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Entropy of the transition state for formation of the peptide bond in the ribosome

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ABSTRACT

In an earlier paper, using quantum mechanics we had investigated the mechanism for peptide bond formation. The calculation was based on a choice of 50 atoms assumed to be important in the mechanism. We used density functional theory to optimize the geometry and energy of the transition state (TS) for the peptide bond formation. That calculation provided the activation energy and mechanism for the TS reaction, but did not consider its thermodynamic parameters. In this paper we calculate entropy $\Delta S^{\ddagger}$, enthalpy $\Delta H^{\ddagger}$, and free energy $\Delta G^{\ddagger}$ for the TS reaction. Using transition state theory (TST) we calculate the rate constant for the TS reaction based upon our calculated $\Delta G^{\ddagger}$. When the rRNA of the peptidyl transfer center (PTC) is considered along with the reactants which pass through it we find that both enthalpy and entropy contribute to the catalysis of the TS reaction.

KEYWORDS

Peptide bond;
Free energy;
Rate constant;
Thermal parameters;
Quantum mechanics.

INTRODUCTION

The ribosome catalyzes the formation of the peptide bond in the production of proteins. The precise details of the mechanism which accomplishes this catalysis has not until recently been known. The crystallization of the ribosome and the X-ray solution of its structure[1] provide the overview idea of the mechanism, and a constraint to which the atomic motions of the mechanism must conform. Our suggestion for the mechanism[2] for formation of the TS gives its atomic geometry and activation energy (E_a). Beginning with the atomic coordinates of a pair of amino acids as they would lie relative to one another in the ribosome, they are allowed to react. Using quantum mechanics (QM) in the form of density functional theory (DFT) a transition state (TS) is found for the reaction and the geometry and a quantitative E_a result. Given such a QM TS we then require that it be "fit" to the environment of the ribosome. The calculated geometry was seen to accommodate well to the physical space available in the ribosome PTC. The 2-fold symmetry of the PTC is suggestive of a rotary

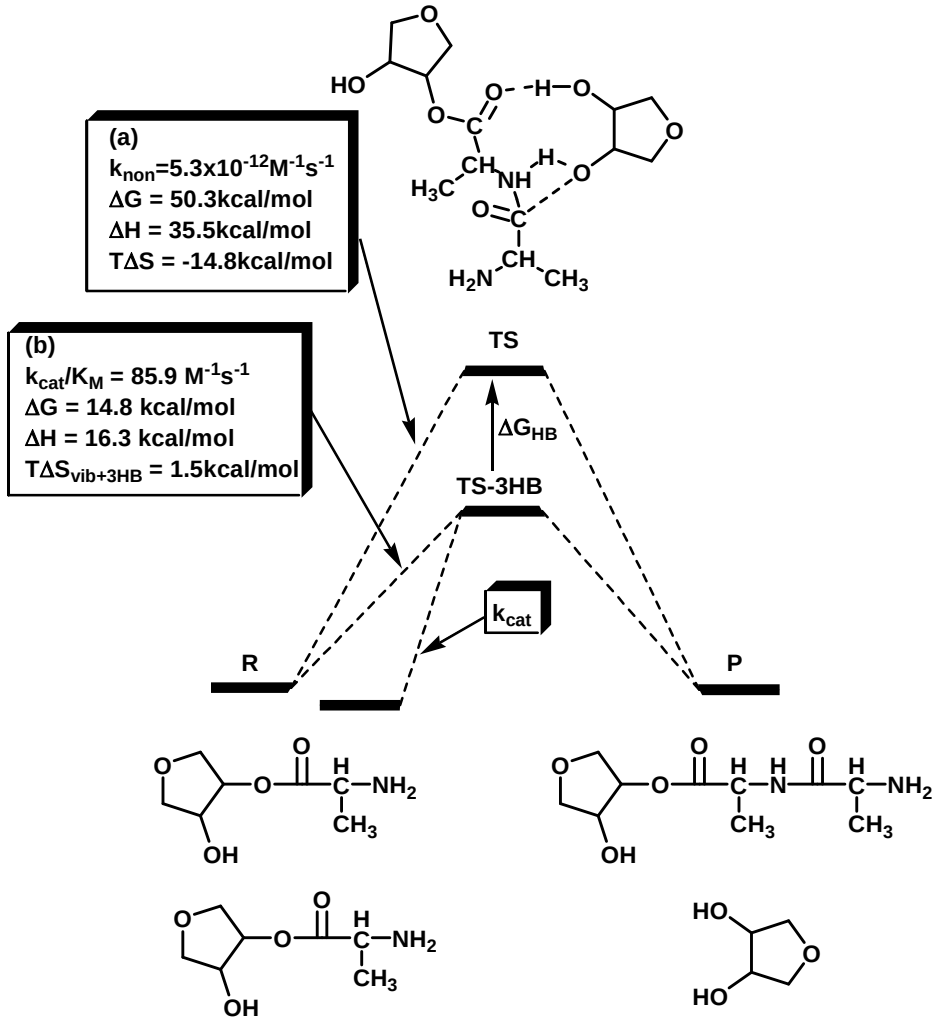

Figure 1 : Calculated activation parameters at 25°C for (a) 2nd order noncatalyzed (k_{non}) and (b) ribosome catalyzed 2nd order peptide bond formation (k_{cat}/K_M)

motion consistent therewith of the A-site amino acid moving towards its partner P-site amino acid. During the course of the rotary reaction pathway the amino acids increase the number of hydrogen bonds to the rRNA nucleotides, which guide their motions. At formation of the TS, the increase in the number of hydrogen bonds is 3[2]. We pointed out that formation of these 3 hydrogen bonds would stabilize the E_a of the TS that had been calculated based upon the gas phase reaction. If each H-bond had an average energy of 6 kcal/mol, the total stabilization would be 18 kcal/mol.

At that time, we had not considered a calculation of the entropy of formation $\Delta S^{\ddagger}$ of the TS, and did not consider the destabilization of $\Delta S^{\ddagger}$ afforded by the presence of the same 3 hydrogen bonds mentioned above, which form between the TS and the rRNA of its surroundings. In this paper we calculate that $\Delta S^{\ddagger}$ and consider its destabilization by hydrogen bonds to its surroundings. We compare our results to experimental measures of $\Delta S^{\ddagger}$ carried out by Wolfenden et al.[3,4] for a similar (but not exactly the same) ribosome reaction. The comparisons are reasonably good. The comparisons of theory and experiment are facilitated by recalling the expressions for the reaction rate constants which occur within Eyring's transition state theory (TST)[5], and separately within the Michaelis-Menten equation[6].

RESULTS

Figure 1 Calculated activation parameters at 25°C for (a) 2nd order noncatalyzed (k_{non}) and (b) ribosome catalyzed 2nd order peptide bond formation (k_{cat}/K_M).

TABLE 1 : Optimized geometry and vibrational frequencies for the reactant molecules shown in figure 1 calculated using B3LYP/6-31+G method**

	XYZ coordinates		
N1	-0.047894	0.012978	-0.196683
C2	1.394250	0.018216	0.033329
C3	1.843146	1.469487	0.141707
O4	1.346880	2.391703	-0.474524
O5	2.899250	1.605109	0.972449
C6	3.415096	2.939275	1.135001
C7	4.293254	3.408958	-0.052357
O8	5.614320	3.586312	0.475081
C9	5.434754	3.968798	1.842127
C10	4.381324	2.982292	2.356330
O11	4.947906	1.727606	2.686804
O12	2.238557	-0.684882	-1.055638
H13	6.390232	3.872114	2.360384
H14	5.080541	5.011087	1.910398
H15	3.875154	3.324377	3.261906
H16	2.569168	3.614095	1.283509
H17	3.918458	4.358519	-0.457289
H18	4.340595	2.674561	-0.859078
H19	5.413217	1.380972	1.912118
H20	1.598723	-0.463102	0.995717
H21	-0.369110	-0.911701	-.467102
H22	1.928147	-1.731803	-1.138635
H23	3.306156	-0.669028	-.810604
H24	2.090773	-0.204424	-2.028929
H25	-0.290979	0.670951	-.933514

TABLE 1 : Optimized geometry and vibrational frequencies for the reactant molecules shown in figure 1 calculated using B3LYP/6-31+G method**

	Vibrational Frequencies[cm^{-1}]				
1	21.61	24	870.36	47	1402.02
2	37.26	25	887.23	48	1403.67
3	64.38	26	918.86	49	1430.11
4	78.84	27	938.03	50	1433.91
5	118.36	28	959.62	51	1496.61
6	201.33	29	1007.56	52	1499.29
7	226.08	30	1032.20	53	1505.93
8	243.50	31	1046.58	54	1510.87
9	250.58	32	1078.55	55	1642.92
10	262.87	33	1080.61	56	1791.99
11	291.67	34	1118.37	57	2985.04
12	331.57	35	1154.37	58	3033.20
13	341.57	36	1160.11	59	3035.96
14	414.25	37	1193.55	60	3070.87
15	433.34	38	1207.98	61	3105.66
16	471.26	39	1251.76	62	3111.68
17	551.16	40	1258.62	63	3117.96
18	673.21	41	1266.34	64	3120.31
19	717.60	42	1274.24	65	3128.91
20	753.88	43	1317.56	66	3140.69
21	792.90	44	1347.59	67	3507.79
22	822.06	45	1351.29	68	3604.23
23	837.07	46	1380.27	69	3804.82

For case (a) the reactants are in gas phase. For case (b) the reactants are positioned and oriented as they would be in the ribosome, and thus translational, rotational, and electronic degrees of freedom are suppressed and do not contribute to the entropy change of the TS reaction. The figure is qualitative and not drawn to scale. The reactants at bottom left are peptides ester bonded to a sugar ring, and the reactants at bottom right are a dipeptide attached to a sugar ring and a free sugar ring which has released an ester bonded peptide, and accepted a hydrogen atom.

A description of the calculated thermodynamic changes associated with the chemical reaction for the formation of the peptide bond within and without the circumstances of the ribosome is organized in figure 1. In our model of the ribosome peptide bond TS reaction we take the electronic, translational, and rotational contributions to entropy to be zero. That is,

$$\Delta S_{el}^{++} = \Delta S_{tr}^{++} = \Delta S_{rot}^{++} = 0 \quad (1)$$

The electronic contribution vanishes because the reaction follows the electronic ground state energy surface, and the electronic levels are widely spaced. The translational and rotational contributions vanish because, in the ribosome, the translational and rotational degrees of freedom of the reaction are suppressed by their attachment to tRNA molecules at the A and P sites. Therefore, the conditions of the ribosome environment reduce the change of entropy to that associated only with the vibrational degrees of freedom. That is,

$$\Delta S_{vib}^{++} = S_{vib}^{++} - S_{vib}^{reactants} \quad (2)$$

In view of equations 1 and 2 only the vibrational frequencies of the normal modes at the optimized geometries for the TS and reactants are required to obtain the entropies. For one mole of molecules, the vi-

TABLE 2 : Theoretical and experimental thermodynamic parameters. The chemical reactions compared are similar, but not exactly the same

k_{cat}/K_M	This work (Figure 1)	Wolfenden et al.[3]
	$85.9\ M^{1}s^{1}$	$10^3\ M^{-1}s^{-1}$
$\Delta G^{\ddagger}$	14.8 kcal/mol	14.0 kcal/mol
$\Delta H^{\ddagger}$	16.3 kcal/mol	16.0 kcal/mol
$T\Delta S^{\ddagger}$	1.5 kcal/mol	2.0 kcal/mol

brational entropy for a non-linear system is[7];

$$S_{vib} = R\sum_{i=1}^{3N-6(7)}\left(\frac{h\nu_i}{2k_BT}\frac{1}{\frac{h\nu_i}{e^{k_BT}}-1} - \ln\left(1-e^{-\frac{h\nu_i}{k_BT}}\right)\right) \qquad (3)$$

where ν is a vibrational frequency, k_B is Boltzman's constant, T is the absolute temperature. The coordinates and frequencies (not previously calculated) for the reactant molecules are listed in TABLE 1. The results presented there have been obtained using the Mulliken program package[8]. The same information for the TS may be obtained in the supplemental tables of our previous work[2].

In figure 1, there are two 2nd order reactions to be noticed. The first is the "noncatalyzed" reaction, with rate constant k_{non}, and the second, with rate constant k_{cat}/K_M, is the reaction "catalyzed" by the nucleotides of the ribosome which surround the TS. As the transition state is formed some 3 hydrogen bonds attach to it, stabilizing the TS.

The noncatalyzed reaction was studied in the gas phase. The TS search is begun with the reactants oriented and placed relative to one another as they would be in the ribosome, in accordance with the known crystallography of the ribosome[1], treating the reactants and the TS as gas phase molecules having translational, rotational as well as vibrational contributions to the entropy, the calculated entropy contribution to the free energy change is $T\Delta S_{total}^{\ddagger}$ = -14.6 kcal/mol, corresponding to an enormous and unfavorable decrease in entropy. This may be compared to the catalyzed reaction in which the TS is stabilized by the formation of 3 hydrogen bonds to the ribosome nucleotides, and in which the translational and rotational degrees of freedom are suppressed by the ribosome. In this case the calculated entropy contribution to the free energy change is $T\Delta S_{vib+3HB}^{\ddagger}$ = 1.5 kcal/mol, corresponding to a favorable increase in entropy. The calculated enthalpy changes for the uncatalyzed and catalyzed reactions are known from previous work[2] to be $\Delta H^{\ddagger}$ = 35.5 kcal/mol and $\Delta H^{\ddagger}$ = 16.3 kcal/mol respectively. Summing the enthalpy and entropy contributions to obtain the free energy, one obtains $\Delta G^{\ddagger}$ = 50.3 kcal/mol and $\Delta G^{\ddagger}$ = 14.8 kcal/mol for the two respective reactions. The transition state theory of Eyring[4] converts $\Delta G^{\ddagger}$ to numerical values for the reaction rate constants, obtaining k_{non} = 5.3×10^{-12} mol^{-1} s^{-1} and k_{cat}/K_M = 86 mol^{-1} s^{-1}. The stabilization of the TS by 3 hydrogen bonds to the rRNA has both an enthalpy and an entropy component. Assuming an average value for each of these components, we have ΔH_{1HB} = 6 kcal/mol[9] and $(T\Delta S)_{1HB}$ = 1.10 kcal/mol[10]. Thus formation of each hydrogen bond contributes through enthalpy a stabilizing effect and through entropy a destabilizing effect on the TS. Notice however that the net entropy effect is a favorable one. In the absence of the hydrogen bond attachments to the TS, the vibrational contribution to the free energy in $T\Delta S_{vib}$ = 4.8 kcal/mol. The 3 H-bonds contribute $(T\Delta S)_{3HB}$ = 3.30 kcal/mol, so that altogether $(T\Delta S)_{vib+3HB}$ = 1.5 kcal/mol, as indicated in figure 1 and TABLE 2. Thus, the reaction within the ribosome is enhanced by both enthalpy and entropy relative to what would be the case for the same reaction in the gas phase.

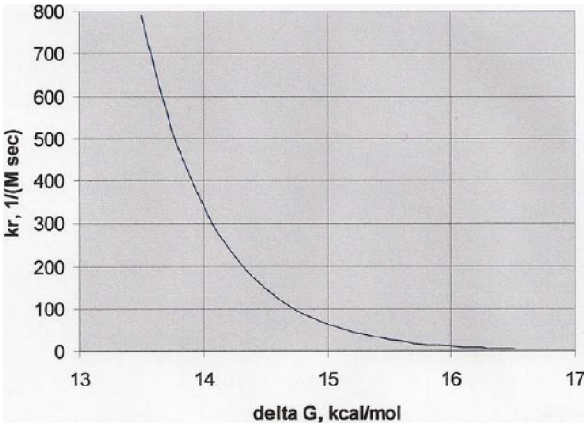

Figure 2 : Transition state theory rate constant, $k_r = \frac{k_BT}{h}e^{\frac{-\Delta G^{++}}{RT}}$

The calculated thermal parameters obtained here for the 2nd order reaction in the ribosome may be compared to the analogous values obtained experimentally

for a similar but not exactly same reaction[3].

TABLE 2 indicates that the compared thermal parameters $T\Delta S^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ are of the same order of magnitude and show similar trends. The calculated second order rate constant depends exponentially upon $\Delta G^{\ddagger}$ so that a relatively small difference between free energies would entail a noticeable difference in the corresponding rate constants (Figure 2).

There is a quantitative and a qualitative aspect to the thermal parameters of the TS displayed in figure 1 shows the quantitative features of the TS are those related to the activation energy and mechanism associated with the mathematically well characterized TS[2]. The qualitative features of the TS considered here are those related to its stabilization by formation of 3 hydrogen bonds. To describe that, we have adopted "average" values for the enthalpy and entropy of hydrogen bond formation. In TST, the exponential dependence of the rate constant upon $\Delta G^{\ddagger}$ means that the calculated rate constant would vary significantly with fluctuations of the free energy away from the average values adopted here (Figure 2).

Calculation methods

Quantum mechanical calculations were carried out with the Mulliken program package (an IBM proprietary software package that implements *ab initio* quantum chemical calculations on the IBM SP/2 supercomputer, The Laboratory for Quantum Crystallography, City University of New York). The Becke three-parameter-hybrid (B3)[12] was used in conjunction with the Lee-Yang-Parr (LYP) functional[13]. For all calculations, a Gaussian-type basis set, 6-31+G(d,p) was used. Thus, geometries of all reactants, products, and TSs have been optimized by using the B3LYP/6-31+G(d,p) of DFT. Vibrational frequencies have been calculated by using the same approximation for characterization of the nature of stationary points and zero-point vibrational energy corrections.

DISCUSSION & CONCLUSIONS

In a previous paper[2] we had calculated the activation energy and the geometry of the TS for formation of the peptide bond, using QM DFT. The calculated activation energy was modified by a qualitative consideration which recognized that as it formed, the TS was stabilized by formation of 3 hydrogen bonds to the ribosome rRNA which formed the PTC.

In this paper we have calculated the activation entropy for formation of the peptide bond. We have considered two 2nd order reactions that are relevant, and indicated in figure 1. The non-catalyzed reaction is associated with an enormous and unfavorable decrease in entropy associated with the translation and rotation degrees of freedom. In the ribosome these degrees of freedom are suppressed, and so the catalyzed reaction shows a favorable increase in entropy. This calculated increase in entropy is only dependent upon knowledge of the normal mode frequencies of the TS and the optimized reactants. These are known from the calculations of this paper and the results of our previous paper[2], and they have been used to calculate the $T\Delta S$ values listed in figure 1, and TABLE 2.

As regards the entropy, if we compare the catalyzed reaction to the non-catalyzed reaction, the ribosome environment affects the reaction in two ways of note. First of all it suppresses the reactant translational and rotational degrees of freedom. In this way the vibrational degrees of freedom only, contribute to the entropy change, which occurs as reactants converge into the transition state. This change entails an increase in the vibrational entropy, calculated to give a value $T\Delta S_{vib}$ = 4.8 kcal/mol. Second, as the TS is formed, there occurs an increase of 3 hydrogen bonds between the TS and rRNA[2]. The effect of the hydrogen bonds is to decrease the entropy of the TS, and by qualitative estimate $T\Delta S_{3HB}$ =3.3 kcal/mol. Overall, $T\Delta S$ = 1.5 kcal/mol, and thus the TS reaction is favored by entropy.

The consideration of activation energy, when the two reactions, non-catalyzed and catalyzed, are compared is as follows. The calculated activation energy for the uncatalyzed gas phase reaction is 35.5 kcal/mol. But formation of 3 hydrogen bonds stabilizes the TS by a qualitative estimate of some 18 kcal/mol, so that the activation energy is reduced to 17.5 kcal/mol.

Using the gas phase reaction as a standard of comparison one sees that the ribosome environment enhances the formation of the peptide bond from both an enthalpy and an entropy point of view. The activation energy for formation of the TS is reduced by formation of 3 hydrogen bonds. The entropy is increased, by the

suppression of translational and rotational degrees of freedom. The remaining vibrational degrees of freedom contribute to an increase of entropy. This is counteracted by a decrease of entropy associated with formation of 3 hydrogen bonds. In the balance there remains a small overall increase of entropy as the TS is formed.

The enthalpy is obtained from the activation energy (see Appendix E), which together with $T\Delta S$ allows calculation of the free energy as listed in figure 1, for both reactions, catalyzed and non-catalyzed. Also listed there are the corresponding rate constants from the expression of transition state theory of Eyring[4].

One may compare our calculated four thermal parameters to the analogous measured parameters obtained for a similar, but not exactly same, reaction[3,4]. The thermal parameters to be compared are listed in TABLE 2. The comparison is reasonably good, as the parameters show similar trends, and $T\Delta S^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta G^{\ddagger}$ are of the same order of magnitude. The transition state theory expression for the rate constant depends exponentially upon $\Delta G^{\ddagger}$, so that the relatively small difference in $\Delta G^{\ddagger}$ implies two orders of magnitude difference in k_{cat}/K_M (Figure 2).

We mention in passing that the four thermal parameters compared in TABLE 2, are obtained somewhat differently, in the two cases shown. In the experiment $\Delta H^{\ddagger}$ follows from the slope of k_{cat}/K_M versus 1/T, obtained by measurements. $T\Delta S^{\ddagger}$ is fixed by the y-intercept of the same line. From $\Delta H^{\ddagger}$ and $T\Delta S^{\ddagger}$ the free energy $\Delta G^{\ddagger}$ follows. In the theory, $\Delta S^{\ddagger}$ and E_a are obtained by calculation. E_a determines $\Delta H^{\ddagger}$ and then $\Delta G^{\ddagger}$ follows from $\Delta H^{\ddagger}$ and $T\Delta S^{\ddagger}$. The rate constant in transition state theory is calculated from knowledge of $\Delta G^{\ddagger}$.

Our calculated 2nd order catalyzed reaction may be viewed in reference to the analogous experimental reaction, as we have done in TABLE 2. However the noncatalyzed reaction calculated here is a gas phase reaction. The analog experimental reaction[3,4] is a solution reaction. The two non-catalyzed reactions, of different phase, are therefore not directly comparable. However, we point out what may be qualitative similarities and differences between the calculated gas phase and measured liquid phase analogous non-catalyzed 2nd order reactions. The activation energy (and therefore the enthalpy) might be expected to be higher in the gas phase reaction because no stabilizing H-bonds to the TS occur in the gas phase reaction. In solution however, H-bonds between water and the TS would stabilize the TS. Therefore the activation energy (and enthalpy) might be expected to be of comparable magnitude for both the solution reaction and that as measured for the ribosome[3,4]. The entropy of activation measured in solution might be expected to decrease because, just as shown by our calculations in this paper, that is the predicted direction of change associated with the translational and rotational degrees of freedom for the reaction. In addition the TS might well increase the order of water molecules attached to it. The ribosome reaction by contrast would be expected to exclude water and to suppress translational and rotational contributions to entropy, and as our calculations here show, the vibration degrees of freedom contribute to positive entropy of activation. Theoretical calculations and experimental measurements concur in a qualitative way in that entropy of activation is enhanced by the peptidyl transfer center of the ribosome.

An additional way in which the ribosome catalyzes the TS reaction is by formation of a hydrogen bond between an O donor on the reactant P-site and a carbonyl O acceptor on the reactant A-site. This has been confirmed experimentally[11] and has been noted in our previous QM theoretical calculations[2].

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