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An Inhibitor of Ribosomal Peptidyl Transferase Using Transition-State Analogy†

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ABSTRACT: The phosphoramidate of CCdAp and puromycin (CCdApPuro) is a potent inhibitor of ribosomal peptidyl transferase, as assayed by the fragment reaction. Inhibition is competitive at the ribosomal A-site. CCdApPuro protects P-site-associated bases in the peptidyl transferase loop region of 23S rRNA from carbodiimide modification. The K_i's of structural homologues of CCdApPuro suggest that both the CCdA and puromycin moieties participate in binding. Thus, CCdApPuro appears to bridge the A- and P-sites of the ribosome, implying that substrates are juxtaposed with a geometry suitable for direct reaction during peptidyl transfer.

The ribosomal peptidyl transferase is not yet defined in molecular terms. This ubiquitous enzyme is an integral part of the ribosomal large subunit (Maden et al., 1968). Experiments have constrained the essential active site constituents to a few proteins and 23S rRNA (Tate et al., 1983). Several lines of evidence suggest 23S rRNA participation (Noller, 1991). Recently, it was observed that the peptidyl transferase activity of the large subunit of *Thermus aquaticus* ribosomes can withstand three successive types of deproteinization (Noller et al., 1992). The resulting ribonucleoprotein contained ≈5% of the original protein ([^{35}S]methionine), yet retained 80% of peptidyl transferase activity. This finding implicates 23S rRNA, although the remaining protein may still be crucial.

Physical selection for active peptidyl transferase may help define the molecular components of the active site. Transition-state analogues have proven useful for enzymic activity selection because they exploit affinity for the reaction transition state (Wolfenden, 1972). Thus, they have allowed the activity enrichment of enzyme pools (Abdel-Aal & Hammock, 1986; Andersson & Wolfenden, 1980) and the selection of catalytic antibodies and RNAs (Lerner et al., 1991; Hirschmann et al., 1994; Prudent et al., 1994). We describe a high-affinity ligand for the peptidyl transferase active site, designed as a peptidyl transfer transition-state analogue.

The simplest, most probable mechanism for peptidyl transfer is nucleophilic addition−elimination (Fersht & Jencks, 1970) (Figure 1A). An alternative mechanism proceeds through an acyl-enzyme intermediate, as does peptide hydrolysis by serine proteases (Fersht, 1985). It has been proposed recently that a pseudouridine of 23S rRNA may play a role in such a mechanism (Lane et al., 1992). However, no acyl-ribosome intermediate has been detected (Krayevsky & Kukhanova, 1979). For our design, we assumed direct reaction of the substrates with a rate-limiting transition state similar to the reaction intermediate (Fersht & Jencks, 1970) shown in Figure 1A—tetrahedral about the attacked carbon with a negative charge on the former carbonyl oxygen.

MATERIALS AND METHODS

Synthesis of CCdApPuro. Phosphoramidates were synthesized via carbodiimide coupling according to previously

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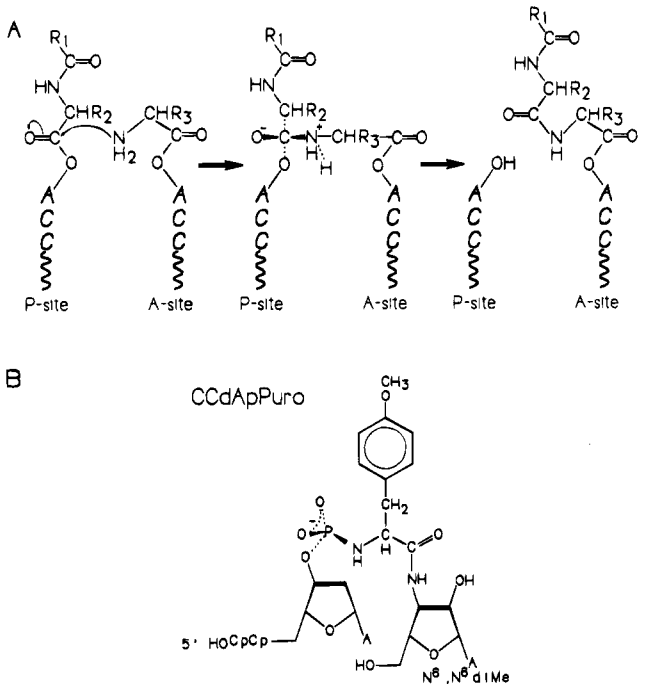

FIGURE 1: (A) Nucleophilic addition–elimination mechanism for peptidyl transfer. (B) The structure of CCdApPuro.

published procedures (Ivanovskaya et al., 1987; Chu et al., 1983). For a typical synthesis of CCdApPuro, the coupling reaction contained 12.3 μmol of neutralized puromycin, 100–200 nmol of CCdAp (Macromolecular Resources, Inc.), 50 μmol of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDAC), and 400 mM MES at pH 5.6 in a total volume of 125 μL. Reaction was carried out at 25 °C for 19 h. The mixture was then diluted with 500 μL of water and applied to a 200 μL bed volume A-25 Sephadex column equilibrated with 30 mM NH_4OAc at pH 6.5. The column was washed with 400 μL of 30 mM NH_4OAc (pH 6.5) to remove excess puromycin and other nonbinding contaminants. Crude product was then eluted with 750 μL of 500 mM NaCl and 30 mM NH_4OAc (pH 6.5). CCdApPuro was purified from this fraction by HPLC on a Microsorb C_{18} column (Rainin) eluted with a 100 mM NH_4OAc (pH 6.5)/acetonitrile gradient (0–50% acetonitrile in 80 min; retention time = 33 min). Fractions containing CCdApPuro were evaporated to dryness, and the product was resuspended in water and stored at −70 °C. Final yields, as determined by UV spectroscopy, were 20–30% based on an extinction coefficient of 49 500 M^{-1} cm^{-1} at a λ_{max} = 267 nm (estimated from the sum of the spectra of CCdAp and puromycin). The product was confirmed as CCdApPuro by UV spectroscopy—its spectrum closely matched that predicted for the sum of the chromophores—and by composition analysis using HPLC. As predicted for phosphodiester bond cleavage, treatment with 0.5 N NaOH for 18 h at 25 °C gave CMP (both 2′ and 3′ isomers) and a product that chromatographed with synthesized dApPuro, in a 2:1 molar ratio (determined by UV absorbance). Incubation of the latter at pH 1 and 37 °C for 3 h gave equimolar adenine and puromycin, as expected for hydrolysis of the P–N bond of the phosphoramidate and depurination of the dA residue.

Synthesis of dApPuro. dApPuro was synthesized from deoxyadenosine 3′-monophosphate and puromycin by a method similar to that described for CCdApPuro. The coupling reaction contained 14 μmol of puromycin, 50 μmol of EDAC, 4.5 μmol of 3′-dAMP (free acid) and 400 mM MES at pH 5.9. Reaction was carried out at 25 °C for 9 h. The product was purified by paper chromatography (Whatmann 3MM, eluted with 5:3 EtOH/NH_4OAc, pH 7.5) (R_f = 0.6) followed by HPLC on a C_{18} column. The overall yield was 12%. The UV spectrum (λ_{max} = 265 nm) closely matched that predicted for the sum of the chromophores. Incubation at pH 1 and 37 °C for 3 h gave equimolar adenine and puromycin.

Preparation of Ribosomes. Salt-washed *Escherichia coli* 70S ribosomes were prepared from MRE600 cells essentially as described (Staehelin & Maglott, 1971) and were stored at −70 °C in 100 mM NH_4Cl, 10 mM MgOAc, 0.5 mM EDTA, 3 mM β-mercaptoethanol, and 20 mM Tris-HCl (pH 7). Prior to all fragment reactions and chemical modification assays, ribosomes were incubated in storage buffer for 10 min at 42 °C to activate peptidyl transferase (Miskin et al., 1968).

Peptidyl Transferase Assay. The fragment, CAACCA-[^{35}S]fMet (specific activity, 1000 Ci/mmol), was prepared from *E. coli* [^{35}S]fMet-tRNA by digestion with RNase T1 and purification by paper electrophoresis according to Moazed and Noller (1991). Assays of the fragment reaction with puromycin were performed as described (Monro, 1971). All reactions were run at 4 °C and contained 267 mM KOAc, 13 mM $MgCl_2$, 33 mM Tris-HCl (pH 7.9 at 4 °C), 33% (v/v) methanol, 20000–30000 cpm of fragment, and puromycin and preactivated 70S ribosomes as indicated in a total volume of 75 μL. Reactions were initiated with methanol addition, except where indicated, and terminated with the addition of 50 μL of 300 mM NaOAc (pH 5.5) saturated with $MgSO_4$. The product of peptide bond formation, [^{35}S]fMet-puromycin, was extracted with 1 mL of ethyl acetate, and the radioactivity in the extract was quantitated by scintillation counting. Radioactivity extracted from control reactions lacking puromycin was subtracted from that of all experimental reactions. Reaction times were such that initial rates were measured.

Chemical Modification of 23S rRNA. Modification of the 23S rRNA of *E. coli* 70S ribosomes with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMCT) was essentially by the method of Moazed and Noller (1991). Reactions were run at 4 °C for 30 min and contained ribosomes and CCdApPuro as indicated in 100 μL of 267 mM KCl, 13 mM $MgCl_2$, 33% methanol, 42 mg/mL CMCT, and 53 mM potassium borate (pH 8.0). Reactions were stopped with the addition of 200 μL of ethanol, and the RNA was extracted and analyzed by primer extension as described (Stern et al., 1988). Extensions utilized AMV reverse transcriptase and were from a primer hybridizing to nucleotides 2640–2656 of 23S rRNA. Nucleotides 2449–2637 were examined extensively for protection by CCdAp-Puro.

Band intensities in primer extension gel autoradiograms were measured by densitometry (LKB Ultroscan XL). Intensity was determined to be linear with radioactivity under our conditions. Band intensity (I) as a function of protecting agent (A) is assumed to be proportional to the concentration of unbound ribosomes plus a constant, according to

$$I = I_{sat} + I_0/(1 + [A]/K_d^A)$$

where I_{sat} is the intensity at saturating protecting agent (>0 due to partial inactivity of ribosomes and/or incomplete

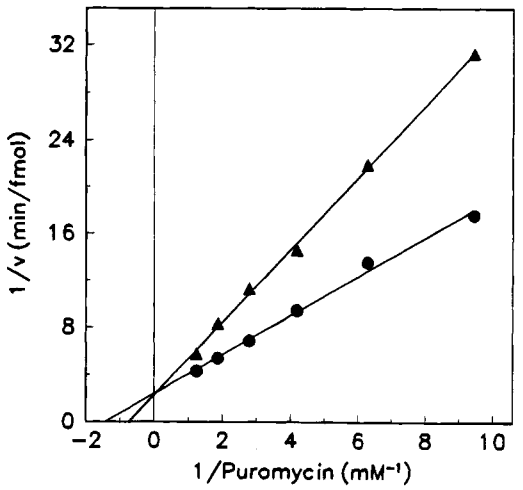

FIGURE 2: Double-reciprocal plot of the initial velocity of the fragment reaction catalyzed by *E. coli* 70S ribosomes as a function of puromycin concentration, in the absence (●) and presence (▲) of 110 nM CCdApPuro. The ribosome concentration was 31 nM.

protection) and I_0 is the intensity in addition to I_{sat} in the absence of agent.

RESULTS AND DISCUSSION

Design of the Analogue. Our peptidyl transfer transition-state analogue is the 3′-phosphoramidate of CCdAp and puromycin (CCdApPuro) (Figure 1B). Included in this molecule are the ubiquitous 3′-terminal trinucleotide of tRNA, CCA, and puromycin, an antibiotic analogue of the 3′-end of aminoacyl-tRNA (Pestka, 1971). Thus, CCdAp-Puro simulates both substrates positioned around a tetrahedral center with a negatively charged oxygen, approximating the presumed transition state.

The remaining tRNA and nascent peptide of the donor (P-site) substrate are not modeled. Previous studies using minimal analogues of peptidyl- and aminoacyl-tRNA (Chládek & Sprinzl, 1985) suggest that residues prior to the 3′-trinucleotide are not important for binding to either the A- or P-site at the active center. There is some evidence, however, for a nascent peptide interaction in the P-site (Chládek & Sprinzl, 1985). dA was used in place of A as the 3′-terminal residue to simplify coupling of the 3′-phosphate to puromycin. The role of the 2′-OH in donor activity is equivocal. tRNA-CCdA-Ac_2Lys is active as a donor (Wagner et al., 1982). In other experiments, CdA-AcLeu was inactive whereas CA-AcLeu was active (Quiggle et al., 1981).

Inhibition of Peptidyl Transferase. To determine whether CCdApPuro can bind the active site, inhibition of peptidyl transferase was assayed. Peptidyl transferase activity was monitored using the fragment reaction with puromycin (Monro, 1971). Our fragment is the 3′-hexanucleotide of $tRNA^{fMet}$ charged with *N*-formylmethionine, which serves as the donor substrate. Transfer of formylmethionine to the amino group of puromycin is detected.

CCdApPuro is a potent inhibitor of peptidyl transferase. Figure 2 shows a double-reciprocal plot of the initial velocity of the fragment reaction as a function of puromycin concentration, in the absence and presence of 110 nM CCdApPuro. Inhibition is competitive at the A-site: the maximal reaction velocities determined are statistically indistinguishable, whereas the apparent K_m's for puromycin

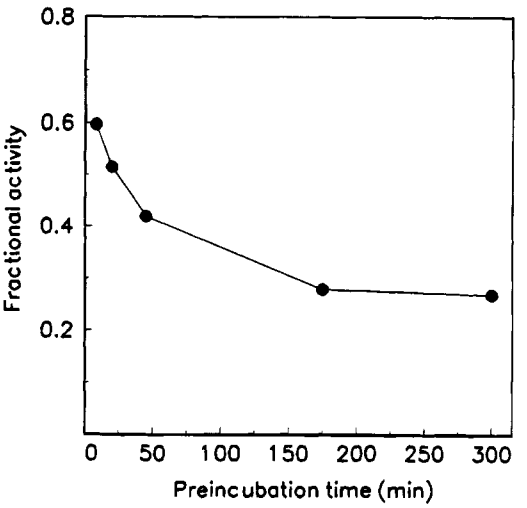

FIGURE 3: Dependence of the inhibition of the fragment reaction on preincubation time. Complete reaction mixtures minus the fragment substrate were incubated for the times shown before initiation of the reaction by fragment addition. Reaction time was 30 min. Plotted is the relative activity of ribosomes preincubated in the presence of inhibitor to that of ribosomes preincubated without inhibitor under otherwise identical conditions. Total ribosome and CCdApPuro concentrations were 21 and 31 nM, respectively.

clearly differ. From three similar experiments, including Figure 2, we determine a K_i for CCdApPuro of 90 ± 4 nM. Inhibition by CCdApPuro is reversed by dilution and therefore is probably noncovalent. Incubation of ribosomes with 280 nM CCdApPuro for 5 h resulted in no irreversible loss of activity with respect to ribosomes incubated under identical conditions without inhibitor (data not shown).

Inhibition increases markedly if ribosomes are preincubated with CCdApPuro before initiation of the fragment reaction. Figure 3 shows inhibition by CCdApPuro with preincubation time. Under the conditions of this experiment, an apparent equilibrium is approached after 3 h. This augmented inhibition is fully reversible (see earlier discussion).

After preincubation with CCdApPuro and varied puromycin, the fragment reaction shows mixed or noncompetitive A-site inhibition (data not shown) and a K_i for CCdApPuro of 2.1 nM, which is 45-fold lower than the immediate K_i. Deviation from competitive A-site inhibition after preincubation may reflect a slow transformation in binding to a second state with a new relation to the puromycin site.

Chemical Protection of 23S rRNA. Peptidyl transferase substrates and antibiotic inhibitors have been shown to protect bases in 23S rRNA from chemical modification (Moazed & Noller, 1987, 1989, 1991), predominantly within the very highly conserved central loop of domain V of the RNA—a region associated with peptidyl transferase by several other criteria (Noller, 1991). We, therefore, have used the chemical footprint of CCdApPuro on 23S rRNA to confirm and characterize binding at the peptidyl transferase center. Moazed and Noller (1989, 1991) identified several 23S rRNA base protections dependent on the 3′-terminus of bound tRNA. To determine whether CCdApPuro occupies the same sites as the tRNA termini, we used CMCT, a reagent that modifies U and to a lesser extent G, to probe nucleotides 2449−2637. In this region, tRNA-dependent protection from CMCT was found at U2506, U2584, and

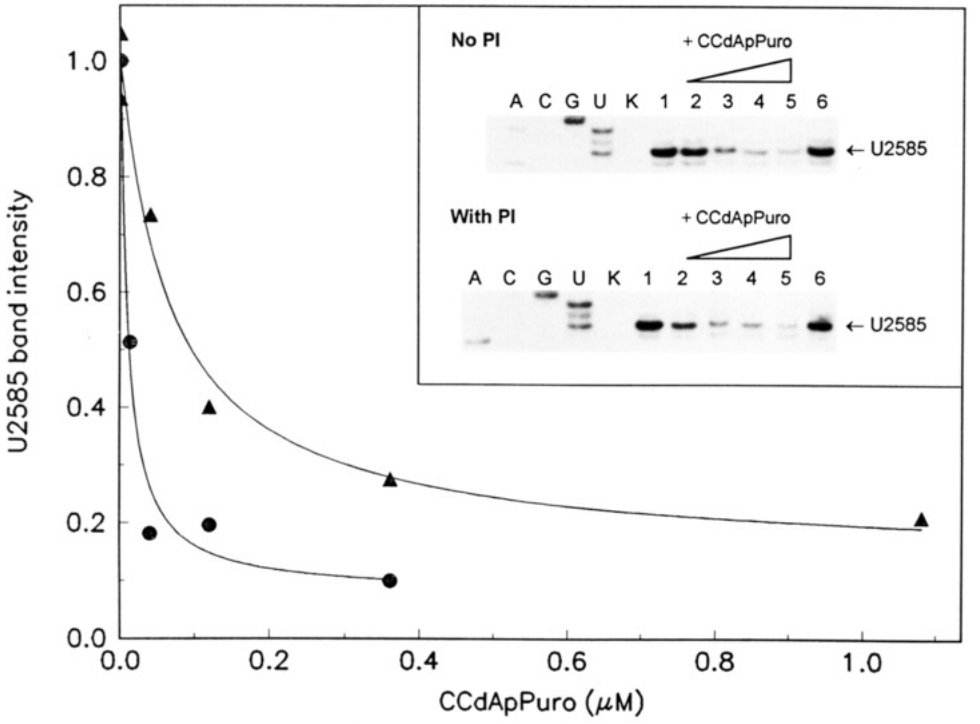

FIGURE 4: Protection of 23S rRNA base U2585 from CMCT as a function of CCdApPuro concentration. CMCT modification was monitored by primer extention as described in Materials and Methods. Data shown are derived from densitometry of the autoradiograms shown in the inset. Curves drawn are least-squares fits of the predicted function for band intensity vs protecting agent (see Materials and Methods). (▲) Modification is initiated without preincubation (no PI) of ribosomes with CCdApPuro. (●) CMCT is added after a 4 h incubation (with PI) of ribosomes with CCdApPuro under modification reaction conditions. Inset: Lanes A, C, G, and U are dideoxy sequencing reactions; lanes K are reverse transcriptions from unmodified control RNA; lanes 1 and 6 are modifications in the absence of CCdApPuro; lanes 2, 3, 4, and 5 are modifications in the presence of (no PI) 40, 120, 360, and 1100 nM, respectively, or (with PI) 13, 40, 120, and 360 nM, respectively.

U2585 for P-site-bound tRNA (all require the tRNA 3′-terminal A) and at Ψ2555 and U2609 for A-site-bound aminoacyl-tRNA (both require the aminoacyl group) (Moazed & Noller, 1989). We observe protection by CCdApPuro of all three P-site-specific positions. Thus, CCdApPuro interacts with the P-site of the active center. No other protection from CMCT was seen.

Although puromycin is an analogue of the 3′-terminal aminoacyl-A of aminoacyl-tRNA, the absence of protection at Ψ2555 and U2609 by CCdApPuro does not contradict occupation of the A-site. Moazed and Noller (1987, 1989) did not detect puromycin-dependent protection of either of these positions. To further characterize the interaction of CCdApPuro with the peptidyl transferase active center, we are currently determining the 23S rRNA protection pattern using various chemical probes.

To associate base protection with inhibition of peptidyl transferase, we determined the degree of protection of U2585 at varying CCdApPuro concentrations (Figure 4). A plot of the data (Figure 4) suggests a K_d of 70 ± 20 nM (without preincubation), in agreement with the K_i for fragment reaction inhibition (above). Protection at U2506 and U2584 shows a similar dependence on the CCdApPuro concentration (not shown). These results suggest that P-site occupation is associated with biochemical inhibition by CCdApPuro at the A-site.

The pattern of 23S rRNA base protection from CMCT is unaltered in the region we have examined when modification follows preincubation. The degree of protection, however, is increased, as is inhibition of the fragment reaction (Figure 4). Thus, both immediate and final binding states involve the P-site. From Figure 4, we derive a K_d for CCdApPuro after preincubation of 10 ± 4 nM.

Inhibition by Structural Homologues of CCdApPuro. The affinities of structural homologues were measured to determine which elements of CCdApPuro support binding to peptidyl transferase (Figure 5). dApPuro (the phosphoramidate of 2′-deoxyadenosine 3′-monophosphate and puromycin) is a weak inhibitor of the fragment reaction, with affinity not significantly better than that of puromycin (Table 1). Thus, the deoxyadenosine moiety and phosphoramidate center do not appear to contribute significantly to binding. The contribution of these elements may be offset by blocking of the puromycin α-amino group. N-Substituted derivatives of puromycin and other A-site substrates frequently have considerably weakened binding affinities (Pestka et al., 1970; Vince et al., 1975; Vogel et al., 1969).

The K_i's observed for dApPuro and CCdApPuro (Table 1) suggest that the two C's contribute 4−5 orders of magnitude to the affinity. This is similar to the contribution of the two penultimate C's to P-site substrate binding (Krayevsky & Kukhanova, 1979), reinforcing the conclusion that CCdApPuro binds to the canonical P-site. The puromycin moiety also contributes to CCdApPuro binding. CCdA and CCdAp have K_i's more than 2 orders of magnitude greater than that of CCdApPuro. Thus, both the CCdA and puromycin halves of CCdApPuro participate in binding. CCdApPuro appears to bridge the active center, occupying the A- and P-sites simultaneously.

CCdApPuro as a Transition-State Analogue. According to transition-state theory, the reaction rate enhancement by an enzyme is approximately the ratio of the product of the

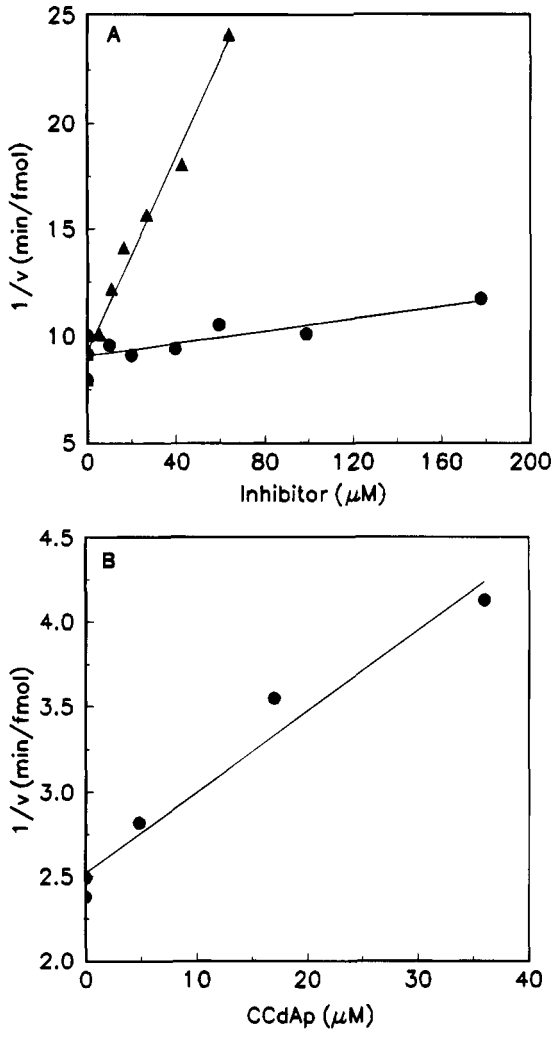

FIGURE 5: Inhibition by partial structural homologues of CCdApPuro. (A) Plot of the reciprocal initial velocity of the fragment reaction in the presence of varied concentrations of dApPuro (●) or CCdA (▲). Ribosome and puromycin concentrations were 630 and 11 μM, respectively. (B) Reciprocal initial velocity of the fragment reaction in the presence of varied concentrations of CCdAp. Ribosome and puromycin concentrations were 490 nM and 27 μM, respectively.

K_d's of ground-state substrates to the K_d for the transition state (Wolfenden, 1972). From the rate of the peptidyl transferase-catalyzed reaction of puromycin with peptidyl-tRNA (Synetos & Coutsogeorgopoulos, 1987) and the approximate uncatalyzed rate (Nierhaus et al., 1980; Weber & Orgel, 1979), we estimate a rate enhancement of 10^3–10^4 M. Using K_d's reported for fragment binding (Krayevsky & Kukhanova, 1979) and the K_m for puromycin (Table 1), we estimate a K_d of 10^{-10}–10^{-9} M^2 for the complex of peptidyl transferase with fragment reaction ground-state substrates. Thus, the K_i for CCdApPuro after preincubation (2.1 ± 0.1 nM) is 10^3–10^5 times lower than the K_d predicted for the transition state. It is possible that some of this difference is due to the lack of an analogue for the transferred peptide in the structure of CCdApPuro. To better quantify the extent to which CCdApPuro resembles the transition state, one could correlate K_i for CCdApPuro and its derivatives with K_m/k_{cat} for analogous substrate derivatives (Bartlett & Marlowe, 1983).

Mechanism of Peptidyl Transferase. The results presented here are consistent with a direct-attack mechanism for peptidyl transfer (Figure 1). Simultaneous occupation of both substrate binding sites by CCdApPuro implies that substrates bind a few angstroms apart and can have a relative geometry suitable to accelerate peptide bond formation. We cannot be certain, however, that CCdApPuro binds the A- and P-sites exactly as do independent substrates.

Table 1: Comparison of Peptidyl Transferase Ligands[a]

substance	K_m[b] (μM)	K_i (μM)	SE[c]
puromycin	740		40
dApPuro		640	180
CCdA		41	4
CCdAp		53	8
CCdApPuro			
no PI[d]		9.0×10^{-2}	0.4×10^{-2}
with PI		2.1×10^{-3}	0.1×10^{-3}

[a] Values and standard errors for puromycin and CCdApPuro are each derived from three independent double-reciprocal plots. All other values are derived from the plots shown in Figure 6. [b] The K_m reported for puromycin is that determined after preincubation for 6 h. The K_m's measured with and without preincubation were statistically indistinguishable. [c] SE is the standard error in micromolar. [d] PI stands for preincubation preceding fragment reaction initiation (see text).

It has been argued that simply positioning the reacting amino and ester groups for addition could account for the observed reaction rate (Nierhaus et al., 1980). However, a chemical involvement of peptidyl transferase in catalysis is possible (Pestka et al., 1972; Nierhaus et al., 1980). For example, reaction may be accelerated by deprotonation of the attacking amino group and/or stabilization of the transitional oxyanion. Our study gives no evidence of stabilization of the transition-state reaction center by peptidyl transferase; the phosphoramidate centers of dApPuro and CCdApPuro do not appear to contribute significantly to binding. This is consistent with a primary template function of the active site. However, the geometry and/or charge distribution of the phosphoramidate may inadequately approximate those of the transition-state center. Furthermore, the phosphoramidate analogues do not account for proton transfer. Thus, stabilization of the transition state through deprotonation of the attacking amino group may not be reflected in analogue binding.

CONCLUDING REMARKS

CCdApPuro is one of the most potent reversible inhibitors of peptidyl transferase known. This rationally designed molecule has a high affinity for the active center of the enzyme and apparently bridges both substrate binding sites. Thus, CCdApPuro and derivatives may prove useful in characterizing the peptidyl transferase active site. Related molecules may also have potential as rational antibiotics.

CCdApPuro may be particularly useful as a selective agent. Partially active enzyme pools may be enriched for activity by selection for binding to immobilized CCdApPuro. Such pools may consist of partially or completely deproteinized large ribosomal subunit RNAs, *in vitro* synthesized derivatives of 23S rRNA, or random-sequence RNA. We are currently using CCdApPuro to search for peptidyl transfer catalysis in both random RNA and 23S rRNA derivative pools.

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REFERENCES

Abdel-Aal, Y. A. I., & Hammock, B. D. (1986) *Science 233*, 1073.
Andersson, L., & Wolfenden, R. (1980) *J. Biol. Chem. 255*, 11106.
Bartlett, P. A., & Marlowe, C. K. (1983) *Biochemistry 22*, 4618.
Chládek, S., & Sprinzl, M. (1985) *Angew. Chem., Int. Ed. Engl. 24*, 371.
Chu, B. C. F., Wahl, G. M., & Orgel, L. E. (1983) *Nucleic Acids Res. 11*, 6513.
Fersht, A. R. (1985) in *Enzyme Structure and Mechanism*, 2nd ed., pp 405–413, W. H. Freeman & Co., New York.
Fersht, A. R., & Jencks, W. P. (1970) *J. Am. Chem. Soc. 92*, 5442.
Hirschmann, R., Smith, A. B., III, Taylor, C. M., Benkovic, P. A., Taylor, S. D., Yager, K. M., Sprengeler, P. A., & Benkovic, S. J. (1994) *Science 265*, 234.
Ivanovskaya, M. G., Gottikh, M. B., & Shabarova, Z. A. (1987) *Nucleosides Nucleotides 6*, 913.
Krayevsky, A. A., & Kukhanova, M. K. (1979) *Prog. Nucleic Acid Res. Mol. Biol. 23*, 1.
Lane, B. G., Ofengand, J., & Gray, M. W. (1992) *FEBS Lett. 302*, 1.
Lerner, R. A., Benkovic, S. J., & Schultz, P. G. (1991) *Science 252*, 659.
Maden, B. E. H., Traut, R. R., & Monro, R. E. (1968) *J. Mol. Biol. 35*, 333.
Miskin, R., Zamir, A., & Elson, D. (1968) *Biochem. Biophys. Res. Commun. 33*, 551.
Moazed, D., & Noller, H. F. (1987) *Biochimie 69*, 879.
Moazed, D., & Noller, H. F. (1989) *Cell 57*, 585.
Moazed, D., & Noller, H. F. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 3725.
Monro, R. E. (1971) *Methods Enzymol. 20*, 472.
Nierhaus, K. H., Schulze, H., & Cooperman, B. S. (1980) *Biochem. Int. 1*, 185.
Noller, H. F. (1991) *Annu. Rev. Biochem. 60*, 191.
Noller, H. F., Hoffarth, V., & Zimniak, L. (1992) *Science 256*, 1416.
Pestka, S. (1971) *Annu. Rev. Microbiol. 25*, 487.
Pestka, S., Hishizawa, T., & Lessard, J. L. (1970) *J. Biol. Chem. 245*, 6208.
Pestka, S., Goorha, R., Rosenfeld, H., Neurath, C., & Hintikka, H. (1972) *J. Biol. Chem. 247*, 4258.
Prudent, J. R., Uno, T., & Schultz, P. G. (1994) *Science 264*, 1924.
Quiggle, K., Kumar, G., Ott, T. W., Ryu, E. K., & Chládek, S. (1981) *Biochemistry 20*, 3480.
Staehelin, T., & Maglott, D. R. (1971) *Methods Enzymol. 20*, 449.
Stern, S., Moazed, D., & Noller, H. F. (1988) *Methods Enzymol. 164*, 481.
Synetos, D., & Coutsogeorgopoulos, C. (1987) *Biochim. Biophys. Acta 923*, 275.
Tate, W. P., Schulze, H., & Nierhaus, K. H. (1983) *J. Biol. Chem. 258*, 12810.
Vince, R., Almquist, R. G., Ritter, C. L., & Daluge, S. (1975) *Antimicrob. Agents Chemother. 8*, 439.
Vogel, Z., Zamir, A., & Elson, D. (1969) *Biochemistry 8*, 5161.
Wagner, T., Cramer, F., & Sprinzl, M. (1982) *Biochemistry 21*, 1521.
Weber, A., & Orgel, L. (1979) *J. Mol. Evol. 13*, 185.
Wolfenden, R. (1972) *Acc. Chem. Res. 5*, 10.

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