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2 and 8-Azido ATP: Photoaffinity Labeling of 2-5A Synthetase, Enzymatic Synthesis of 2 and 8-Azido Anamgs of 2-5A for Use as Photoaffinity Probes of Rnase L

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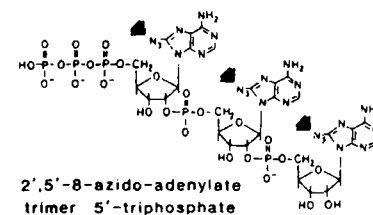
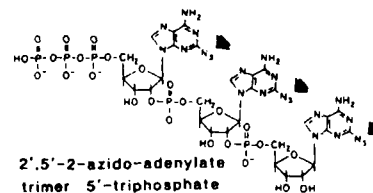
2- AND 8-AZIDO ATP: PHOTOAFFINITY LABELING OF 2-5A SYNTHETASE,
ENZYMATIC SYNTHESIS OF 2- AND 8-AZIDO ANALOGS OF 2-5A FOR USE AS
PHOTOAFFINITY PROBES OF RNASE L

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The 2-5A/RNase L system is widely accepted to be part of the antiviral mechanism of interferon^{1,2} and may also regulate cell growth³, where 2-5A exerts its biological effects by activating RNase L. Numerous 2-5A analogs have been synthesized with the goal of binding to, but not activating, RNase L. However, these analogs have had limitations when studied *in vitro*. We have reported on the unique properties of 2-5A molecules in which Rp and Sp chirality have been introduced into the 2-5A backbone to form the phosphorothioate analogs of 2-5A⁴⁻⁶. By chiral modification of the 2-5A backbone, we have examined the stereochemical requirements for binding to and activation of RNase L. In order to elucidate the mechanism by which 2-5A binds to and activates RNase L, it is essential to ascertain the interactions in the nucleotide binding domain of RNase L and/or other 2-5A binding proteins. By employing photoaffinity labeling using enzymatically synthesized 2- and 8-azido photoprobes of 2-5A, we have characterized the 2- and 8-azido trimer 5'-triphosphate photoprobes of 2-5A and described the biological properties of these photoprobes (Figure 1) of 2-5A and their application in photolabeling of RNase L and/or other 2-5A binding proteins⁷ have been reported. 2- and 8-azidoATP are substrates for the 2-5A synthetase from IFN- β -treated HeLa cell extracts and from rabbit reticulocyte lysates, but not for highly purified 2-5A synthetase from rabbit reticulocyte lysates⁸. UV irradiation results in the photoinsertion of 2- and 8-azidoATP into the catalytic site of the 2-5A synthetase. Analysis of Scatchard plots of the 2-5A synthetase suggests the presence of high affinity and low affinity binding sites that may correspond to the acceptor and the 2'-adenylation sites of the enzyme.

The azido 2-5A photoprobes bind to and activate RNase L as well as does authentic 2-5A as shown by radiobinding, core-cellulose and rRNA cleavage assays using RNase L of L929 cell extracts⁷. The 2-azido photoprobe photolabels one protein (M_r 185000), whereas the 8-azido photoprobe photolabels six proteins (M_r 46000, 63000, 80000, 89000, 109000, and 158000). Under the same conditions, $p_3A_4[^{32}P]pCp$ photolabels only one protein (M_r 80000). Because the photosensitive groups on the 2- and 8-azido 2-5A photoprobes are attached to C-2 and C-8 of the adenine rings and because the biological properties of the 2- and 8-azido 2-5A trimer 5'-triphosphates are similar to those of authentic p_3A_3 , they will be powerful probes for mapping the amino acids in the domain of RNase L and/or other 2-5A binding proteins. Our eventual goal is to elucidate the role(s) of 2-5A in cell metabolism. Supported in part by NSF grant DMB84-15002 and NIH grant P01 CA29545.



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