and is currently under intensive investigation, it is becoming increasingly clear that in many tissues this enzyme plays a key role in the regulation of purine nucleotide concentrations and interconversions, as well as in the deamination of amino acids (17). Therefore, potent inhibition of this enzyme could have deleterious effects on cellular survival. It seems likely that, since many vital normal tissues may be highly dependent upon 5'-AMP deaminase for crucial metabolic regulations, inhibition of this enzyme might result in severe toxicity. If these speculations are valid, one might predict that toxicity resulting from inhibition of 5'-AMP deaminase would be much greater with coformycin than with deoxycoformycin and would be negligible with compounds such as EHNA or DHMPR.

In preliminary studies with human erythrocytic 5'-AMP deaminases, differences in the inhibitory effects of coformycin have been observed with the soluble and membrane-bound enzymes, with little inhibition of the membrane-bound enzyme detected. Thus, it is possible that inhibitors such as coformycin may be useful biochemical tools for examining the properties and physiological functions of 5'-AMP deaminases.

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