Vol. 83, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 28, 1978 Pages 414-420

INHIBITION OF RIBOSOMAL PEPTIDYLTRANSFERASE WITH CYTIDYLYL-3'→5'- /2'(3')-O-L-PHENYLALANYL /-L-ADENOSINE

Prakash Bhuta and Jiří Žemlička*

Michigan Cancer Foundation and Department of Oncology

Wayne State University School of Medicine

Detroit, Michigan 48201

Received May 9,1978

SUMMARY

The title compound 1e, obtained by chemical synthesis, is an inhibitor of *E. coli* ribosomal peptidyltransferase. A 50% inhibition of peptidyltransferase-catalyzed N-Ac-Phe-puromycin formation at puromycin concentration 1×10^{-4} M with 70 S ribosome-poly U-N-Ac[^{14}C]-Phe-tRNA complex occurred at 5×10^{-4} M of 1e. In contrast, the parent compound 2'(3')-O-L-phenylalanine-L-adenosine (1b) is a much weaker inhibitor causing only 5% inhibition at 1×10^{-3} M. Alkaline hydrolysis of compound 1e to cytidylyl-3'→5'-L-adenosine (1c) results in a greatly diminished inhibition which, however, exceeds that of 1b by a factor of two. The inhibition of peptidyltransferase with 1e can be reversed by puromycin. The latter effect levels off at 40% inhibition.

INTRODUCTION

Inhibition of ribosomal peptidyltransferase activity is of interest in connection with the mechanism of protein synthesis and mode of action of certain antibiotics. Available inhibitors can be grouped in two classes: compounds structurally related to the 3' terminal sequence of aminoacyl tRNA which are able to accept a peptide chain from peptidyl tRNA (e.g., antibiotic puromycin) and derivatives which inhibit the peptide chain elongation process by binding to the appropriate ribosomal site without subsequent reaction with peptidyl tRNA (e.g., antibiotics chloramphenicol, gougerotin, etc.). Attachment of a cytidylyl-3' residue greatly enhances the acceptor activity and/or inhibitory properties of puromycin and similar aminoacylated compounds

* To whom correspondence should be addressed at the Michigan Cancer Foundation, 110 E. Warren Avenue, Detroit, MI 48201 (USA).

0006-291X/78/0832-0414$01.00/0

derived from natural nucleosides (1-5). The presence of a 3'→5' internucleotide linkage is essential for the enhancement of acceptor (inhibitory) activity. Thus, attachment of cytidylyl-2' residue to puromycin resulted in a significant decrease in inhibition of polypeptide synthesis in a eucaryotic ribosomal system (3). However, a protein synthesis inhibitor with a cytidylyl moiety joined to an aminoacyl derivative of unnatural nucleoside by 3'→5' internucleotide linkage has not yet been described. Recent findings (6) with carbocyclic nucleoside analogs of L-puromycin indicate that the latter or similar 2'(3')-O-aminoacyl-L-adenosines may also act as inhibitors of ribosomal peptidyltransferase. The carbocyclic analogs of puromycin described to date (6), are not suitable for derivatization with cytidine 3'-phosphate because of the lack of the function equivalent to the 5' hydroxy group in nucleosides. We have therefore studied the inhibition of ribosomal peptidyltransferase with 2'(3')-O-L-phenylalanyl-L-adenosine (Ib) and the corresponding cytidylyl-3'→5' derivative Ie.

MATERIALS AND METHODS

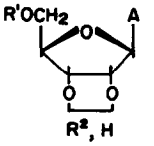
L-Adenosine was obtained through courtesy of Dr. Harry B. Wood, Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, Maryland, $[\alpha]_D^{25}$ + 52.5°, adenosine - 53.4° (c 0.05, H_2O, pH 7), CD spectrum (H_2O, pH 7), max 261 nm ([θ] + 3 100), adenosine (7) max 265 nm ([θ] - 2 970).

N-Dimethylaminomethylene-L-adenosine was prepared as described (8) for the corresponding D-compound, yield 93%, mp 196 -202°; lit. (8) 205 - 207° (D-derivative), CD spectrum (H_2O, pH 7) max 305 nm ([θ] + 2 600).

5'-O-(4-Methoxytrityl)-L-adenosine (9) was obtained by tritylation of N-dimethylaminomethylene-L-adenosine according to the procedure (10) used for D-compound, yield 74%, mp 196 - 202°, lit. (11) 189 - 191° (D-derivative).

2'(3')-O-(N-Benzyloxycarbonyl)-L-phenylalanyl-L-adenosine (Ia) was prepared from 5'-O-(4-methoxytrityl)-L-adenosine as described for the corresponding D-nucleoside (12), yield 46%, NMR spectrum (13) indicated the presence of 73.5% 3'-isomer and 26.5% 2'-isomer. Anal. Calcd. for $C_{27}H_{28}N_6O_7$: C, 59.12; H, 5.15; N, 15.32. Found: C, 58.85; H, 5.15; N, 15.10. Compound Ia was homogeneous on tlc (13,14) using silica gel (F 254) coated aluminum foil (Merck, Darmstadt, Germany) in solvent CH_2Cl_2-CH_3OH (9:1), CD spectrum (ethanol) max 266 nm ([θ] + 4 350).

2'(3')-O-L-Phenylalanyl-L-adenosine (Ib) was obtained as described (12) for the corresponding D-nucleoside by hydrogenolysis of Ia in 80% yield. UV spectra and electrophoretical mobility in 1 M CH_3COOH were identical to those of 2'(3')-O-L-phenylalanyl-D-adenosine (12).



I, A = adenine

Ia : R' = H, R^2 = $C_6H_5CH_2OCOPhe$

Ib : R' = H, R^2 = Phe

Ic : R' = cytidylyl-3', R^2 = H

Id : R' = cytidylyl-3', R^2 = $C_6H_5CH_2OCOPhe$

Ie : R' = cytidylyl-3', R^2 = Phe

Cytidylyl-3'→5'-/2'(3')-O-(N-benzyloxycarbonyl)/-L-phenylalanyl-L-adenosine (Id) was prepared according to the general procedure (10) used for the corresponding D-ribostereoisomer using 2',5'-di-O-tetrahydropyranylcytidine 3'-phosphate (4) and nucleoside Ia as starting materials in 34% yield. Compound Id was homogeneous on tlc (Avicel microcrystalline cellulose, solvent 1-butanol saturated with 10% acetic acid (10)) and its UV spectrum corresponded to that of the D-ribostereoisomer (10).

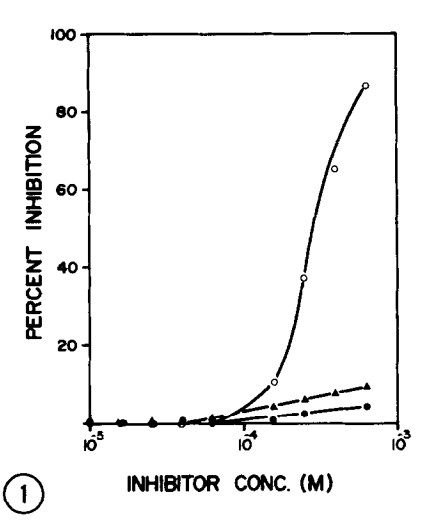
Cytidylyl-3'→5'-/2'(3')-O-L-phenylalanyl/-L-adenosine (Ie) was obtained by hydrogenolysis of Id in 60% yield according to a general procedure (10). UV spectrum and electrophoretic mobility in 1 M acetic acid were identical to those of D-ribostereoisomer (10). Pancreatic ribonuclease degradation of Ie gave cytidine 3'-phosphate / L-adenosine ratio of 0.9 leaving 7% of intact material (2'→5' isomer). Compound Ie (or Ic because of the hydrolysis of 2'(3')-O-L-phenylalanyl residue during the incubation) was stable towards Russell's viper venom phosphodiesterase. This is in accord with previous results on other dinucleoside phosphates containing a 3' terminal L-nucleoside which showed either a complete (15) or a predominant (9) resistance.

Cytidylyl-3'→5'-L-adenosine (Ic) was prepared by alkaline hydrolysis of Id during chromatography in 2-propanol - conc. NH_4OH - H_2O (7 : 1 : 2) on Whatman 3 MM paper. UV spectrum of Ic eluted from the paper with water corresponded to that of cytidylyl-3'→5'-adenosine.

Samples for ribosomal assays were prepared as described (16). Assay of peptidyltransferase activity followed the established procedure (13). Inhibition of puromycin reaction by Ib and Ie was performed as described for chloramphenicol (17). Experimental details are given in legends to the corresponding figures.

RESULTS AND DISCUSSION

Although neither nucleoside Ib nor dinucleoside phosphate Ie accepts Ac-Phe from N-Ac-Phe-tRNA in a peptidyltransferase catalyzed reaction (data not shown), compound Ie is a comparatively strong inhibitor of the puromycin reaction (50% inhibition at ca. 5×10^{-4} M) as indicated in Fig. 1. The



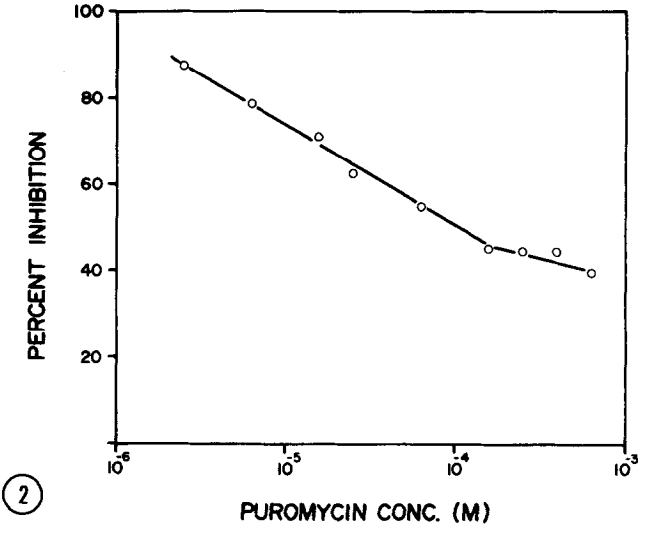


Figure 1. The inhibition of peptidyltransferase-catalyzed N-Ac-Phe-puromycin formation, by Ie (○), Ib (●) and Ic (▲). For this purpose, Ic was obtained by hydrolysis of Ie for 1 h in methanolic NH_3 saturated at 0°C. Compound Ic alone or in the presence of an equimolar amount of phenylalanine gave the same extent of inhibition (data not shown). The latter was also not changed after further purification of Ie by preparative paper electrophoresis on Whatman 3 MM paper in 1 M CH_3COOH. Each reaction mixture contained in 0.1 ml: 0.05 M Tris-HCl (pH 7.4), 0.1 M NH_4Cl, 0.01 M $MgCl_2$, 4.0 A_{260} units of NH_4Cl-washed 70 S ribosomes from *E. coli* MRE 600 cells, 10 µg poly U, 0.46 A_{260} units of N-Ac-^{14}C-Phe-tRNA (approximately 5000 cpm, specific activity 0.213 nmoles of ^{14}C-phenylalanine per mg tRNA), puromycin 1 x 10^{-4} M. Following the incubation at 37°C for 30 min., the reaction was stopped by addition of 3.0 ml of 2.5% trichloroacetic acid at 0°C. The reaction mixture was worked up as described (16). Percent inhibition represents the difference between the radioactivity retained on the filter with both puromycin and inhibitor and that retained in the presence of only puromycin. The amount of radioactivity remaining in the presence of puromycin (1 x 10^{-4} M) was 1485 cpm.

Figure 2. Reversal of inhibition of peptidyltransferase-catalyzed N-Ac-Phe-puromycin formation. The reaction conditions were the same as those described in Fig. 1, except that the concentration of puromycin varied as indicated on the abscissa and the concentration of Ie was kept at 3 x 10^{-4} M. For calculation of percent inhibition, cf. Fig. 1.

parent nucleoside Ib is a very weak inhibitor (5% inhibition at ca 1 x 10^{-3} M) which may seem surprising because analogous carbocyclic analogs of puromycin exhibit a fair degree of inhibition in a poly-UC-directed polyphenylalanine synthesis (6). However, it was noted that with poly U as a messenger the comparative effects were smaller (6). Inhibitory (but not acceptor) activity of Ie and Ib in which the absolute configuration of the 3' terminal ribofura-

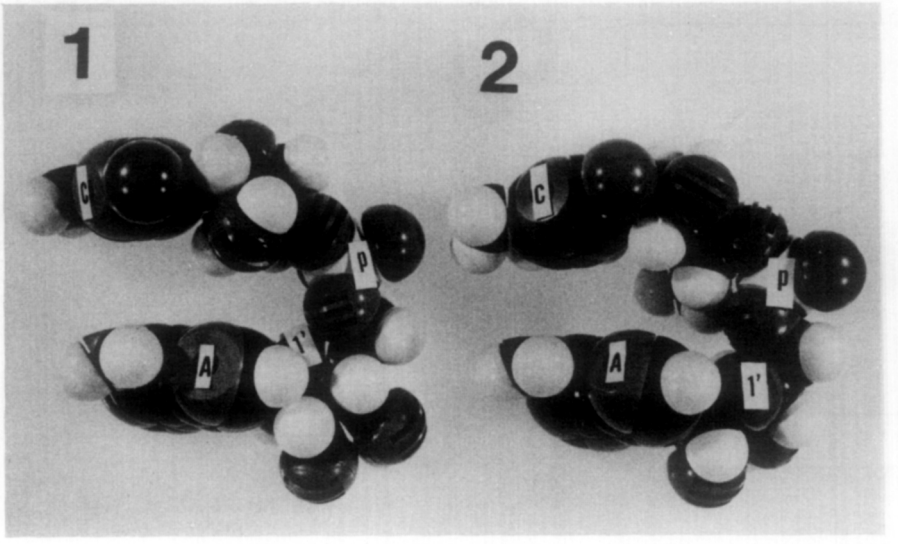


Figure 3. Space-filling models of cytidylyl-3'→5'-L-adenosine (1) and cytidylyl-3'→5'-D-adenosine (2). Nucleoside residues are in a stacked anti-g,g conformation. Letters A and C denote adenine and cytosine residues, p phosphodiester bridge and 1' (D- or L-) ribofuranose oxygen.

nose moiety (L) is known, can be taken as evidence for the configurational assignment of carbocyclic analogs of puromycin ("series b," 6) as corresponding to the L-ribose.

It is also of interest that a small fraction of inhibitor activity (ca 10% at 1 x 10^{-3} M) remains even after alkaline hydrolysis of Ie (Fig. 1). Pure cytidylyl-3'→5'-L-adenosine (Ic) alone or after addition of an equimolar amount of phenylalanine exhibited approximately the same level of inhibition. Thus, the inhibitory activity of Ic exceeds that of nucleoside Ib by a factor of 2. The inhibition of the peptidyltransferase-catalyzed transfer of Ac-Phe from N-Ac-Phe-tRNA can be reversed by the addition of puromycin (Fig. 2). Thus, with an increasing concentration of puromycin the inhibition decreases to ca. 40% where it levels off. This can be taken as an indication that compound Ie is capable of acting at the same ribosomal site (presumably the A-site) as puromycin. However, from an incomplete reversal with puromycin (Fig. 2), a "mixed" type of inhibition may be presumed. It

is noteworthy that carbocyclic analogs of puromycin ("series b," 6) are assumed to be "competitive inhibitors of peptidyltransferase" although little supportive evidence is available.

In this respect, a comparison of space filling models of cytidylyl-3'→5'-L-adenosine (Ic) and "natural" cytidylyl-3'→5'-D-adenosine is of interest. As can be seen from Fig. 3, both compounds can assume very similar stacking patterns, arrangement of bases and position of the phosphodiester bridge. The only major difference is the orientation of the ribofuranose ring. Thus, the planes of these rings in both derivatives are roughly perpendicular to each other. The latter factor is possibly the most important for a "misfit" of compound Ie on peptidyltransferase.

ACKNOWLEDGEMENTS

Thanks are due to Dr. H. L. Chung for spectroscopic measurements and optical rotations. Elemental analysis of compound Ia was performed by Micro-Tech Laboratories, Inc., Skokie, Illinois. This investigation was supported in part by U.S.P.H.S. Research Grant No. CA 21388 from the National Cancer Institute and in part by an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit.

REFERENCES

1. Rychlík, I., Chládek, S. and Žemlička, J., Biochim. Biophys. Acta 138, 640-642 (1967).
2. Harris, R.J., Hanlon, J.E. and Symons, R.H., Biochim. Biophys. Acta 240, 244-262 (1971).
3. Hengesh, E.J. and Morris, A.J., Biochim. Biophys. Acta 299, 654-661 (1973).
4. Chládek, S., Ringer, D. and Quiggle, K., Biochemistry 13, 2727-2735 (1974).
5. Ringer, D., Quiggle, K. and Chládek, S., Biochemistry 14, 514-520 (1975).
6. Vince, R. and Daluge, S., J. Med. Chem. 17, 578-583 (1974).
7. Teng, N.N.H., Itzkowitz, M.S. and Tinoco, I., Jr., J. Am. Chem. Soc. 93, 6257-6264 (1971).
8. Žemlička, J. and Holý, A., Collect. Czech. Chem. Commun. 32, 3159-3168 (1967).
9. Tazawa, I., Tazawa, S., Stempel, L.M. and Ts'o, P.O.P., Biochemistry 9, 3499-3514 (1970).

10. Chládek, S. and Žemlička, J., J. Org. Chem. 39, 2187-2193 (1974).
11. Lohrmann, R. and Khorana, H.G., J. Am. Chem. Soc. 86, 4188-4194 (1964).
12. Žemlička, J., Chládek, S., Haladová, Z. and Rychlík, I., Collect. Czech. Chem. Commun. 34, 3755-3768 (1969).
13. Žemlička, J., Chládek, S., Ringer, D. and Quiggle, K., Biochemistry 14, 5239-5249 (1975).
14. Chládek, S., Ringer, D. and Žemlička, J., Biochemistry 12, 5135-5138 (1973).
15. Holý, A. and Šorm, F., Collect. Czech. Chem. Commun. 34, 3383-3401 (1969).
16. Bhuta, P., Li, C. and Žemlička, J., Biochem. Biophys. Res. Commun. 77, 1237-1244 (1977).
17. Pestka, S., Arch. Biochem. Biophys. 136, 80-88 (1970).