NOVEL SUBSTRATE OF ADENOSINE DEAMINASE

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SUMMARY

Adenosine deaminase (from calf intestinal mucosa) converts 8,2'-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine (II)) into 8,2'-anhydro-8-mercapto-9-β-D-arabinofuranosylhypoxanthine (III) with Km of 2.0×10^{-4}M and V max equal to 7% that of adenosine. This conversion serves as a useful preparative synthesis of III. Further the fact that II (where the purine base is locked in the anti conformation) is a substrate for the enzyme while 8-bromoadenosine (where the base is in the syn conformation) is not a substrate supports the idea that substrates for adenosine deaminase must exist in the anti conformation.

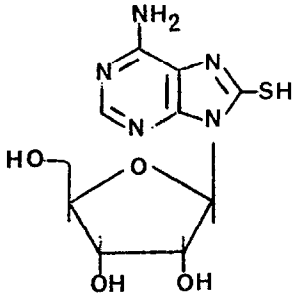
INTRODUCTION

Several enzymes have recently been shown to require as substrates nucleosides which exist in the anti conformation. Experimental evidence suggests that the anti conformation is required of purine and pyrimidine nucleoside di- and triphosphates for enzymic incorporation into polynucleotides (1 - 3). While reviewing the literature on adenosine deaminase we noted that this enzyme may well require the anti conformation in nucleoside substrates. If so, we hoped to use this enzyme as a synthetic tool.

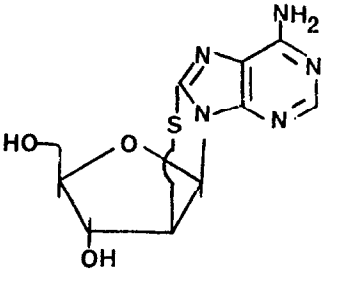
A great deal of work has been carried out concerning substrates for adenosine deaminase. It is known that the 2'- and 3'-OH groups of adenosine do not play a critical role in substrate activity but that a 5'-OH or other hydroxyl capable of acting in place of the 5'-OH is essential for substrate activity (4). A variety of substituents at the 6-position of purine ribosides are hydrolyzed by adenosine deaminase (5). Robins and his coworkers (6) have found that 2-substituted purine nucleosides bind to the enzyme while

bulky substituents in the 8-position prevent adenine nucleosides from being substrates. This latter work indicates that molecules such as 8-bromoadenosine are not substrates because they exist in the syn conformation (3). Other molecules such as 8-aminoadenosine where the substituent is small enough to permit the purine ring to remain in an anti conformation act as substrates.

Robins also found that 8-thioadenosine (I) did not act as a substrate for adenosine deaminase. If this is due to the existence of I in the syn conformation,



I



II

then the 8-thioadenine ring in the anti conformation should be a substrate for the enzyme. Since we have recently developed a novel short synthetic route (7) to 8,2'-thioanhydroadenosine (II) we had available the ideal molecule where the 8-thioadenine ring is constrained in an anti conformation. Further if II were a substrate of adenosine deaminase we would have an exceedingly convenient preparative route to 8,2'-thioanhydroinosine (III).

MATERIALS AND METHODS

8,2'-Thioanhydroadenosine was prepared by the method of Ogilvie and Slotin (7). Paper chromatography was carried out on Whatman 3MM paper in solvent A (i-propyl alcohol: ammonium hydroxide : water in vol. ratio 7:1:2) by the descending technique. Ultraviolet spectra were recorded on a Cary 14 spectrophotometer.

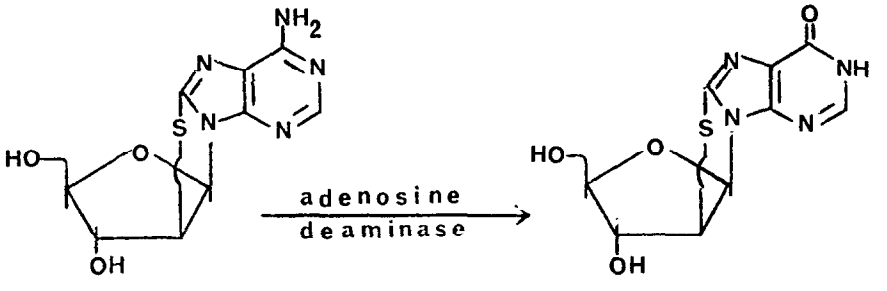
Adenosine deaminase was purchased from the Sigma Chemical Co. The enzyme experiments were carried out at pH 7.5 in 0.05M phosphate buffer

at 25°C. The Km and V max were determined by the procedure of Lineweaver and Burk (8). Products were identified by chromatography in solvent A.

On a preparative scale, II (250mg) was treated with adenosine deaminase (10mg) in 25 ml of buffer solution (pH 7.5, 0.05M phosphate) at 25°C for 75 min. The solution was applied directly to Whatman 3MM paper and chromatographed in solvent A. The product (R_f^A 0.34 compared to R_f^A 0.46 for II) was eluted with water and identified as 8,2'-thioanhydroinosine (III); mp. dec>217°C, mass spec of trimethylsilyl derivative had a parent peak at M/e = 498, UV showed $\lambda_{max}^{H_2O}$ 265.5 (ε = 10,080), the infrared (KBr disc) showed a carbonyl peak at 1700 cm^{-1}. The elemental analysis was consistent with structure III and the yield of III was quantitative both on a weight basis and by spectroscopic determination. The product III was further identified by Raney nickel conversion to 2'-deoxyinosine.

RESULTS AND DISCUSSION

8,2'-Thioanhydroadenosine is a novel substrate for adenosine deaminase with a Km of 2.0 x 10^{-4}M (9) and a V max of 7% that of adenosine. This reaction serves as a very useful preparative route to the hitherto unreported 8,2'-thioanhydroinosine.



II III

The purine ring in II is constrained in an anti conformation while the purine ring in 8-thioadenosine and 8-bromoadenosine is most likely in a syn conformation. Since these latter two molecules are neither substrates

nor inhibitors of adenosine deaminase, while II is a substrate, it would appear that adenosine deaminase should be added to the growing number of enzymes which require as substrates, nucleosides possessing the anti conformation.

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REFERENCES

1. A.M. Kapuler, Ph.D. Dissertation, The Rockefeller University (1969).
2. A.M. Kapuler, C. Monny and A.M. Michelson, Biochim, Biophys. Acta. 217 18 (1970).
3. S.S. Tavale and H.M. Sobell, J. Mol. Biol. 48, 109 (1970).
4.a) H.J. Schaeffer, S. Gurwara, R. Vance, and S. Bittner, J. Med. Chem. 14 367 (1971).
b) R.H. Shah, H.J. Schaeffer, and D.H. Murray, J. Pharm. Sci. 54, 15 (1965).
c) J.L. York and G.A. LePage, Can. J. Biochem. 44, 331 (1966) and references therein.
5.a) R.V. Wolfenden and J.F. Kirsch, J. Am. Chem. Soc. 90, 6849 (1968).
b) R. Wolfenden, J. Am. Chem. Soc. 88, 3157 (1966).
6. L.N. Simon, R.F. Bauer, R.L. Tolman and R.K. Robins, Biochemistry 9, 573 (1970).
7. K.K. Ogilvie and Slotin, submitted for publication.
8. H. Lineweaver and D. Burk, J. Am. Chem. Soc. 56, 658 (1934).
9. For comparison Km of adenosine is 4.7×10^{-5} M.