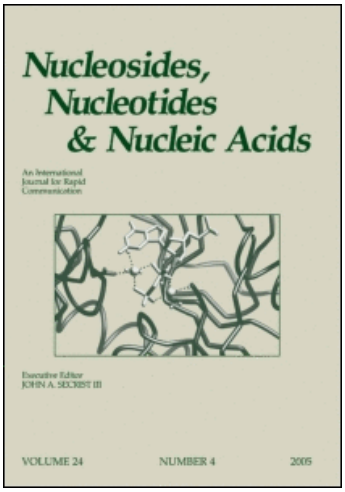

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2 and 8-Azido ATP: Photoaffinity Labeling of 2-5A Synthetase, Enzymatic Synthesis of 2 and 8-Azido Anamgs of 2-5A for Use as Photoaffinity Probes of Rnase L

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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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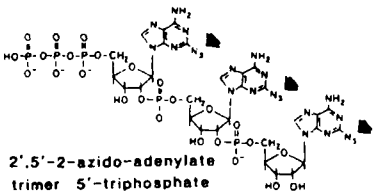
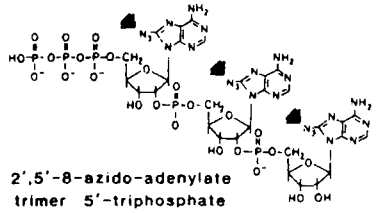
The 2-5A/RNase L system is widely accepted to be part of the antiviral mechanism of interferon[1,2] and may also regulate cell growth[3], where 2-5A exerts its biological effects by activating RNase L. Numerous 2-5A analogs have been synthesized with the goal of binding to, but not activating, RNase 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 2-5A[4-6]. 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'-triphoshate 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 proteins[7] 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 lysates[8]. 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 extracts[7]. 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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