

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: $n\text{ATP} \rightarrow \text{pppA}(\text{pA})_{n-1}^+ (n-1)\text{ppi}$. However, this enzyme can also add adenylate residues, in 2',5'-linkage, to a variety of nucleotides or oligonucleotides supplied as primers. In one such reaction, originally reported by Ball and White, diadenosine tetraphosphate becomes 2'-adenylated to give the products $\text{A5'ppp5'A2'}(p5'A)_n$. 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 $\text{A5'pp5'A2'pA2'pA}(\text{Ap}_2\text{A}_3)$ and $\text{A5'ppp5'A2'pA2'pA}(\text{Ap}_3\text{A}_3)$ were prepared by reaction of AMP or ADP, respectively, with the 5'-phosphorimidazolide of A2'pA2'pA . $\text{A5pppp5'A2'}(p5'A)_n$ ($n = 1, \text{Ap}_4\text{A}_2$; $n=2, \text{Ap}_4\text{A}_3$; $n=3, \text{Ap}_4\text{A}_4$) 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 ($\text{IC}_{50} \sim 10^{-9}\text{M}$). The oligomers Ap_3A_3 and Ap_4A_2 were approximately 100 x less active than 2-5A, and Ap_2A_3 was without discernable activity. Thus the order $\text{Ap}_4\text{A}_4 \sim \text{Ap}_4\text{A}_3 \sim 2-5A \gg \text{Ap}_3\text{A}_3 \sim \text{Ap}_4\text{A}_2 \gg \text{Ap}_2\text{A}_3$ was obtained. When affinity for the 2-5A-dependent endonuclease was determined (by displacement of $2-5A[^{32}\text{P}]\text{-pCp}$ 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_4 confirm the earlier assigned structures and the biological activity of the products of the 2-5A synthetase reaction.