CHEMICAL SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5'-"CAPPED" 2',5'-OLIGOADENYLATES

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The interferon-induced enzyme, 2-5A synthetase, effects the conversion: $nATP \rightarrow pppA(pA)_{n-1} + (n-1)ppi$. However, this enzyme can also add adenylate residues, in 2',5'-linkage, to a variety of nucleotides or oligonulceotides supplied as primers. In one such reaction, originally reported by Ball and White, diadenosine tetraphosphate becomes 2'-adenylated to give the products $A5'pppp5'A2'(p5'A)_n$. In order to provide an independent chemical confirmation of the structure of these enzymatic products, and to provide analogues that would be useful in further delineating the relationship between 2-5A structure and activation of the 2-5A-dependent endoribonuclease, we have chemically synthesized a number of derivatives of 2',5'-oligoadenylates "capped" at the 5'-terminus with adenosine polyphosphates.

The oligonucleotides A5'pp5'A2'pA2'pA(Ap_2A_3) and A5'ppp5'A2'pA2'pA (Ap_3A_3) were prepared by reaction of AMP or ADP, respectively, with the 5'-phosphoroimidazolide of A2'pA2'pA. $A5pppp5'A2'(p5'A)_n$ (n = 1, Ap_4A_2; n=2, Ap_4A_3; n=3, Ap_4A_4) were synthesized by reaction of $p5'A2'(p5'A)_n$ (n=1∿3) with adenosine 5'-trimetaphosphate. All structures were confirmed with ^1H and ^{31}P NMR, and by direct comparison with enzymatic products.

In extracts of mouse L cells programmed with encephalomyocarditis virus RNA, Ap_4A_4 and Ap_4A_3 were approximately equipotent with 2-5A as inhibitors of protein synthesis ($IC_{50} \sim 10^{-9}$M). The oligomers Ap_3A_3 and Ap_4A_2 were approximatley 100 x less active than 2-5A, and Ap_2A_3 was without discernable activity. Thus the order $Ap_4A_4 \sim Ap_4A_3 \sim$ 2-5A $>> Ap_3A_3 \sim Ap_4A_2 >> Ap_2A_3$ was obtained. When affinity for the 2-5A-dependent endonuclease was determined (by displacement of 2-5A[^{32}p]-pCp from endonuclease), all of the analogues as well as 2-5A itself has similar affinities for the endonuclease except for Ap_4A_2 which was bound to the endonuclease ∿100 x less effectively.

These results show that blocking of the β or γ phosphates of the translational inhibitors ppA2'pA2'pA or pppA2'pA2'pA leads to a loss of ability to activate the 2-5A-dependent endonuclease even though these oligomers bind to the endonuclease as well as 2-5A itself. The results obtained with the tetraphosphate Ap_4A_3 and Ap_4A_3 confirm the earlier assigned structures and the biological activity of the products of the 2-5A synthetase reaction.