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What Are the Roles of Substrate-Assisted Catalysis and Proximity Effects in Peptide Bond Formation by the Ribosome?†

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ABSTRACT: The action of the peptidyl transferase center of the large ribosomal unit presents a fundamental step in the evolution from the RNA world to the protein world. Thus, it is important to understand the origin of the catalytic power of this ancient enzyme. Earlier studies suggested that the ribosome catalyzes peptide bond formation by using one of its groups as a general base, while more recent works have proposed that the catalysis is due to proximity effects or to substrate-assisted catalysis. However, the actual nature of the catalytic mechanism remains controversial. This work addresses the origin of the catalytic power of the ribosome by using computer simulation approaches and comparing the energetics of the peptide bond formation in the ribosome and in water. It is found that a significant part of the observed activation entropy of the reference solution reaction is due to solvation entropy, and that the proximity effect is smaller than previously thought. It is also found that the 2′-OH of the A76 ribose, which is associated with a large rate acceleration in the ribosome reaction, does not catalyze peptide bond formation in water. Thus, the catalytic effect cannot be attributed to substrate-assisted catalysis but rather to the effect of the ribosome on the reacting system. Overall, our calculations indicate that the reduction of the activation free energy is mainly due to electrostatic effects. The nature of these effects and their relationship to catalytic factors in modern enzymes is analyzed and discussed.

The spectacular advance in the structural evaluation of the ribosome (*1*−*5*) has led to a molecular view of the elements that participate in peptide bond formation (*4*, *5*). However, the details of this fundamental step are still unclear (*6*). Several proposals have been put forward for the mechanism and the origin of the catalytic power of the ribosome. An early proposal that a group on the ribosome serves as a general base (*7*) was found to be in conflict with more recent experiments (*8*), and attention has now been shifted to new proposals (*9*−*12*). The most detailed recent proposals involved two different directions. One of these proposals addressed the possibility that the catalytic power is due to a reduction in the activation entropy (*9*, *13*), while the other focused on a substrate-assisted catalysis (*11*).

The idea that entropic effects can contribute to enzyme catalysis is clearly not new and has particularly been advanced by Jencks and co-workers (*14*). However, this idea has been found to be inconsistent with theoretical estimates (*15*, *16*) and experimental studies (*17*), in the case of "modern" enzymes. The problem with this proposal has been associated with the fact that the activation entropy for the reference reaction in solution is much smaller than what is usually assumed because the formation of the transition state in solution only restricts a small number of degrees of freedom (*15*). However, in the case of the reference reaction of peptide bond formation by the ribosome, it was found by Wolfenden and co-workers (*9*) that the activation entropy is exceptionally

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large, and thus, it is possible that in this case the catalysis involves a large entropic contribution. It was proposed that this entropic contribution might be associated with the restriction of substrate motion as was envisioned in Jencks' proposal for enzymes (*14*), and as proposed by Moore and Steitz (*12*) and others (*10*). However, as was also recognized by Wolfenden and co-workers (*9*), the observed entropic contribution may reflect changes in solvation entropy rather than the restriction of the substrate configurational space. Now, as stated above, careful analysis of the entropic proposal in regular enzymes (*15*, *18*) indicated that this effect was quite small. Thus, it would be interesting to find out if the ribosome uses a strategy different from those used by more modern enzymes.

The proposal that ribosome catalysis is associated with the substrate itself is also less established than what may be concluded at first glance. That is, Weinger et al. (*11*) found that removal of the P-site tRNA A76 2′-OH group reduces k_{cat} by a factor of 10^6, which is close to the estimate of the catalytic effect of the ribosome [3.5×10^6 for $k_{cat}/(K_M k_{non})$ and 1.6×10^6 for k_{cat}/k_{non}, according to ref *13*], and thus proposed that we have here a case of substrate-assisted catalysis (*11*). However, in this case, one may also question the simple interpretation, since if the catalysis is just due to the 2′-OH group of the substrate, then the same group can catalyze the reaction without the ribosome and then we will not have any catalytic effect.

The issue of the catalytic power of the ribosome is also fascinating because this system represents a very ancient enzyme that perhaps could not exploit the principles of more modern enzymes. Thus, it is important to determine if the ribosome reaction involves nonstandard factors, or whether the rules of biocatalysis were already established with the earliest catalytic machineries.

In this work, we address the above questions by using modern computer simulation approaches. We found in a preliminary study that a significant part of the activation entropy for the reference solution reaction is due to changes in solvation entropies. A similar conclusion was reached by Trobro and Åqvist (*19*), who performed much more extensive calculations and simulated the reaction in the ribosome itself. The present study will start with a systematic evaluation of the free energy surface for the 2′-OH-assisted mechanism in solution. The surface will be used in exploring the entropic effects and the nature of the overall catalysis. The surface will also be used in examining the overall validity of the substrate-assisted catalysis proposal.

METHODS AND SYSTEMS

Our challenge is to examine different options for the catalysis of peptide bond formation in the ribosome. This reaction can be described by the sequence of steps depicted in Figure 1 and, as much as the reaction in the ribosome is concerned, the orientation of the reacting fragments probably corresponds to the *S*-configuration described in the left panel of Figures 1 and 2 (see refs *20* and *31*).

Since we are dealing here with different catalytic options, it is important to use different computational methods capable of modeling the different alternatives. Some of the methods that will be used here will only be mentioned in passing in the corresponding sections. However, other methods will be considered briefly below.

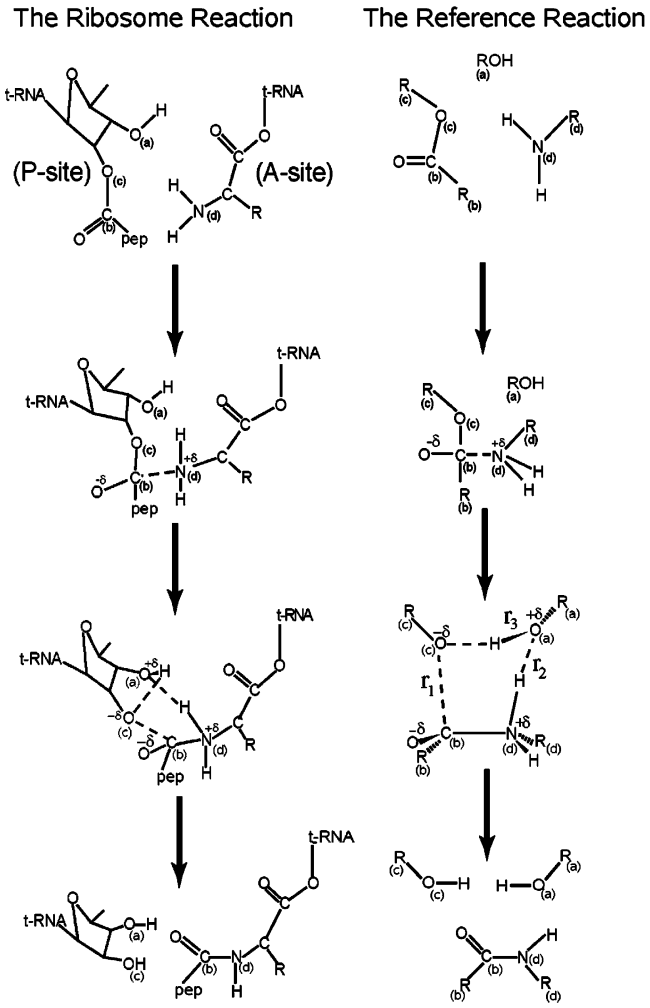

FIGURE 1: Schematic description of the steps in peptide bond formation for the ribosome (left) and in a reference solution reaction (right). The letter code on some atoms is only given to orient the reader to the identity of different groups.

Probably the optimal starting point for studies of enzymatic reactions is the establishment of the energetics of the corresponding reference reactions in solution. This is clearly essential for the elucidation of the difference between the catalyzed and unanalyzed reactions. In the present case, we can use the results of our early extensive study of the free energy surface for methanolysis of amines in solution. Apparently, the surface for regular peptide bond formation is obtained from the same surface. The original evaluation of the surface for the methanolysis reaction (*21*) was based on careful ab initio calculations coupled with the Langevin dipole (LD)[1] solvent model (a QM/LD model) in the program Chem/Sol (*22*). The final "consensus" surface was obtained by subjecting the calculated proton transfer energies to experimental calibration, thus guaranteeing as close as possible a connection between the relevant and known experimental observations. In this work, we used the same approach and evaluated the surfaces for substrate-assisted catalysis in water and also re-evaluated the surface for water-catalyzed peptide bond formation in water. We also repeated the calculations with the more widely available COSMO

[1] Abbreviations: EVB, empirical valence bond; LD, Langevin dipole; RR, restraint release; LRF, local reaction field; LRA, linear response approximation.

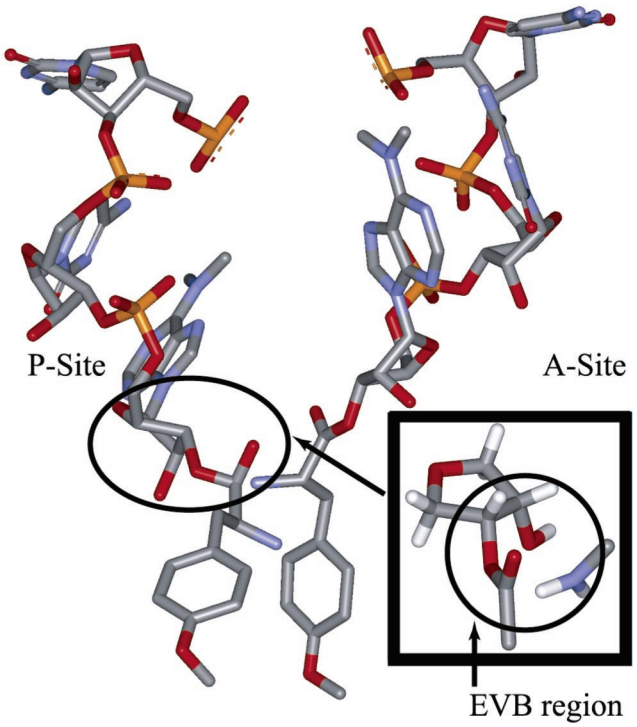

FIGURE 2: Reacting system inside the ribosome and the part included in the EVB active space.

solvent model (*23*). The actual calculations started by scanning the surface in the gas phase as a function of r_1 and r_3 for different values of r_2 (see the notation of Figure 1), where at each point we constrained the system at the specific r_1 and r_3 values and minimized the energy with regard to all the other coordinates. Next we considered the effect of the solvent by performing a single-point calculation of the energy in solution at each of the scanning points. The resulting surface served as an approximation for the energetics of the reaction in solution. In doing so, we assumed implicitly that minimization with respect to the solute coordinates orthogonal to r_1, r_3, and r_2 will give similar results in the gas phase and in solution. This assumption was verified for some specific cases. It seems to us that the scanning procedure described above is at present more effective than the alternative option of performing a transition state search in the solution surface using analytical derivatives (which is, as of now, still problematic). At any rate, our calculated COSMO surfaces are described in Figure 3 (see also the Supporting Information), and the transition state for the substrate-assisted reaction in water is depicted in Figure 4. The LD surface (not shown) was found to be quite similar to the COSMO surfaces, with no more than 3 kcal/mol difference at some points, and with the same trend in the difference between the waster-assisted and 2′-OH-assisted reactions.

The next tool in our modeling study has been the empirical valence bond (EVB) model. This model, which has been described extensively elsewhere (*24*, *25*), represents the overall potential surfaces of the system in solution or proteins by mixing resonance structures that correspond to the reactant, the product, and different intermediates in the presence of the given environment. The free energy surface that corresponds to the given potential surface is evaluated by umbrella sampling free energy perturbation (US/FEP) calculations (*24*). The reliability of the EVB calculations is ensured by forcing the solution calculations to reproduce the best estimates of the energetics of the solution reaction, and then using the same parameters without any change while replacing the effect of the solution environment in the EVB Hamiltonian by the protein or ribosome environment.

Since one of the main issues in this paper is the nature of the entropic contributions to the catalytic effect, we have to be able to estimate activation entropies. This task, whose challenging nature is reviewed in ref *26*, is accomplished here by combining two approaches. The entropic effects associated with the proximity of the reacting fragments ($\Delta S^{\ddagger}_{prox}$)

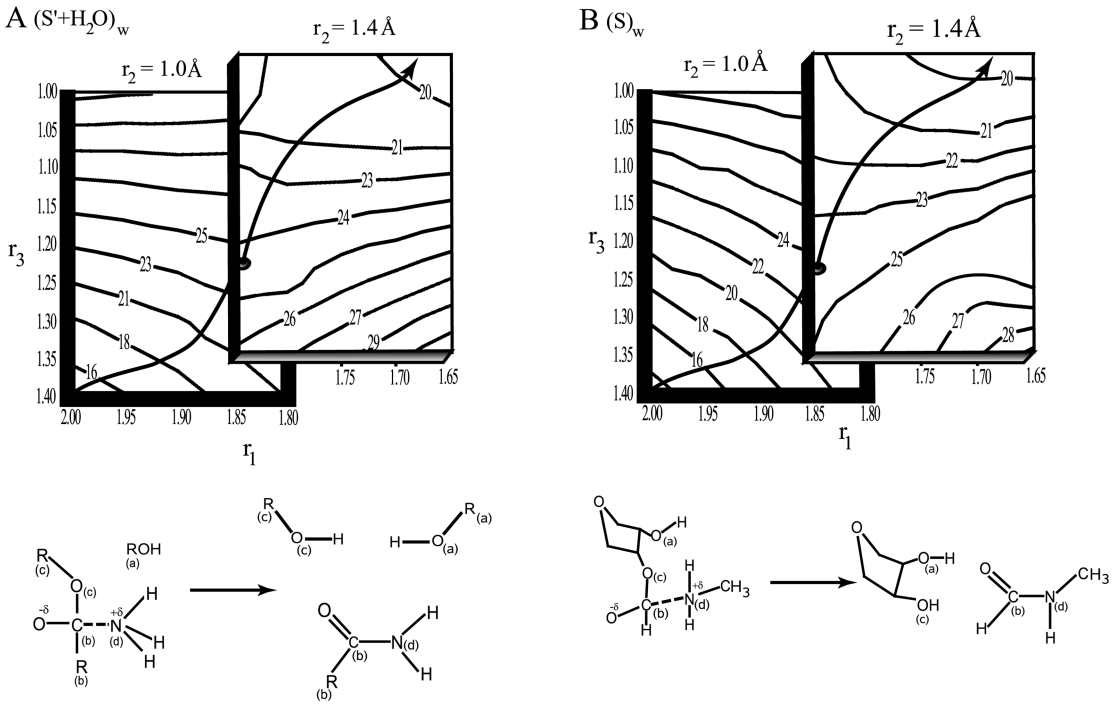

FIGURE 3: (A) Free energy surface for peptide bond formation in water, for the water-assisted reactions of ammonia and methyl methanoate. (B) Free energy surface for peptide bond formation in water, for the 2′-OH-assisted reactions of methylamine and a ribosyl ester. The surfaces are drawn as functions of r_1 and r_3 for several values of r_2 (r_1, r_2, and r_3 are defined in Figure 1).

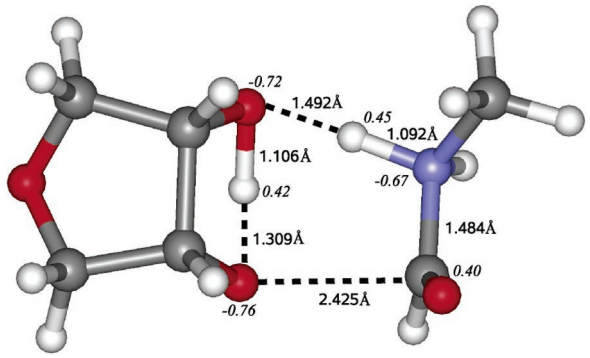

FIGURE 4: Calculated structure of the transition state (TS) for the substrate-assisted reaction in water. The numbers in italics are the Mulliken charges, obtained using the COSMO solvent model at the B3LYP/6-31G* level.

are evaluated by our restraint release approach [RR (*15*)]. This approach is based on subjecting the given system in its reactant state (RS) or transition state (TS) to a strong harmonic Cartesian restraint and then evaluating the free energy of releasing this restraint. This procedure is repeated for different sets of Cartesian restraint coordinates, and the lowest RR free energy obtained is taken as the best estimate of the configurational entropy of the system. This variational minimization reflects the fact that all the RR free energies contain enthalpic contributions (*15*) and that these contributions approach zero for restraint coordinates that give the lowest RR contribution.

Thus, we can write

$$-T\Delta S^{\ddagger}_{\text{prox}} = \min(\Delta G^{\text{TS}}_{\text{RR}}) - \min(\Delta G^{\text{RS}}_{\text{RR}}) + T\Delta S^{\text{RS}}_{\text{cage}} \quad (1)$$

where min indicates the minimum value of the indicated ΔG_{RR}. Here, $\Delta S^{\text{RS}}_{\text{cage}}$ is the entropy associated with bringing the reacting fragment from a molar volume to the same solvent cage. This term is evaluated analytically and rigorously as a function of the restraint used to keep the fragment at a contact distance (see ref *15* for details).

The contributions of the solvent molecules are much simpler to evaluate than the solute configurational entropies since they represent a simple electrostatic effect, where the orientation of the solvent dipole toward the solute charges leads to a reduction in their movements and to negative entropic contributions. This type of effect has been shown to be captured quantitatively by a calibrated LD model that assesses the entropic contribution of each solvent dipole by examining the field on these dipoles (*27*).

This is done by using

$$\Delta S_{\text{sol}} = C_i \sum_j \Delta S'_j + \Delta S_{\text{phob}} \quad (2)$$

where C_i is an empirical constant, j runs over the solvent dipoles, and $\Delta S'_j$ is given by

$$\Delta S'_j = R \ln\left\{\frac{\pi}{2 \arccos[L(x_j)]}\right\} - 5R \arctan\left(\frac{x_j}{27}\right) \exp\left(-\frac{x_j^6}{32}\right) \quad (3)$$

$$x = \frac{\mu_0 \zeta}{3kT}$$

and μ_0, ζ, k, and T denote the magnitude of the solvent dipole, the total electrostatic field as the position of the dipole, the Boltzmann constant, and the thermodynamic temperature, respectively. The term ΔS_{phob} is the field-dependent hydrophobic estimate described in ref *27*.

In the analysis of the catalytic power of enzymes, it is frequently important to evaluate the electrostatic energy of the RS and TS. This is done here by using the linear response approximation [LRA (*28*)]. This approach uses the relationship

$$\Delta G_{\text{ele}} \cong \frac{1}{2}[\langle U(Q{=}Q_0) - U(Q{=}0)\rangle_{Q=Q_0} + \langle U(Q{=}Q_0) - U(Q{=}0)\rangle_{Q=0}] \quad (4)$$

where the $\langle\ \rangle_Q$ expressions designate the averages over trajectories of the solvent under the influence of substrate charge Q. The substrate is considered with its actual residual charges ($Q = Q_0$) and in the fully nonpolar form ($Q = 0$) (see ref *28* for more discussion). The contribution from the second term is due to the preorganization of the active site, and it is zero for reactions in water (see ref *29*).

The EVB force field was constructed by fitting it to the solution potential surface. To simplify the calculations, we constructed EVB states for the reactant, the tetrahedral intermediate, and the transition state. This simplified treatment was used in ref *30*, and it provides a convenient tool for accelerated convergence when the reactant and product states are very different. Furthermore, the treatment provided us with a simple and effective way of comparing the electrostatic energy of the TS in water and in the enzyme.

The structures used for the EVB calculations were constructed as follows. In one model (model A), we merged the structures of the A site [PDB entry 1FG0 (*7*)] and the P site [PDB entry 1M90 (*31*)] of the Yale group and then built the actual substrate on the corresponding CC-puromycin and CCA-Phe-caproic parts. In the other model (model B), we took the Weizmann Institute structure composed of ASM-D50 structure 9 (PDB entry 1NJP) and the P site coordinates, described in ref *20* and kindly provided by A. Yonath, and again modified the end groups in the P and A sites to a model of the actual substrate. Of course, all the initial structures were later relaxed and allowed to undergo the chemical transformation by the EVB mapping procedure (see below). The coordinates of the substrate at a snapshot of the simulation in the reactant and the transition states of the ribosome in model A are given in the Supporting Information. The reactant state structure (RS) is not so far from the original PDB (rmsd of 0.9 Å for the entire system). Model A was used for the full EVB calculations, while both models A and B were used for the evaluation of the electrostatic contributions to catalysis.

The simulation systems were solvated by a 22 Å SCAAS solvent model (*32*) centered around the selected EVB region (see Figure 2) with the SCAAS special polarization constraints that guarantee proper electrostatic boundary conditions. Long-range electrostatic effects were treated by the local reaction field (LRF) model (*33*). The LRF treatment, which is equivalent to a treatment without any cutoff, involved a 10 Å inner sphere around each group (where the electrostatic interactions were evaluated at every step) and a fourth-order expansion of the potential from the rest of the system, which is updated every 40 steps (see ref *33* for details). To minimize the problems in treating the highly

Table 1: Calculated Contributions (in kilocalories per mole) to the Activation Barrier of Peptide Bond Formation in Water and in the Ribosome[a]

system	reaction	$-T\Delta S^{\ddagger}_{sol}$	$-T\Delta S^{\ddagger}_{prox}$	$\Delta H^{\ddagger}$[c]	$\Delta g^{\ddagger}$
$(S'+H_2O)_w$	H_2O as the base	7	$4.5 + 2.3$[b]	10	24
$(S)_w$	2′-OH as the base	7	$3.5 + 2.3$[b]	11	24
ribosome (A)	2′-OH as the base	4[d]	≥ 4.0[d]		16

[a] The systems considered are (i) the water-assisted reactions of ammonia and methyl methanoate in water [$(S'+H_2O)_w$], (ii) the 2′-OH-assisted reactions of methylamine and 1,5-dideoxy-3-*O*-acetyl-β-D-ribofuranose in water [$(S)_w$], and (iii) the ribosome reaction in model A. The $-T\Delta S^{\ddagger}_{sol}$ and $-T\Delta S^{\ddagger}_{prox}$ values are the contributions of solvation entropy and orientational entropy to the activation barrier, respectively. [b] The orientational entropy includes an ~2.3 kcal/mol contribution from the work of bringing the reactants to the same solvent cage (see ref *15*). This contribution does not include the effect of the water molecule that serves as a base since the reaction takes place in 55 M water. [c] The enthalpic contributions were obtained using the relation $\Delta H^{\ddagger} = \Delta g^{\ddagger} - T\Delta S^{\ddagger}$. [d] The separation to entropy and enthalpy is problematic for the reaction inside the ribosome, since it depends significantly on what is taken as the reacting system (a slightly larger region I gave a lower $-T\Delta S^{\ddagger}_{prox}$); on the other hand, the electrostatic contribution (Table 2) remains constant.

charged system, we kept the overall system neutral by adding counterions that balanced the charges due to phosphate.

The simulations were started with a 40 ps relaxation, and then we continued with EVB simulations consisting of 51 windows each of a simulation time of 20 ps, with 0.5 fs time steps. The simulations were conducted at 298 K. We also ran linear response approximation (LRA) calculations of the electrostatic energies of the transition state and the ground state running 40 ps in each state. All the actual calculations were carried out with the MOLARIS package (*34*) and the Chem/Sol program (*35*).

RESULTS AND DISCUSSION

Analyzing the Entropic Contributions. As a first step in our analysis, we evaluated the solvation entropies for the fragments involved in the water-catalyzed reaction water using the LD model. The calculations were performed with Chem/Sol using the ab initio Mulliken charges obtained from B3LYP/6-31G* level calculations with the COSMO solvent model using the GAUSSIAN03 program package (*36*). The results are summarized in Table 1 and in the Supporting Information. As seen from the table, the contribution of the solvation entropy, $\Delta S^{\ddagger}_{sol}$, to $-T\Delta S^{\ddagger}$ is quite significant.

Evaluating the contributions from the solute configurational entropy, $-T\Delta S^{\ddagger}_{prox}$, to the activation barrier is quiet challenging, and was done here by the RR approach (*15*). The RR calculations led to the results presented in Tables 1. As seen from the table, $\Delta S^{\ddagger}_{prox}$ for the reaction in water amounts to ~5 kcal/mol from the RR calculations. The additional ~2.3 kcal/mol accounts for the effect of having the reactants in the same solvent cage, or of moving from 1 M to the effective concentration of water (55 M). This $-T\Delta S_{cage}$ effect, which is evaluated rigorously in our RR approach, represents a well-known factor that is not considered as a part of the catalytic puzzle. At any rate, our calculation gives a $-T\Delta S^{\ddagger}$ of ~12 kcal/mol for the water-catalyzed reaction in water, which is in good agreement with the corresponding observed effect of ~13 kcal/mol (*13*). It is also possible that our calculations overestimated $\Delta S^{\ddagger}_{prox}$ since the RR approach provided an upper limit (in case the lower limit is not reached).

While the calculations of the entropy contribution to the activation energy of the reference reaction in water are directly comparable to the corresponding experimental results, this does not apply to the ribosome reaction. That is, in the case of the ribosome, the E + S → ES step involves the entire A chain as a substrate (e.g., see ref *9*). However, our entropy calculations consider the RR process for a small part of the reacting system. In this case, we do not consider explicitly the entropy associated with the change in interaction between the tRNA and its surroundings. Evidently, these entropy effects are complicated and hard to quantify since they involve a change in solvation and strong ionic interactions (where entropy−enthalpy compensation can play a major role). This point becomes quite clear when one notes that the binding entropy of the overall substrate is positive instead of negative; this is, in fact, in conflict with what is expected of an entropy trap.

With the considerations described above in mind, we find it more instructive to focus on the origin of the overall activation free energy of the ribosome reaction rather than on its entropies and enthalpic contributions. It is important to recognize in this respect that the catalytic effect is related in our analysis to the comparison of the chemical step in the ribosome and in solution [k_{cat}/k_{non} rather than $k_{cat}/(K_M k_{non})$] and that the observed value of k_{cat}/k_{non} is 1.6×10^4 (this corresponds to $\Delta g_w^{\ddagger} - \Delta g_{cat}^{\ddagger} \approx 5.7$ kcal/mol).

Substrate-Assisted Catalysis. While the analysis presented above can rationalize the observed entropic effects, we should also address the observed effect of the 2′-OH group and the substrate-assisted catalytic proposal. To explore this problem, we started by considering the chemical advantage of having the 2′-OH group as the base instead of a water molecule, in the reference reaction in water. This was done by repeating the QM/LD and COSMO calculations with the 2′-OH group as a base.

Although we expected some chemical catalysis due to the change in the pK_a between H_3O^+ and ROH_2^+, it appears that the effect only manifests itself in the intermediate step and basically disappears at the rate-limiting transition state (see Figure 3). It was found that there was no special enthalpic or strain penalty for having the 2′-OH group serve as a base relative to only methanol as a base. Thus, we could not find any significant advantage in having a substrate-assisted catalysis in water (see Table 1).

Since the substrate assistance in water does not explain its effectiveness in the ribosome, we had to examine the catalytic effect of the ribosome in a systematic EVB study that compares the substrate-assisted reaction in water and in the ribosome. The results of these calculations are also summarized in Table 1. As seen from the table, we were able to account semiquantitatively for the ribosomal catalysis (8 kcal/mol calculated vs 6 kcal/mol observed for $\Delta g_w^{\ddagger} - \Delta g_{cat}^{\ddagger}$), as accomplished previously in the careful study of Trobro and Åqvist (*19*). The difference is associated with our emphasis on analyzing the substrate-assisted effect and in our analysis of the entropy and electrostatic contributions. Furthermore, our LRA analysis (see below) appears to provide an additional important insight.

Electrostatic Contributions to Catalysis. Although the calculated catalytic effect reported in Table 1 and Figure 5 is in good agreement with the observed effect [~6 kcal/mol (*13*)], our main emphasis is on the analysis of the origin of this effect. Analyzing Table 1, we find that the calculated 8 kcal/

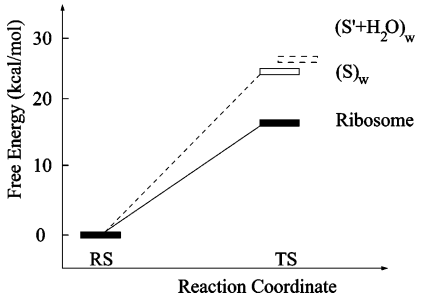

FIGURE 5: Calculated energetics of the ribosome reaction (in model A), the reference reaction of ammonia and methyl methanoate in water, and of methylamine and a ribosyl ester in water.

Table 2: Electrostatic Contributions to Catalysis[a]

structure	$\Delta\langle\Delta U\rangle_{Q=Q_0}$	$\Delta\langle\Delta U\rangle_{Q=0}$	$\Delta\Delta g^{\ddagger}_{elec}$
model A	−11	−6	−8
model B	−10	−4	−7

[a] The electrostatic contributions (in kilocalories per mole) were evaluated by the LRA approach of eq 4. $\Delta\langle\Delta U\rangle_{Q=Q_0}$ and $\Delta\langle\Delta U\rangle_{Q=0}$ are the contributions to the activation barrier from the first and second terms of eq 4, respectively. Models A and B are defined in Methods and Systems.

mol difference between the $\Delta g^{\ddagger}$ of the ribosome reaction and that of the substrate-assisted catalysis involves ∼3 kcal/mol of solvation entropy and 2 kcal/mol of orientational entropy. Here we note, however (see below), that while the entropic contributions in the solution reaction are well-defined, it is harder to analyze and classify the entropic effects in the ribosome. At any rate, to explore the origin of the overall catalytic effect, we performed the LRA calculations of eq 4. These calculations (Table 2) indicated that $\Delta G^{\ddagger}_{ele} = \Delta G_{ele}(TS) - \Delta G_{ele}(RS)$ is ∼−8 kcal/mol, and that approximately half of this effect comes from the preorganization term and the other half from the $\langle\ \rangle_{Q=Q_0}$ term in eq 4. The nature of the electrostatic stabilization can also be illustrated by considering the potential (U) from the surrounding (water or ribosome) at the location of each substrate atom and writing

$$\Delta g^{RS\rightarrow TS}_{elec} \cong \sum_k \Delta V_k = \sum_k U^{TS}_{elec,k} Q^{TS}_k - U^{RS}_{elec,k} Q^{RS}_k \quad (5)$$

where Q_k is the residual charge on the k^{th} atom.

Figure 6 depicts ΔV ($=V^{TS} - V^{RS}$) at each of the substrate atoms for the reaction in both the ribosome and water. Note that we include, in eq 5, only those atoms whose charges change upon moving from the RS to the TS. Evidently, the ΔV values are larger in the ribosome, and thus, the corresponding electrostatic contribution to catalysis is larger.

While we expect an error range of ∼2 kcal/mol in the LRA analysis, this analysis indicates that most of the catalytic effect is due to electrostatic contributions. Hence, it is important to recognize that most of the solvation entropy effect is simply an electrostatic free energy contribution.

CONCLUDING REMARKS

Since the ribosome represents a very ancient enzyme that evolved during the transition from the RNA world to the protein world, it is reasonable to wonder whether it exploited the principles of more modern enzymes or used more primitive tricks. Several instructive proposals have recently been put forward, including the idea that the enzyme uses

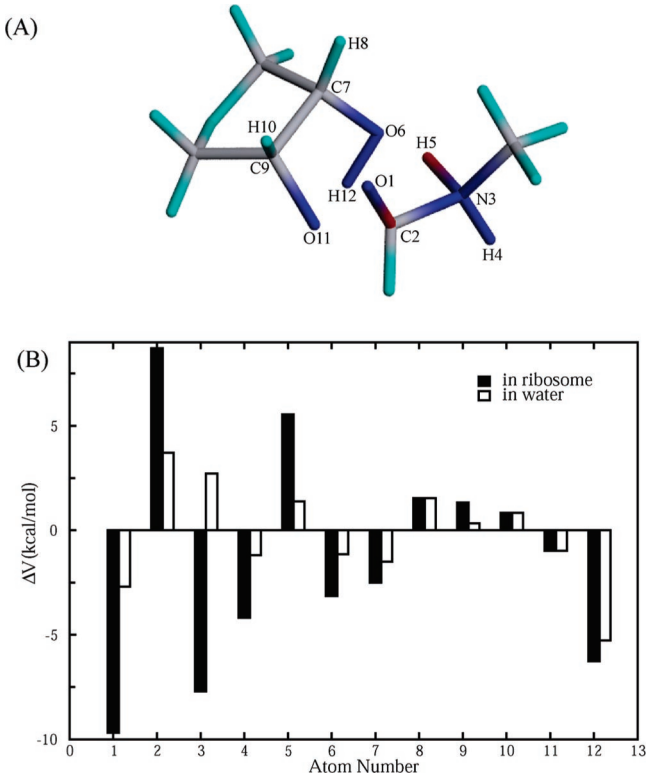

FIGURE 6: (A) ΔV values of eq 5 at the different atoms of the substrate in the ribosome (red and blue designate positive and negative contributions, respectively). (B) ΔV values at each of the substrate atoms for the reaction in the ribosome (black) and in water (white). O_6 and O_{11} correspond to $O_{(a)}$ and $O_{(c)}$, respectively, in Figure 1.

proximity effects (*9*, *10*, *12*), and substrate-assisted catalysis (*11*). While these ideas are based on clear experimental findings, it is hard to quantify them, since it is not clear how to relate the structure and function of the ribosome in a unique way, while using currently available experimental methods. Here we tried to address the question of the catalytic power of the ribosome by giving the structural information and state-of-the-art computational methods. We first required the computational results to reproduce the overall experimental kinetic and then decomposed the contributions to the overall activation barriers and analyzed their origin.

It was found that the proximity effects are much smaller than what is usually assumed, and that a large part of the catalytic effect is due to the reduction in the solvation entropy. Furthermore, it was also found that the overall reduction in the activation free energy is due to electrostatic effects. As discussed in the previous section, it is hard to quantify the separation of the activation free energy into entropic and enthalpic contributions, since the substrate involves large tRNA chains in addition to the actual reacting system. However, the overall catalytic free energy is reproduced almost quantitatively by the corresponding changes in the contributions from the electrostatic free energy of the system.

Regardless of the exact contribution of the enthalpic and entropic effects, we find here that the appealing idea that substrate-assisted catalysis (*37*, *38*) is the origin of the catalytic power of the ribosome (*11*) may be an oversimplification. That is, the calculations show that the substrate-

assisted reaction in water is not faster than the corresponding water-assisted reaction in water. However, inside the ribosome and with the ribosome electrostatic effect, the reaction uses the 2′-OH group of the substrate.

At this stage, it is still hard to determine the exact catalytic consequence of the evolutionary stage of the ribosome as compared to other enzymes. The difference might be partially due to the fact that the ribosome binds a very large substrate, where the bound reacting part is larger than that in most enzymes. In this case, the binding of the nonreacting parts of the substrate may contribute to pulling the substrate into the active site. On the other hand, in many modern enzymes, it is found that the ground state of the reacting part is already stabilized in the active site more than in water. However, it is clear that in both cases electrostatic effects play a major role and that the preorganization effect was already incorporated in the ribosome machinery.

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SUPPORTING INFORMATION AVAILABLE

Four tables and four figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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