AFFECT OF SPLEEN AND SNAKE VENOM PHOSPHODIESTERASES ON NUCLEOTIDES CONTAINING NUCLEOSIDES IN THE SYN CONFORMATION

Kelvin K. Ogilvie
Department of Chemistry
McGill University
Montreal, Quebec H3C 3G1

Frank H. Hruska
Department of Chemistry
University of Manitoba
Winnipeg, Manitoba

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SUMMARY: Dinucleotides containing 6-methyldeoxyuridine and inosine have been prepared and subjected to spleen and snake venom phosphodiesterases. Spleen enzyme degrades all nucleotides tested while snake venom causes very little degradation of nucleotides having 6-methyldeoxyuridine in the 3'-terminal position. The clear implication is that snake venom will not recognize nucleoside units in the syn conformation.

Recently several authors have shown (1-3) that certain enzymes catalyzing polynucleotide synthesis, such as polynucleotide phosphorylase and RNA polymerase, will not use as substrates nucleoside di- or triphosphates having the bases in the syn conformation. This has caused considerable interest in determining the conformation of nucleosides and several, such as 8-halopurine nucleosides (4,5) and 6-methylcytidine and 6-methyluridine (6,7) have been shown to exist in the syn conformation in solution. In addition the "wobble" nucleoside inosine has been shown to exist in both syn and anti conformations in the crystalline state (8) although calculations indicate that the barrier to rotation is low (9). In this paper we show that snake venom phosphodiesterase causes very little degradation of deoxyadenylyl-(3'-5')-6-methyl-2'-deoxyuridine(A) but readily degrades thymidylyl-(3'-5')-inosine(D) and

thymidylyl-(3'-5')-isopropylidineinosine(C). Spleen phosphodiesterase degrades all of the above nucleotides and also 6-methyl-2'-deoxyuridylyl-(3'-5')-thymidine(B).

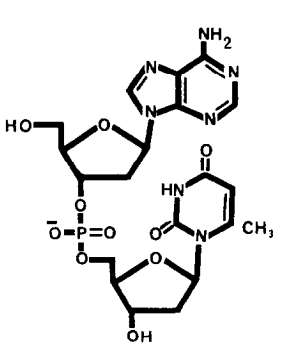
MATERIALS AND METHODS

All nucleotides described were synthesized from the appropriately protected nucleoside units using the phosphotriester method of Letsinger and Ogilvie (10). The methoxytrityl group was used to protect the 5'-hydroxyl positions of all nucleosides during synthesis and the t-butyldimethylsilyl group(II) was used to protect the 3'-hydroxyl of deoxynucleosides. Isopropylidineinosine was used during the synthesis of C and D. Protecting groups were removed prior to treatment with enzymes and the pure nucleotides were characterized by paper chromatography and electrophoresis.

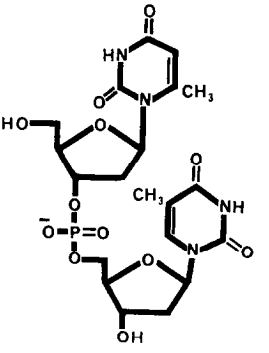
Spleen phosphodiesterase was obtained from several sources including Nutritional Biochemicals Corp., Worthington Biochemicals Corp., and a highly purified sample kindly donated by Dr. J. Spencer of the Department of Biochemistry of McGill University. Enzyme from all the above sources gave the same results. Degradations were carried out using standard procedures at pH 6.5 and 37°C (12,13). Snake venon phosphodiesterase was obtained from Calbiochem. Enzymatic reactions were carried out using standard procedures at pH 9 and 37°C.

RESULTS AND DISCUSSION

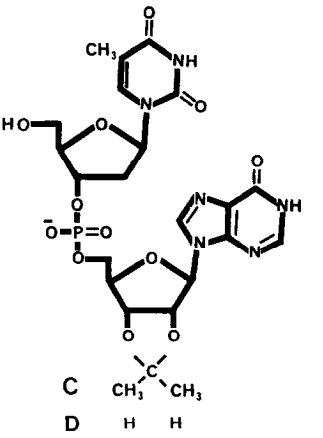
We have recently shown (14) by PMR that 6-methyl-2'-deoxyuridine possess the syn-conformation in solution, both in the free nucleoside and when incorporated into nucleotides such as A. As a result it was clearly of interest to determine the effect of enzymes on this nucleotide. Spleen phosphodiesterase caused complete and rapid degradation of the molecule. However snake venom enzyme caused only 14% degradation after nine hours at 37°C and 54% after



A



B



C CH_3 CH_3

D H H

24 hrs. We therefore synthesized 6-methyl-2'-deoxyuridylyl-(3'-5') thymidine (B) and found that spleen enzyme completely degraded the molecule as did snake venom phosphodiesterase.

Because of the obvious implication of the effect of syn-conformation nucleoside in the 3'-terminal position of nucleotides on snake venom phosphodiesterase, we decided to use the enzyme to test the conformation of inosine when attached to a nucleotide in solution. Several X-ray studies have shown that inosine can exist in either syn or anti-conformations in the crystalline state (8,15) and have implied importance to the observation since inosine is a "wobble" nucleoside. Theoretical studies have shown that the barrier to rotation about the C-N glycoside bond in inosine is very low and our studies on nucleotides C and D show that snake venom rapidly and completely degrades these nucleotides.

The fact that snake venom did cause some degradation of A is consistent with the idea that the barrier to rotation between syn and anti in 6-methyldeoxyuridine is high but that it is nevertheless an equilibrium and over a period of time those molecules of A having the terminal nucleoside in the anti-conformation will be

degraded. Thus this observation of an anti requirement for snake venom phosphodiesterase will have many important implications and has shown here that inosine, at least in the dinucleotides studied, has freedom to exist in the anti-conformation in solution. Spleen phosphodiesterase does not appear to have a similar conformational requirement.

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REFERENCES

1. Kapuler, A.M., Monny, C., and Michelson, A.M. (1970) Biochem. Biophys. Acta, 217, 18-29.
2. Kapuler, A.M., and Reich, E. (1971) Biochemistry, 10, 4050-4061.
3. Ikehara, M., Tazawa, I., and Fukui, T. (1969) ibid, 8, 736-743.
4. Tavale, S.S., and Sobell, H.M. (1970) J. Mol. Biol., 48, 109-123.
5. Ikehara, M., Uesugi, S., and Yoshida, K. (1972) Biochemistry, 11, 830-836.
6. Suck, D., and Saenger, W. (1972) Nature, 235, 333-334.
7. Schweizer, M.P., Banta, E.B., Witkowski, J.T., and Robins, R.K. (1973) J. Amer. Chem. Soc., 95, 3770-3778.
8. Subramanian, E., Madden, J.J., and Bugg, C.E. (1973) Biochem. Biophys. Res. Comm., 50, 691-696.
9. Jordan, F. (1973) Biopolymers, 12, 243-255.
10. Letsinger, R.L., and Ogilvie, K.K. (1967) J. Amer. Chem. Soc., 89, 4801-4803.
11. Ogilvie, K.K. (1973) Can. J. Chem., 51, 3799-3807.
12. Ralph, R.K., Connors, W.J., Schaller, H., and Khorana, H.G. (1963) J. Amer. Chem. Soc., 85, 1988-1997.
13. Razzell, W.F., and Khorana, H.G., (1961) J. Biol. Chem., 236, 1144-1149.
14. Manuscript in preparation.
15. Munns, A.R.I., and Tollin, P. (1970) Acta. Cryst., B26, 1101-1113.