# The ribosome as an entropy trap

**Annette Sievers[a,b], Malte Beringer[b,c], Marina V. Rodnina[c], and Richard Wolfenden[a,d]**

[a]Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC 27599; and [c]Institute of Physical Biochemistry, University of Witten/Herdecke, Stockumer Strasse 10, 58448 Witten, Germany

Contributed by Richard Wolfenden, April 7, 2004

**To determine the effectiveness of the ribosome as a catalyst, we compared the rate of uncatalyzed peptide bond formation, by the reaction of the ethylene glycol ester of *N*-formylglycine with Tris(hydroxymethyl)aminomethane, with the rate of peptidyl transfer by the ribosome. Activation parameters were also determined for both reactions, from the temperature dependence of their second-order rate constants. In contrast with most protein enzymes, the enthalpy of activation is slightly less favorable on the ribosome than in solution. The $2 \times 10^7$-fold rate enhancement produced by the ribosome is achieved entirely by lowering the entropy of activation. These results are consistent with the view that the ribosome enhances the rate of peptide bond formation mainly by positioning the substrates and/or water exclusion within the active site, rather than by conventional chemical catalysis.**

The peptidyl transferase (PT) center of the ribosome is located on the 50S subunit in domain V of 23S rRNA. Crystal structures showed that the PT center consists of RNA exclusively (1–5), indicating that the ribosome is a ribozyme. *In vitro* selection experiments have demonstrated that RNA is indeed capable of catalyzing peptide bond formation, in that RNA fragments containing an eight-base binding motif matching the PT loop (A2448-G2455) of 23S rRNA exhibit (low) activity in peptide synthesis (6, 7). At the PT center, the amino group of aminoacyl-tRNA bound at the A site attacks the ester linkage between the carboxylate group of the growing peptide chain and the 3′-OH group of peptidyl-tRNA bound at the P site.

Although the catalytic mechanism of peptide bond formation on the ribosome is not known in detail, the intrinsic reactivity of esters with amines is so great that ester aminolysis is one of the more familiar procedures in preparative organic chemistry (8). In an early attempt to study uncatalyzed peptide bond formation in solution, Weber and Orgel (9) showed that 2′(3′)-*O*-glycyladenosine 5′-*O*-methylphosphate and free amino acids react to form dipeptides at ambient temperatures. In view of the facility of this reaction it seems reasonable to inquire whether chemical catalysis is required at the ribosome's active site, or whether juxtaposition of the ester and amine within the active site of the ribosome might suffice to explain the observed rates of peptidyl transfer. The rate of peptide bond formation is known to be inhibited ≈100-fold by protonation of a ribosomal group with a $pK_a$ value of 7.5 (10). That observation would appear to be consistent with a number of possibilities, including general-acid/base catalysis and/or a pH-dependent conformation change within the active site. Based on the crystal structure, a chemical role in catalysis was originally ascribed to a highly conserved adenine residue (A2451 in *Escherichia coli*), and a charge relay system involving G2447 was postulated to bring about an extensive shift in the $pK_a$ value of A2451 (2). In later experiments, however, point mutations showed that A2451 could be replaced without a complete loss of function (10–12). Moreover, replacement of G2447 by A was found not to affect the ionization of the ribosome group with the apparent $pK_a$ value of 7.5 (13). Although these results do not appear to support a charge relay mechanism involving G2447, with A2451 acting as general base, the participation of a ribosomal group with a lower $pK_a$ value cannot be excluded experimentally because ribosomes lose activity at pH $< 5.2$.

Many enzymes act by lowering the heat of activation of the reactions that they catalyze, consistent with the frequent involvement of polar forces of attraction (H-bonds and electrostatic interactions) in stabilizing the altered substrate in the transition state. These "enthalpic" sources of transition state affinity are expected for enzymes that employ general acid-base or nucleophilic catalysis to promote substrate transformation (14). The role of entropy in transition-state stabilization for these reactions is small and variable, with an average value near zero. If the ribosome, like these enzymes, acted as a chemical catalyst, then the rate enhancement produced by the ribosome might be expected to arise from a reduction in the enthalpy of activation. If, on the other hand, the ribosome served mainly to position the substrates in the active site during peptidyl transfer, then the rate enhancement produced by the ribosome might be expected to be largely entropic in origin.

In the present experiments, we sought to test these alternatives by comparing the activation parameters for ester aminolysis in the ribosome and in solution (Fig. 1). As models for peptidyl-tRNA, we used the methyl ester of *N*-acetylglycine and the ethylene glycol ester of *N*-formylglycine. The $pK_a$ value of the carboxylic acid that is transferred in these reactions (3.43 for *N*-formylglycine) is similar to that of the carboxyl terminus of a growing peptide chain [3.42 (15)], so that the electrophilic character of this group would be expected to be similar as well. The ethylene glycol ester of *N*-formylglycine was used to incorporate a vicinal hydroxyl group, like that which is present in the terminal adenosine residue of peptidyl-tRNA. As a model for the acceptor amine, we used the primary amine Tris(hydroxymethyl)aminomethane (Tris) ($pK_a = 8.1$, 25°C) (16), whose $pK_a$ value resembles that of aminoacyl-tRNA ($pK_a = 8.1$, 25°C).[e] Because the $pK_a$ values of their conjugate acids are virtually identical, their nucleophilicities are expected to be similar as well. In the case of the ribosome reaction, a dipeptidyl-tRNA (fMetPhe-$tRNA^{Phe}$) was used as the donor substrate at the P site, and puromycin (Pm) ($pK_a = 6.9$) (10), an analog of the 3′ end of aminoacyl-tRNA, was used as the acceptor substrate at the A site. By comparing the effective second-order rate constant, $k_{cat}/K_M$, for peptide bond formation on the ribosome with the second-order rate constant for the model reaction in solution, we sought to establish the rate enhancement produced by the ribosome.

## Materials and Methods

**Synthesis of *N*-Formylglycine Ethylene Glycol Ester (NGFF).** *N*-formylglycine (100 mg) was dissolved in ethylene glycol (1 ml) and stirred at 20°C under vacuum for 20 h.

**Synthesis of *N*-Acetylglycine Methyl Ester (NAGM).** *N*-acetylglycine (1 g) and methanol/HCl (10 ml, 3.8 M) were stirred at room

Abbreviations: Pm, puromycin; PT, peptidyl transferase; Tris, tris(hydroxymethyl)aminomethane.

[b]A.S. and M.B. contributed equally to this work.

[d]To whom correspondence should be addressed. E-mail: water@med.unc.edu.

[e]Estimated at 25°C from the free energy of ionization (≈11 kcal/mol) of glycine (32) and the identical $pK_a$ values of leucyl-tRNA and leucine ethyl ester at 37°C (33).

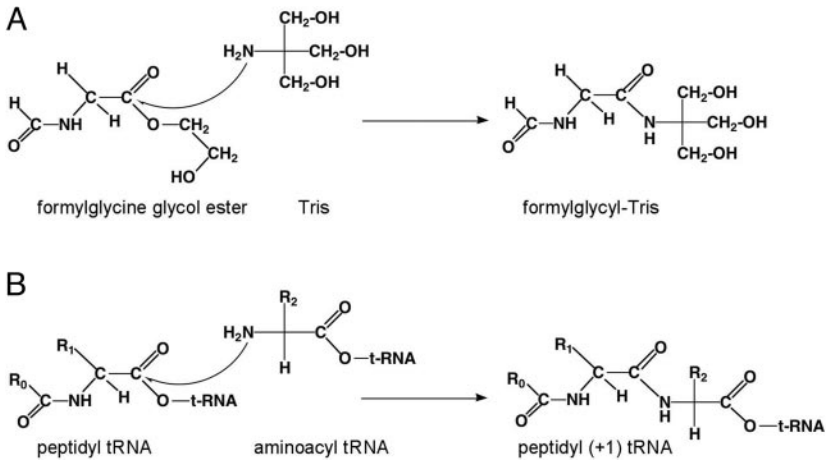


**Fig. 1.** Chemistry of peptide bond formation. (*A*) Model ester aminolysis reaction between *N*-formylglycine ethylene glycol ester and Tris(hydroxymethyl)-aminomethane in aqueous solution. (*B*) Ester aminolysis in the PT center of the ribosome. Substrates are peptidyl-tRNA in the P site and aminoacyl-tRNA in the A site.

temperature for 1 h. NAGM was purified by a chloroform extraction and reextracted into water. The purity of both compounds was confirmed by proton NMR.

**Uncatalyzed Peptide Bond Formation.** In a typical experiment, 10 mM NAGM (or NFGG) and 250 mM Tris·HCl at various pH values (7.4–8.4) were incubated in a heating block at different temperatures (45–65°C) for varying time intervals. The samples were diluted with $D_2O$ containing added pyrazine as an integration standard, and the integrated intensities of the nonexchangeable protons were measured by NMR. The rate of ester hydrolysis and aminolysis were monitored by observing the integrated intensities of carbon-bound protons, using the chemical shifts shown in Table 1.[f]

**Ribosome-Catalyzed Peptide Bond Formation.** Materials were prepared as described in refs. 17 and 18. Ribosome complexes containing formyl [$^3$H]Met[$^{14}$C]Phe-tRNA$^{Phe}$ in the P site were prepared and purified according to ref. 10. Quench-flow assays were performed at 15–37°C in a KinTek apparatus, mixing equal volumes (12 $\mu$l) each of ribosome (0.15 $\mu$M after mixing) and Pm solutions in buffer A (50 mM Tris·HCl/20 mM Bis-Tris/70 mM $NH_4Cl$/30 mM KCl/7 mM $MgCl_2$) at pH 7.5. Reactions were quenched with 25% formic acid. Formyl [$^3$H]Met[$^{14}$C]Phe-Pm formed by the PT reaction was extracted into ethyl acetate in the presence of 1.5 M sodium acetate (pH 4.5), saturated with $MgSO_4$, and quantified by double-label radioactivity counting. Rate constants were estimated by exponential fitting of time courses with TABLECURVE software (Jandel, San Rafael, CA).

**Viscosity Studies.** Trehalose dihydrate (Sigma) solutions of varying concentrations (relative viscosities of 1.36, 2.30, and 4.70) were prepared in buffer A at pH 7.5. Viscosities of buffered solutions were measured by using a Cannon-Fenske (CANNON Instrument, State College, PA) kinematic viscometer at 25°C and are relative to buffer containing no trehalose. Both ribosome complexes and Pm were dissolved in trehalose solutions before the start of the reaction to avoid mixing effects. Peptide bond formation with 0.15 $\mu$M ribosome complexes and 1 mM Pm was measured at 25°C and pH 7.5 in the quench-flow apparatus.

**Calculations of Heats and Entropies of Activation.** Adopting the usual convention (19), the activation energy, $E_a$, was calculated from the slope of the Arrhenius plot, $E_a = -\text{slope} \times 2.3\,R$ and the enthalpy of activation as $\Delta H^{\neq} = E_a - RT$, where $R$ is the gas constant and $T$ is the absolute temperature. The free energy of activation at 25°C was calculated from $\Delta G^{\neq} = -2.3\,RT \log((k_{cat}/(K_M\ h))/(k_B{}^*T)$, with $h$ = Boltzmann's constant and $k_B$ = Planck's constant, and $T\Delta S^{\neq}$ was obtained from the difference between $\Delta G^{\neq}$ and $\Delta H^{\neq}$.

## Results and Discussion

The time course of uncatalyzed peptide bond formation between the glycine esters and Tris was measured by NMR (Table 1) at temperatures between 25°C and 60°C. The dependence of the rate of peptide bond formation on pH, between pH 6.5 and 10, corresponded to that expected if the unprotonated form of the amine were the reactive species for the uncatalyzed reaction, just as it appears to be for the ribosome-catalyzed reaction (10). The rate of ester aminolysis was directly proportional to the concentration of free amine (Fig. 2), indicating that only one molecule

**Table 1. Proton NMR chemical shifts in $D_2O$**

| Species | Chemical shift, ppm |
|---|---|
| *N*-acetylglycine methyl ester (pH 6.5) | |
| $\mathbf{CH_3}CONHCH_2COOCH_3$ | 2.02 |
| $CH_3CONH\mathbf{CH_2}COOCH_3$ | 3.96 |
| $CH_3CONHCH_2COO\mathbf{CH_3}$ | 3.72 |
| *N*-acetylglycine (pH 6.5) | |
| $\mathbf{CH_3}CONHCH_2COO^-$ | 1.98 |
| $CH_3CONH\mathbf{CH_2}COO^-$ | 3.95 |
| *N*-acetylglycine-Tris (pH 6.5) | |
| $\mathbf{CH_3}CONHCH_2CONHC(CH_2OH)_3$ | 2.00 |
| $CH_3CONH\mathbf{CH_2}CONHC(CH_2OH)_3$ | 3.94 |
| $CH_3CONHCH_2CONHC(\mathbf{CH_2}OH)_3$ | 3.88 |
| *N*-formylglycine ethylene glycol ester | |
| $\mathbf{H}CONHCH_2COOCH_2CH_2OH$ | 8.14 |
| *N*-formylglycine-Tris (cis/trans peptide bond) | |
| $\mathbf{H}CONHCH_2CONHC(CH_2OH)_3$ | 8.2 |
| | 8.0 |
| *N*-formylglycine | |
| $\mathbf{H}CONHCH_2COO^-$ | 8.4 |

Chemical shift values refer to the atom depicted in bold type.

[f]First-order rate constants $k_{hydr}$ for the ester hydrolysis reaction increase linearly with increasing hydroxide concentration above pH 4, consistent with hydroxide attack. At lower pH values the rate of ester hydrolysis reaches a minimum at pH 3.5 and increases again at lower pH, consistent with acid catalysis.

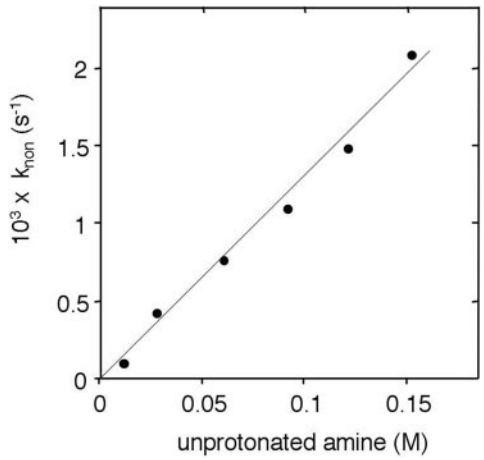


**Fig. 2.** Apparent first-order rate constant of peptide bond formation, calculated from integrated $^1$H NMR intensities of the reactant and product, plotted as a function of unprotonated amine, with a fixed concentration (0.25 M) of total amine at varying pH values at 40°C. Similar results were obtained at fixed pH by varying the concentration of total amine (data not shown). The linear dependence of the rate shows that only one molecule of Tris participates in the ester aminolysis reaction.

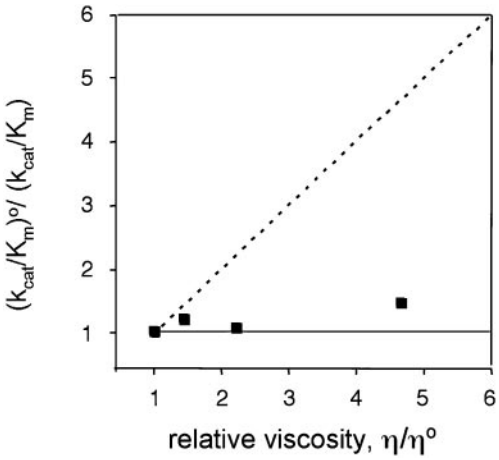


**Fig. 3.** Effect of viscosity on $k_{cat}/K_M$ of peptide bond formation on the ribosome. $k_{cat}/K_M$ was measured in the absence [$(k_{cat}/K_M)°$] or presence of increasing trehalose concentrations. $\eta°$ and $\eta$ are viscosities of the reaction solutions in the absence and presence of trehalose, respectively. The dashed line (slope = 1) shows the behavior expected if the rate of reaction were limited by binding of the substrate (Pm).

of Tris seems to participate in this uncatalyzed reaction. If Tris, in addition to being a substrate, catalyzed this reaction, the reaction rate would be expected to depend on the second (or higher) power of the amine concentration (20, 21).

Because peptide bond formation on the ribosome is rapid, with a first-order rate constant of 5 $s^{-1}$ in the presence of saturating Pm (pH 7.5, 25°C), the time course of the reaction was measured by quench-flow. Earlier work has shown that the chemical step is likely to be rate-limiting for the PT reaction on the ribosome at saturating concentrations of Pm (10). However, to obtain a second-order rate constant, $k_{cat}/K_M$, comparable with the second-order rate constants measured in the uncatalyzed reaction, it was necessary to conduct the reaction at subsaturating concentrations of Pm. Under these conditions, the observed rate of peptide bond formation might in principle be limited either by slow binding of the substrate or by a subsequent chemical process. We used viscosity variation to distinguish between these alternatives (22). If the rate of reaction were limited by binding of the substrate, then the rate of reaction would be expected to decrease with viscosity, yielding a slope of unity in a plot of the ratio $(k_{cat}/K_M)°/(k_{cat}/K_M)$ as a function of relative viscosity (Fig. 3). If a chemical step were rate-limiting, then the reaction rate would not be expected to depend on viscosity. In the presence of the viscosogen trehalose, the rate constant of peptide bond formation in the PT reaction was found to be nearly invariant with changing viscosity up to a relative viscosity of 4.7 (Fig. 3). Thus, binding does not appear to be rate-limiting in the PT reaction.[g]

Next, we determined the influence of temperature on the rates of the uncatalyzed (25–65°C) and ribosome-catalyzed (15–37°C) reactions. Both reactions yielded linear Arrhenius plots (Fig. 4). The uncatalyzed reaction of Tris with *N*-formylglycine ethylene glycol ester and with *N*-acetylglycine methyl ester showed activation parameters that were identical within experimental error, $\Delta H^{\neq} = +12.7 \pm 0.3$ kcal/mol and $T\Delta S^{\neq} = -11.8 \pm 0.4$ kcal/mol (25°C). Thus, the presence of a vicinal hydroxyl group adjacent to the ester linkage in the glycol ester does not affect reactivity significantly. An Arrhenius plot of $k_{cat}/K_M$ for peptidyl transfer by the ribosome yielded $\Delta H^{\neq} = +16.0 \pm 0.4$ kcal/mol and $T\Delta S^{\neq} = +2.0 \pm 0.2$ kcal/mol. In separate experiments, values for $k_{cat}/K_M$ were determined from the dependence of the rate of peptide bond formation on the concentration of Pm at 25°C and pH 7.5. Fig. 5 *Inset* shows that the velocity of the reaction reaches

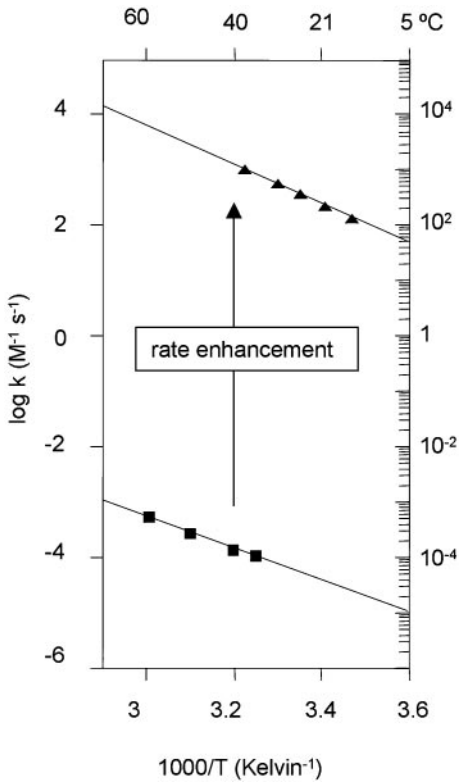


**Fig. 4.** Temperature dependence of the second-order rate constants of uncatalyzed (squares) and ribosome-catalyzed (triangles) peptide bond formation. The second-order rate constants are calculated for the concentration of total amine at pH 7.5.

[g]The absence of any apparent effect of trehalose on $k_{cat}/K_M$ could mask the presence of compensating effects on $k_{cat}$ and $K_M$. Under Pm saturating conditions, increasing concentrations of trehalose were found to have no effect on $k_{cat}$, tending to rule out that possibility.

BIOCHEMISTRY

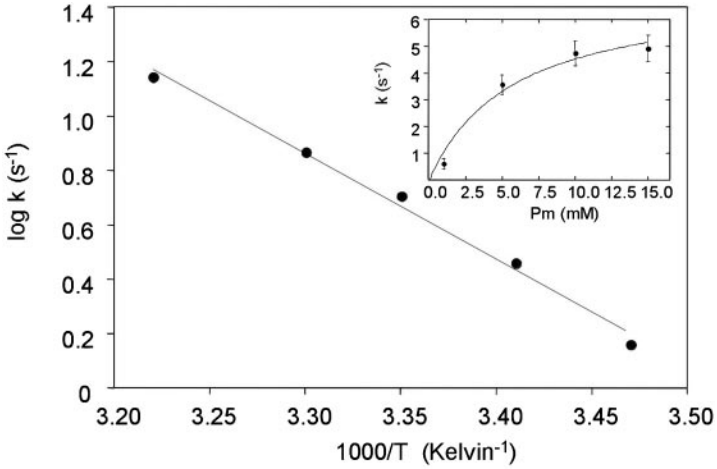


**Fig. 5.** Temperature dependence of the first-order rate constant of ribosome-catalyzed peptide bond formation. (*Inset*) Dependence of the rate of ribosome-catalyzed peptide bond formation on Pm concentration at 25°C.

a plateau at Pm concentrations ≥10 mM with a rate constant of 5 $s^{-1}$ and yields a $K_M$ of 5 mM. Similar measurements of the temperature dependence (15–37°C) of the first-order rate constant in the presence of saturating Pm also yielded a linear Arrhenius plot, with $\Delta H^{\neq} = +17.2 \pm 0.9$ kcal/mol and $T\Delta S^{\neq} = +0.7 \pm 0.2$ kcal/mol (Fig. 5).

The enthalpy of activation for the second-order reaction ($k_{cat}/K_M$) taking place on the ribosome, at nonsaturating con-

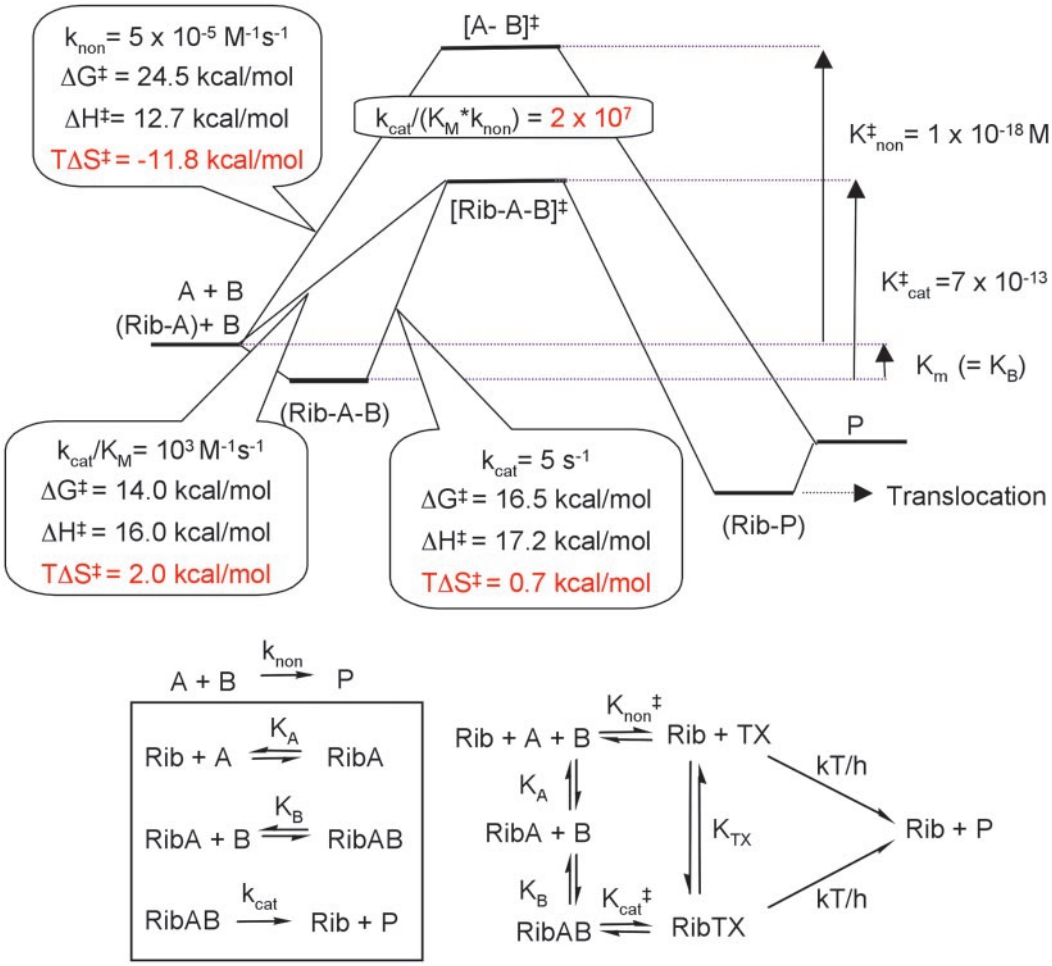


**Fig. 6.** Activation parameters at 25°C for the second-order uncatalyzed ($k_{non}$) and ribosome catalyzed peptide bond formation ($k_{cat}/K_M$), calculated from the concentration of total amine at pH 7.5. The broken line shows the first-order reaction in the ribosomal active site ($k_{cat}$). $K^{\ddagger}$ values represent the equilibrium constant between the ground state and transition state calculated from the differences in free energy.

centrations of Pm (16.0 kcal/mol), is somewhat more positive than the enthalpy of activation for uncatalyzed ester aminolysis (12.7 kcal/mol), not lower as would be expected if chemical catalysis were at work. The entropy of activation for peptidyl transfer within the ribosome (+2.0 kcal/mol) is much more favorable than for ester aminolysis in solution (−11.8 kcal/mol at 25°C). That difference in entropy is sufficient to account for the $2 \times 10^7$-fold rate enhancement ($[k_{cat}/K_M]/k_{non}$) that the ribosome produces (Fig. 6). It seems reasonable to speculate that this entropic advantage might arise from the positioning of substrates in the active site, or from the removal of the reaction from bulk water.[h] The zwitterionic intermediate in ester aminolysis (24) might be expected to organize water in its own vicinity, rendering the entropy of activation more negative. No such penalty would be incurred in a water-poor environment such as the active site of the ribosome whose structure is presumably organized to accommodate this species. Our findings suggest that general acid/base catalysis does not play a significant role in peptidyl transfer in the ribosome. The observed pH-dependence of the rate constant of peptide bond formation (10) may arise instead from conformational changes in the PT center. Consistent with that possibility, pH-dependent rearrangements at the active site of 23S rRNA have been demonstrated (25).

For single-substrate reactions, $k_{cat}$ can be compared with $k_{non}$ to obtain a dimensionless rate enhancement ($k_{cat}/k_{non}$). In a two-substrate reaction, the rate ratio has a different meaning. Because $k_{cat}$ is a first-order rate constant (expressed in units of $s^{-1}$), whereas $k_{non}$ is a second-order rate constant (expressed in units of $M^{-1} \cdot s^{-1}$), their ratio is expressed in units of molarity and represents the "effective concentration" of the second substrate that would be needed in solution to mimic the reactivity of the second substrate that is present in the active site (26). In the two-substrate process of peptide bond formation, the ratio of $k_{cat}$ to $k_{non}$ yields a value of $10^5$ M.

If it is designed to bind and align the two substrates, an "entropy trap" catalyst may also tend to bind the reaction products tightly because of their similarity in structure to the substrates. On the ribosome, the resulting barrier is overcome at least in two ways. First, the binding affinity of the A-site substrate (aminoacyl-tRNA) is decreased >1,000-fold upon its conversion to product (peptidyl-tRNA) (27), perhaps because of the loss of H-bonding interactions involving the amino group (5, 28). As a result, the 3′ ends of both peptidyl-tRNA and deacylated tRNA would be expected to gain some degree of freedom to move from the A and P sites to the P and E sites, respectively, resulting in "hybrid" binding states, as shown by footprinting (29) and functional tests (30, 31). Second, in the translocation reaction that is brought about by the action of elongation factor G, the tRNAs are moved from their "product positions" into their respective posttranslocation positions. That processive event moves the peptidyl-tRNA into the P site and prepares the A site of the PT center for the entry of the next aminoacyl-tRNA.

We thank Wolfgang Wintermeyer for valuable comments on the manuscript, Vladimir Katunin and Yuri Semenkov for tRNA preparations, and Petra Striebeck, Astrid Böhm, Carmen Schillings, and Simone Möbitz for expert technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (M.V.R.), the Alfried Krupp von Bohlen und Halbach-Stiftung (M.V.R.), the Fonds der Chemischen Industrie (M.V.R.), National Institutes of Health Grant GM-18325 (to R.W.), and National Institutes of Health Training Grant GM-08570 (to A.S.).

[h]For an intramolecular reaction in water, it has been shown that proximity alone does not appear to produce as large a rate enhancement as that which is achieved by the ribosome (23).

1. Ban, N., Nissen, P., Hansen, J., Moore, P. B. & Steitz, T. A. (2000) *Science* **289,** 905–920.
2. Nissen, P., Hansen, J., Ban, N., Moore, P. B. & Steitz, T. A. (2000) *Science* **289,** 920–930.
3. Schlunzen, F., Zarivach, R., Harms, J., Bashan, A., Tocilj, A., Albrecht, R., Yonath, A. & Franceschi, F. (2001) *Nature* **413,** 814–821.
4. Harms, J., Schluenzen, F., Zarivach, R., Bashan, A., Gat, S., Agmon, I., Bartels, H., Franceschi, F. & Yonath, A. (2001) *Cell* **107,** 679–688.
5. Hansen, J. L., Schmeing, T. M., Moore, P. B. & Steitz, T. A. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 11670–11675.
6. Zhang, B. & Cech, T. R. (1997) *Nature* **390,** 96–100.
7. Zhang, B. & Cech, T. R. (1998) *Chem. Biol.* **5,** 539–553.
8. Fieser, L. F. & Fieser, M. (1956) *Organic Chemistry* (Reinhold, New York).
9. Weber, A. L. & Orgel, L. E. (1979) *J. Mol. Evol.* **13,** 185–192.
10. Katunin, V. I., Muth, G. W., Strobel, S. A., Wintermeyer, W. & Rodnina, M. V. (2002) *Mol. Cell* **10,** 339–346.
11. Thompson, J., Kim, D. F., O'Connor, M., Lieberman, K. R., Bayfield, M. A., Gregory, S. T., Green, R., Noller, H. F. & Dahlberg, A. E. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 9002–9007.
12. Polacek, N., Gaynor, M., Yassin, A. & Mankin, A. S. (2001) *Nature* **411,** 498–501.
13. Beringer, M., Adio, S., Wintermeyer, W. & Rodnina, M. (2003) *RNA* **9,** 919–922.
14. Wolfenden, R., Snider, M., Ridgway, C. & Miller, B. (1999) *J. Am. Chem. Soc.* **121,** 7419–7420.
15. Zief, M. & Edsall, J. T. (1937) *J. Am. Chem. Soc.* **59,** 2245–2248.
16. Bates, R. G. & Hetzer, H. B. (1961) *J. Am. Chem. Soc.* **65,** 667–671.
17. Rodnina, M. V., Fricke, R., Kuhn, L. & Wintermeyer, W. (1995) *EMBO J.* **14,** 2613–2619.
18. Rodnina, M. V., Savelsbergh, A., Matassova, N. B., Katunin, V. I., Semenkov, Y. P. & Wintermeyer, W. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 9586–9590.
19. Wynne-Jones, W. F. K. & Eyring, H. (1935) *J. Chem. Phys.* **3,** 492–502.
20. Bruice, T. C. & Benkovic, S. J. (1964) *J. Am. Chem. Soc.* **86,** 418–426.
21. Jencks, W. P. & Gilchrist, M. (1966) *J. Am. Chem. Soc.* **88,** 104–108.
22. Snider, M. J., Gaunitz, S., Ridgway, C., Short, S. A. & Wolfenden, R. (2000) *Biochemistry* **39,** 9746–9753.
23. Tamura, K. & Schimmel, P. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 1393–1397.
24. Gresser, M. J. & Jencks, W. P. (1977) *J. Am. Chem. Soc.* **99,** 6963–6967.
25. Bayfield, M. A., Dahlberg, A. E., Schulmeister, U., Dorner, S. & Barta, A. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 10096–10101.
26. Kirby, A. J. (1980) *Adv. Phys. Org. Chem.* **17,** 183–278.
27. Semenkov, Y. P., Rodnina, M. V. & Wintermeyer, W. (2000) *Nat. Struct. Biol.* **7,** 1027–1031.
28. Schmeing, T. M., Seila, A. C., Hansen, J. L., Freeborn, B., Soukup, J. K., Scaringe, S. A., Strobel, S. A., Moore, P. B. & Steitz, T. A. (2002) *Nat. Struct. Biol.* **9,** 225–230.
29. Moazed, D. & Noller, H. F. (1989) *Nature* **342,** 142–148.
30. Semenkov, Y., Shapkina, T., Makhno, V. & Kirillov, S. (1992) *FEBS Lett.* **296,** 207–210.
31. Sharma, D., Southworth, D. R. & Green, R. (2004) *RNA* **10,** 102–113.
32. Owen, B. B. (1937) *J. Am. Chem. Soc.* **56,** 24–27.
33. Wolfenden, R. (1963) *Biochemistry* **2,** 1090–1092.