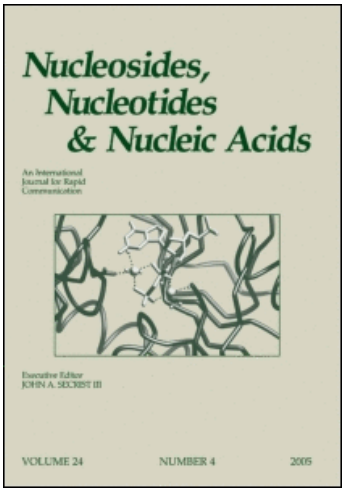

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Cordycepin Analogs of 2-5 a as Activators of RNase L: Study of the Structural Requirements for RNase L Activation

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CORDYCEPIN ANALOGS OF 2-5A AS ACTIVATORS OF RNASE L: STUDY OF THE STRUCTURAL REQUIREMENTS FOR RNASE L ACTIVATION

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Enzymatically and chemically synthesized cordycepin analogs of 2-5A[+] trimer and tetramer were found to be biologically active as protein synthesis inhibitors in intact cultured human fibroblast and murine L929 cells[1,2]. In rabbit reticulocyte lysates, the cordycepin tetramer analog of 2-5A inhibits protein synthesis through binding to and activation of RNase L[3]. Our present results using L929 cell extracts provide direct evidence that the cordycepin analogs of 2-5A can bind to and activate RNase L.

Radiobinding assay. The chemically and enzymatically synthesized cordycepin trimer and tetramer analogs of 2-5A were able to bind to RNase L in murine L929 cell extracts (FIG. 1A). The concentration of cordycepin trimer and tetramer analogs required to displace 50% of the 2-5-$p_3A_4[^{32}P]pCp$ probe from RNase L was 4×10^{-8} and 1×10^{-8} M, respectively. Both analogs were about ten times less effective than their authentic counterparts, in good agreement with a previous report[4].

Core-cellulose assay. The chemically and enzymatically synthesized cordycepin trimer and tetramer analogs of 2-5A were able to activate immobilized and partially purified RNase L (FIG. 1B). Hydrolysis of poly$U[^{32}P]pCp$ was monitored to determine the extent of RNase L activation. The cordycepin analogs activated RNase L as low as 2×10^{-8} M.

[+]2-5A, 2',5'-linked oligoadenylates -- ppp5'A2'$(p5'A)_n$, where n = 2,3; the cordycepin analogs of 2-5A, ppp5'(3'dA)2'$(p5'3'dA)_n$.

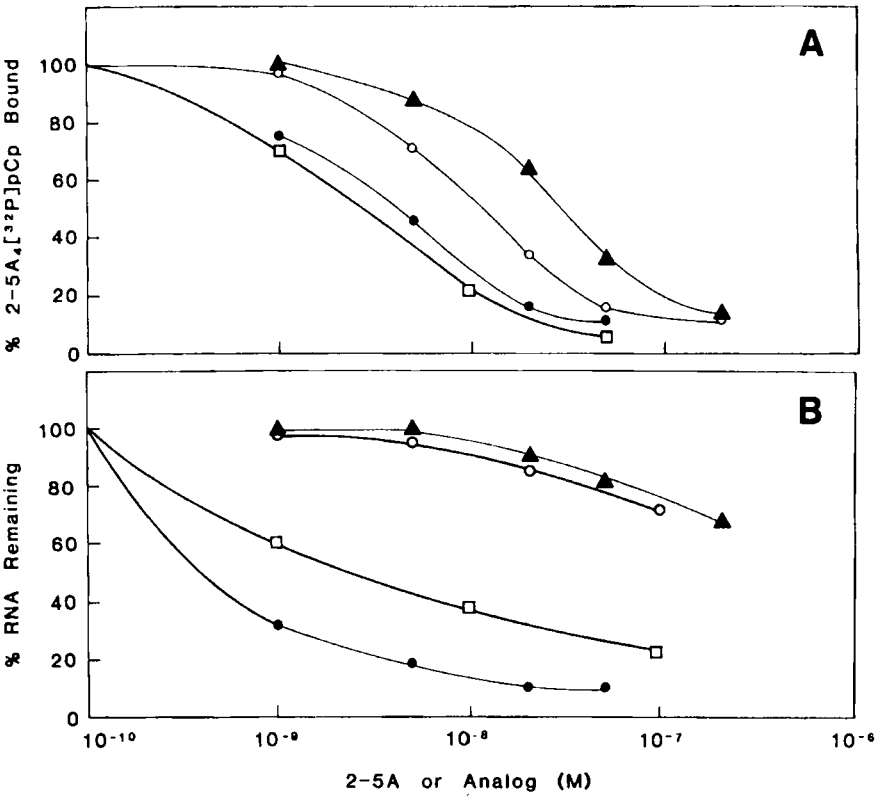

FIGURE 1. A. The ability of the cordycepin analogs to displace 2-5-$p_3A_4[^{32}P]pCp$ from RNase L in mouse L929 cell extracts was determined in radiobinding assays[5]. (100% represents 12,000 cpm; input 2-5-$p_3A_4[^{32}P]pCp$ was 21,000 cpm). B. The ability of the cordycepin analogs to activate purified RNase L was determined by hydrolysis of poly$U[^{32}P]pCp$ in core-cellulose assays[6]. (100% represents 5,000 cpm poly$U[^{32}P]pCp$; total input was 27,000 cpm). p_3A_3, ●; p_3A_4, □; $p_3 3'dA_3$, ▲; $p_3 3'dA_4$, o.

Ribosomal cleavage assay. In functional ribosomal cleavage assays, 2-5A or its analogs activated RNase L to cleave rRNA present in intact ribosomes to yield specific cleavage products[7]. The cordycepin analogs of 2-5A can activate RNase L in the L929 cell-free system as low as 1×10^{-8} M (FIG. 2).

The findings described here are consistent with our earlier reports[1,3] demonstrating that the inhibition of protein synthesis by the 5'-triphosphate cordycepin trimer and tetramer analogs is directed through activation of RNase L and not other effects as has been suggested[9]. In the radiobinding and rRNA cleavage assays with extracts

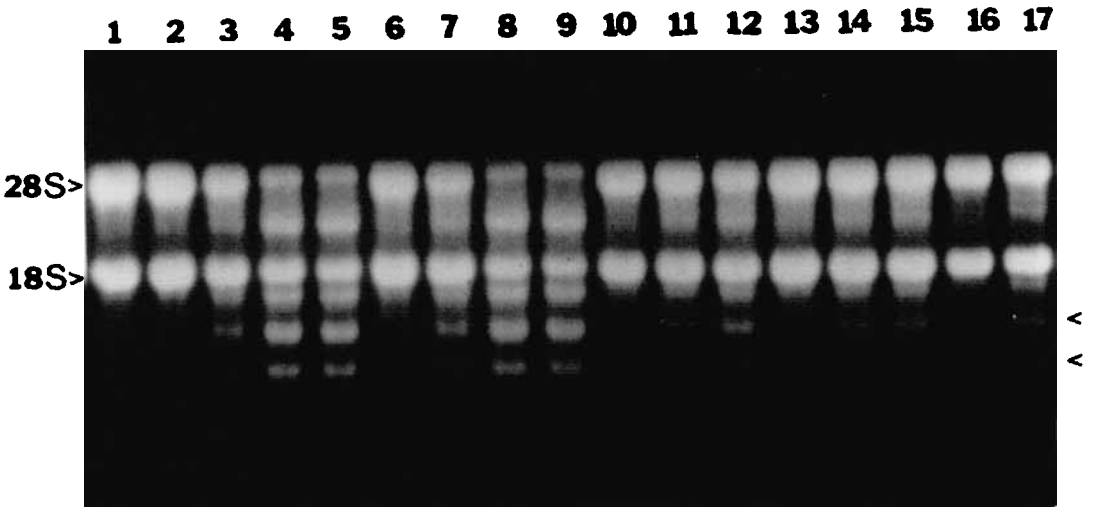

FIGURE 2. rRNA cleavage assays with enzymatically and chemically synthesized cordycepin analogs of 2-5A were performed as described[8]. L929 cell extracts were incubated in the absence (lane 1) or presence of p_3A_3 and p_3A_4 at 10^{-10}, 10^{-9}, 10^{-8} and 10^{-7} M (lanes 2-5 and 6-9, respectively); chemically synthesized $p_3 3'dA_3$ and $p_3 3'dA_4$ at 10^{-9}, 10^{-8} and 10^{-7} M (lanes 10-12 and 13-15, respectively); enzymatically synthesized $p_3 3'dA_4$ at 10^{-9} and 10^{-8} M (lanes 16-17) at 30°C for 1 hr. Total RNA was extracted, denatured and analyzed on 1.8% agarose gels after staining with ethidium bromide. The RNA bands are visualized with uv light. The positions of 28S and 18S rRNA as well as specific cleavage products (arrows) are indicated.

of L929 cells (FIG. 1A, 2), the cordycepin analogs were ten times less active than authentic 2-5A, which differs from our earlier report on the inhibition of protein synthesis in intact L929 cells in which the cordycepin analogs were more active than 2-5A[1]. The reason for this difference may be that one hour incubation (Fig. 1A, 2) is not sufficient to utilize the advantage of the prolonged effect of the much more stable cordycepin analogs, as was observed hours or days after initial treatment of L929 cells[1]. This apparent discrepancy is emphasized in the core-cellulose assay, in which phosphodiesterases are removed.

Variation between the observations from this and other laboratories[1-4,9] that the cordycepin analogs of 2-5A have their effects through activation of RNase L may be explained by the suggestion of Kerr and coworkers[10] that, unlike all other analogs tested to date, the 5'-triphosphate is required for activation of RNase L by the cordycepin ana-

logs. This suggestion has been confirmed in our laboratory, i.e., we have demonstrated that the cordycepin trimer and tetramer 5'-monophosphate analogs can bind to RNase L equally as well as the 5'-triphosphates; however, the 5'-monophosphates can not activate RNase L in functional assays[11]. Our data show that the 3'-hydroxyls of 2-5A are not critical in binding to and activation of RNase L; however, the 5'-triphosphate of the cordycepin analogs of 2-5A is required for activation of RNase L.

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REFERENCES

1. Lee, C., & Suhadolnik, R.J. (1983) FEBS Lett. 157, 205-209.
2. Eppstein, D.A., Van Der Pas, M.A., Schryver, B.B., Sawai, H., Lesiak, K., Imai, J., & Torrence, P.F. (1985) J. Biol. Chem. 260, 3666-3671.
3. Suhadolnik, R.J., Devash, Y., Reichenbach, N.L., Flick, M.B., & Wu, J.M. (1983) Biochem. Biophys. Res. Commun. 111, 205-212.
4. Sawai, H., Imai, J., Lesiak, K., Johnston, M.I., & Torrence, P.F. (1983) J. Biol. Chem. 258, 1671-1677.
5. Knight, M., Wreschner, D.H., Silverman, R.H., & Kerr, I.M. (1981) Methods Enzymol. 79, 216-227.
6. Silverman, R.H. (1985) Analyt. Biochem. 144, 450-460.
7. Wreschner, D.H., James, T.C., Silverman, R.H., & Kerr, I.M. (1981) Nucleic Acids Res. 9, 1571-1581.
8. Karikó, K., & Ludwig, J. (1985) Biochem. Biophys. Res. Commun. 128, 695-698.
9. Krause, D., Lesiak, K., Imai, J., Sawai, H., Torrence, P.F., & Silverman, R.H. (1986) J. Biol. Chem. 261, 6836-6839.
10. Haugh, M.C., Cayley, P.F., Serafinowska, H.T., Norman, D.G., Reese, C.B., & Kerr, I.M. (1983) Eur. J. Biochem. 132, 77-84.
11. Karikó, K., Li, S.W., Sobol, R. W., Jr., Reichenbach, N.L., Charubala, R., Pfleiderer, W., & Suhadolnik, R.J., manuscript submitted.