Hydrolysis of an RNA dinucleoside monophosphate by neomycin B

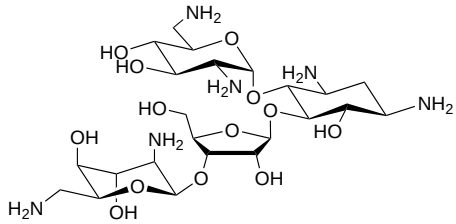
Sarah R. Kirk and Yitzhak Tor*

Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093-0358, USA

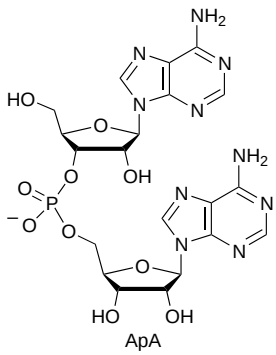
Neomycin B is shown to accelerate the phosphodiester hydrolysis of adenylyl(3′–5′)adenosine (ApA) more effectively than a simple unstructured diamine.

Current approaches to site-directed RNA hydrolysis involve the conjugation of a reactive moiety capable of cleaving phosphodiester bonds to a recognition element capable of sequence-specifically hybridizing to RNA.[1] In most cases, a metal complex is covalently attached to a DNA strand which forms a stable heteroduplex. Upon hybridization, a Lewis acid is placed in close proximity to the RNA backbone to effect hydrolysis.[2] In a similar fashion, DNA–polyamine conjugates have been demonstrated to induce site-directed RNA strand scission.[3] While these high molecular-weight conjugates are promising sequence-specific RNA cleavers, combining recognition and catalysis in a single small organic molecule has remained a challenging problem.[4]

The discovery that aminoglycoside antibiotics interact specifically with diverse RNA molecules such as group I introns,[5] hammerhead ribozymes[6] and the HIV-1's TAR[7] and RRE[8] sites has attracted considerable interest and stimulated studies attempting to identify the elements involved in these recognition phenomena.[9–11] As highly functionalized polycationic oligosaccharides, interactions between the aminoglycosides' polar residues (*i.e.* amino and hydroxy groups) and the RNA backbone and/or heterocyclic bases are likely to occur. Since simple unstructured polyamines[12,13] as well as basic polypeptides[14] have been shown to catalyze RNA hydrolysis, we hypothesized that aminoglycoside antibiotics may exhibit similar effects. As a first step toward developing small molecules capable of both recognizing RNA and cleaving it, we demonstrate that neomycin B is able to accelerate the hydrolysis of a ribodinucleoside monophosphate.



Neomycin B



ApA

The hydrolysis rate of adenylyl(3′–5′)adenosine (ApA) in the presence of 0.3 M neomycin B, 1,3-diaminopropane and 3-aminopropan-1-ol was investigated at pH 8.0 and 50 °C.† Reversed-phase HPLC was employed to measure the rate of appearance of adenosine (A) against the loss of ApA.‡ Neomycin B hydrolyzes ApA with a pseudo-first-order rate constant of $1.6 \pm 0.1 \times 10^{-5}$ min^{-1}, approximately three times faster than 1,3-diaminopropane ($5 \pm 1 \times 10^{-6}$ min^{-1}) and more than 50 times faster than Tris buffer alone or 3-aminopropan-1-ol ($3–4 \pm 2 \times 10^{-7}$ min^{-1}) (Fig. 1).§ The observed hydrolysis rates were not significantly altered when 2 mM EDTA was added, excluding metal ion-mediated hydrolysis. Furthermore, the observed formation of adenosine 2′,3′-cyclic monophosphate (A > p) and its subsequent degradation to adenosine 2′-phosphate (A′2p) and 3′-phosphate (A′3p) rules out the possibility of nuclease-mediated hydrolysis that would generate A′3p exclusively.[15] These observations clearly show that an aminoglycoside antibiotic is capable of significantly accelerating the hydrolysis of a ribodinucleoside phosphate.

The rate of ApA hydrolysis by neomycin B is pH dependent. When the pH of the buffered 0.3 M neomycin B was lowered from 8.0 to 7.0, the rate of hydrolysis was slowed down by a factor of three. As demonstrated earlier for simple diamines, the hydrolysis rate acceleration is dependent on the relative abundance of the neutral, monoprotonated and diprotonated forms.[13] While the uncharged form has the highest hydrolytic activity and the dicationic form is essentially inactive, the monocationic form—which is the most populated form at pH 7—is the major contributor to the observed catalysis. Neomycin B contains six amino groups with pK_a values between 5.7 and 8.8.[16] As the pH is lowered and neomycin B becomes highly protonated, the rate of ApA hydrolysis is expected to decrease, which indeed is observed.

Neomycin B has three times as many amines as 1,3-diaminopropane, yet the three-fold rate enhancement observed for ApA hydrolysis may be coincidental. *A priori*, several factors may contribute to the observed rate acceleration: the number of amino groups, their stereochemical relationships, as well as

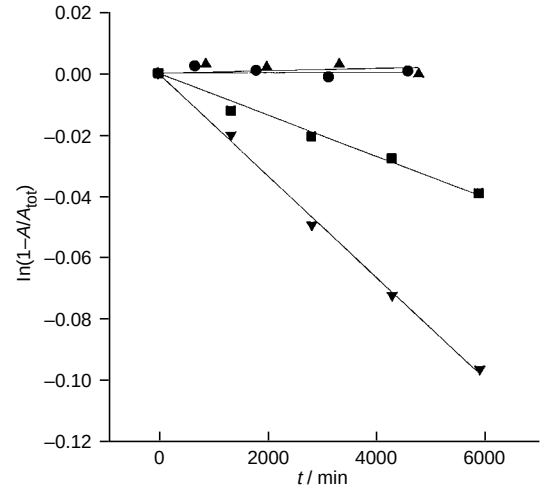


Fig. 1 Kinetic analysis of ApA hydrolysis by (▼) 0.3 M neomycin B, (■) 0.3 M 1,3-diaminopropane, (▲) 0.3 M 3-aminopropan-1-ol and (●) 50 mM Tris·HCl at pH 8.0 and 50 °C (see footnotes † and §)

their individual pK_a values. As reported earlier,[13] simple oligoamines such as diethylenetriamine and triethylenetetramine are essentially as effective as 1,3-diaminopropane in hydrolyzing ApA. This suggests that the number of amino groups may not be the major factor distinguishing neomycin B from 1,3-diaminopropane. Since the observed rate is a combination of individual hydrolysis rates mediated by various pairs of amino groups, it is likely that some pairs are much more active than others. This may be due to their spatial positioning and individual pK_a values, as well as the lower conformational freedom of neomycin B when compared to simple unstructured diamines. Since cyclohexane-1,3-diamine has been found to be as effective as 1,3-diaminopropane in hydrolyzing ApA,[13] we favor the modulation of individual pK_a values as the likely source for the rate enhancement observed with neomycin B. While the amines in 1,3-diaminopropane are rather basic, (pK_a 8.1 and 9.8),[13] certain pairs in neomycin B are less basic and display a wider difference in their pK_a. For example, the pK_a values reported for N^1 and N^3 are 8.04 and 5.74, respectively.[16] This may lead to a higher population of a monocationic form at a given pH compared to 1,3-diaminopropane, and therefore to a faster hydrolysis.

Neomycin B has been shown to bind to various RNA sequences[5–8] and now to hydrolyze an RNA dinucleoside phosphate. These observations provide new leads for the design of small molecules that combine RNA recognition with hydrolysis. Such molecules may become useful chemical probes for RNA structure and folding.[17] We are currently exploring these possibilities with larger and well-defined RNA structures.

This work was supported by the Universitywide AIDS Research Program, University of California, grant No. R96-SD-067, and by the Hellman Faculty Fellowship (Y. T.).

Footnotes and References

* E-mail: ytor@ucsd.edu

† All samples contained 50 mM Tris and 0.1 mM ApA. The pH was adjusted to 8.0 at room temperature (21 °C) with highly pure HCl (low trace metals: iron < 1 ppb, lead < 0.01 ppb, lutetium < 0.005 ppb, zinc < 0.02 ppb). At 50 °C, there is a drop of approximately 0.5 pH unit.

‡ HPLC analysis was performed using a C18 reversed-phase column with 25 mM sodium phosphate buffer, pH 3.5, containing 2% MeCN as the initial eluent, at a flow-rate of 1 ml min^{-1}. Gradient elution up to 50% MeCN was used to optimize separation.

§ The chromatograms were monitored at 260 nm and the resolved peaks were integrated and corrected for any volume changes against a peak of 2′-deoxyadenosine used as an internal standard. Plotting $\ln(1 - A/A_{tot})$ *vs.* t (min), where A = integration for adenosine, and A_{tot} = sum of integrals for all species, gives a straight line with a slope of $-k$. The linear correlation coefficient was > 0.99 for samples with substantial hydrolysis (*e.g.* neomycin B and 1,3-diaminopropane) and somewhat lower for control samples with very little hydrolysis, as expected. The rate constants reported for the neomycin B and 1,3-diaminopropane samples are the average of four independent runs.

1 For general references discussing RNA hydrolysis, see J. Chin, *Acc. Chem. Res.*, 1991, **24**, 145; R. Breslow, E. Anslyn and D.-L. Huang, *Tetrahedron*, 1991, **47**, 2365; J. Vincent, M. Crowder and B. Averill, *TIBS*, 1992, **17**, 105; E. Anslyn and D. Perreult, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 430 and references cited therein.

2 D. Magda, R. A. Miller, J. L. Sessler and B. L. Iverson, *J. Am. Chem. Soc.*, 1994, **116**, 7439; K. Matsumura, M. Endo and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, 1994, 2019; J. Hall, D. Hüsken, U. Pieles, H. E. Moser and R. Häner, *Chem. Biol.*, 1994, **1**, 185; J. K. Bashkin, E. I. Frolova and U. Sampath, *J. Am. Chem. Soc.*, 1994, **116**, 5981; J. Hall, D. Hüsken and R. Häner, *Nucleic Acids Res.*, 1996, **24**, 3522; D. Magda, S. Crofts, A. Lin, D. Miles, M. Wright and J. L. Sessler, *J. Am. Chem. Soc.*, 1997, **119**, 2293; D. Magda, M. Wright, S. Crofts, A. Lin and J. L. Sessler, *J. Am. Chem. Soc.*, 1997, **119**, 6947.

3 M. Endo, Y. Azuma, Y. Saga, A. Kuzuya, G. Kawai and M. Komiyama, *J. Org. Chem.*, 1997, **62**, 846.

4 For the hydrolytic cleavage of $tRNA^{Phe}$ by metal-free bleomycin, see M. V. Keck and S. M. Hecht, *Biochemistry*, 1995, **34**, 12 029.

5 U. von Ahsen, J. Davies and R. Schroeder, *Nature*, 1991, **353**, 368; U. von Ahsen, J. Davies and R. Schroeder, *J. Mol. Biol.*, 1992, **226**, 935; J. Davies, U. von Ahsen and R. Schroeder, *The RNA World*, ed. R. F. Gesteland and J. F. Atkins, Cold Spring Harbor Laboratory Press, New York, 1993, pp. 185–204.

6 T. K. Stage, K. J. Hertel and O. C. Uhlenbeck, *RNA*, 1995, **1**, 95.

7 H.-Y. Mei, A. A. Galan, N. S. Halim, D. P. Mack, D. W. Moreland, K. B. Sanders, H. N. Troung and A. W. Czarnik, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 2755.

8 M. L. Zapp, S. Stern and M. R. Green, *Cell*, 1993, **74**, 969; G. Werstuck, M. L. Zapp and M. R. Green, *Chem. Biol.*, 1996, **3**, 129.

9 M. Hendrix, P. B. Alper, E. S. Priestley and C.-H. Wong, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 95.

10 H. Wang and Y. Tor, *J. Am. Chem. Soc.*, 1997, **119**, 8734; H. Wang and Y. Tor, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1951; H. Wang and Y. Tor, *Angew. Chem.*, in the press.

11 T. Hermann and E. Westhof, *J. Mol. Biol.*, in the press.

12 K. Yoshinari, K. Yamazaki and M. Komiyama, *J. Am. Chem. Soc.*, 1991, **113**, 5899. See also: K. N. Dalby, A. J. Kirby and F. Hollfelder, *Pure Appl. Chem.*, 1994, **66**, 687.

13 M. Komiyama and K. Yoshinari, *J. Org. Chem.*, 1997, **62**, 2155.

14 B. Barbier and A. Brack, *J. Am. Chem. Soc.*, 1992, **114**, 3511.

15 H. Dugas, *Bioorganic Chemistry, A Chemical Approach to Enzyme Action*, 2nd edn., Springer Verlag, New York, 1989, pp. 123–137.

16 R. E. Botto and B. Coxon, *J. Am. Chem. Soc.*, 1983, **105**, 1021. See also: D. E. Dorman, J. W. Paschal and K. E. Merkel, *J. Am. Chem. Soc.*, 1976, **98**, 6885; L. Szilágyi, Z. Sz. Pusztahelyi, S. Jakab and I. Kovács, *Carbohydr. Res.*, 1993, **247**, 99.

17 C. P. H. Vary and J. N. Vournakis, *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 6978; J. M. Kean, S. A. White and D. E. Draper, *Biochemistry* 1985, **24**, 5062; C. Ehresmann, F. Baudin, M. Mougel, P. Romby, J.-P. Ebel and B. Ehresmann, *Nucleic Acids Res.*, 1987, **15**, 9109; G. Knapp, *Methods Enzymol.*, 1989, **180**, 192; D. S. Sigman and C.-H. Chen, *Annu. Rev. Biochem.*, 1990, **59**, 207; D. S. Sigman, A. Mazumder and D. M. Perrin, *Chem. Rev.*, 1993, **93**, 2295.

Received in Corvallis, OR, USA, 15th August 1997; 7/06023H