

Conformational Study of Y-Base in Yeast tRNA<sup>phe</sup>

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To understand the importance of Y-base adjacent to the anticodon stabilizing codon-anticodon interaction, a study has been undertaken for the model compound involving the interaction between Y-base and anticodon as well as the participation of water molecule by calculating the conformational free energy using an empirical potential function. We restrict our analysis to sites directly associated with Y-base by varying only the backbone torsion angles of Y-base. The hydration and Mg<sup>2+</sup> binding effects on the conformational stability of Y-base are calculated and discussed. The free Y-base is proved to be less stable than the hydrated one. The free energy change due to the hydration of Y-base amounts to -119.5 kcal/mole, in which the conformational energy change is -142.4 kcal/mole and the configurational entropy change is -76.9 e. u. It is found that the water molecules bound to Y-base and Mg<sup>2+</sup> contribute to the conformation of Y-base dominantly.

## Introduction

Transfer ribonucleic acid (tRNA) plays an important role in protein synthesis in all living systems.<sup>1,2</sup> Specially, in the process of the translation of the messenger ribonucleic acid (mRNA) at the ribosome, the particularly modified nucleoside adjacent to the anticodon of tRNA is essential for the amino acid transfer function and enhances the binding effect of the complementary oligonucleotide (codon) to the anticodon.<sup>3-5</sup> Since hypermodified base, Y, adjacent to the anticodon of yeast tRNA<sup>phe</sup> is naturally fluorescent, this base may have been used to study the conformation of this region of tRNA selectively.<sup>6-9</sup> The conformation of Y-base is sensitive to the overall shape and electrostatic property of the surrounding ion-water atmosphere and conformational changes can occur with the hydration and Mg<sup>2+</sup> binding. Recently, the importance of Mg<sup>2+</sup> and hydration in stabilizing the anticodon loop has been recognized without considering the hydration effect of Y-base and its side chain.<sup>10,11</sup> To investigate the important role of Y-base within the anticodon loop, the conformational free energy change in various environments is calculated by using an empirical potential function.<sup>12-15</sup> To recognize the factors involved in the stability of a given conformation and in the change of one conformation to another, the configurational entropies of Y-base and water molecules bound to it is evaluated. In addition, the conformational change of Y-base due to the hydration and Mg<sup>2+</sup> binding is analyzed and the influence of Y-base in the anticodon loop of tRNA is also discussed.

## Methods

## A. Potential Functions

Interaction energies have been calculated using the empirical potential energy functions described by Kang *et al.*<sup>12-15</sup> that are composed of the electrostatic energy  $E_{el}$ , polarization energy  $E_{pol}$ , nonbonded energy  $E_{nb}$ , torsional energy

$E_{tor}$ , and hydrogen bond energy  $E_{hb}$ .

The total energy is given by the following expression:

$$E_{tot} = \sum_{nb \text{ pairs}} (E_{el} + E_{pol} + E_{nb}) + \sum_{torsions} E_{tor} + \sum_{hb \text{ pairs}} E_{hb} \quad (1)$$

Atomic partial charges used for the calculation of the electrostatic and polarization energies have been calculated using the Del-Re method<sup>16,17</sup> for  $\sigma$  charges and the Hückel method for  $\pi$  charges.<sup>18</sup> In the case of the free (vacuum) state, a dielectric constant of 1.0 was used. For the hydrated states, a distance-dependent dielectric constant,  $\epsilon(r_{ij})$ , was proposed as follows;<sup>15</sup>

$$\epsilon(r_{ij}) = (\epsilon_0 - 1) [(r_{ij} - r_{con}) / (r_{eff} - r_{con})]^n + 1 \quad (2)$$

where at the contact distance  $r_{con}$ ,  $\epsilon(r_{ij}) = 1.0$  and for some particular interaction distance  $r_{eff}$ , the dielectric constant  $\epsilon(r_{ij}) = \epsilon_0$ , the bulk dielectric constant of the system. Only in the region  $r_{con} < r_{ij} < r_{eff}$ ,  $\epsilon(r_{ij})$  is considered to be a function of  $r_{ij}$ . Boundary conditions such as  $\epsilon(3\text{\AA}) = 1.0$  and  $\epsilon(7\text{\AA}) = 4.0$  were taken from the work of Hopfinger.<sup>19</sup>

The nonbonded energy was approximated using the Lennard-Jones type, in which the dispersion coefficient was calculated by the London formula<sup>20</sup> and the equilibrium distance was assigned to be a distance approximately 0.2Å greater than the sum of the van der Waals radii of the interacting atoms.<sup>21</sup> The atomic static polarizabilities used in the polarization and nonbonded terms were taken from the work of Kang and Jhon.<sup>22</sup> The torsional potential was composed of usual periodic function.<sup>23</sup> Potential parameters of hydrogen bond were obtained optimally fitted to the results of *ab initio* computation.<sup>15</sup> Details of the nature of the potential energy functions and various parameters used are appeared in ref 15.

## B. Conformational Entropy

1. *Conformational Entropy of Biomolecule.* If the joint probability distribution function for a set of torsion angles satisfies a normalized multivariate Gaussian distribution func-

tion,<sup>24</sup> the conformational entropy at the  $i$ th minimum is given by<sup>25</sup>

$$S_q^{(i)} = 1/2 nR + 1/2 R \ln[(2\pi)^n \sigma^{(i)}] \quad (3)$$

where  $n$  is the number of degrees of freedom in a set of torsion angles and  $\sigma^{(i)}$  is the determinant of the covariance matrix at the  $i$ th minimum. Hence the entropy difference associated with the conformational change of biomolecule is given by

$$\Delta S_q(i \rightarrow j) = R/2 \ln(\sigma^{(j)}/\sigma^{(i)}) \quad (4)$$

where  $\sigma^{(i)}$  and  $\sigma^{(j)}$  are the determinants of the covariance matrices at the  $i$ th and  $j$ th minima, respectively.

Karplus *et al.*<sup>25,26</sup> obtained the elements of the covariance matrix from the harmonic analysis results. However, it is not easy to obtain the harmonic analysis results for each conformation at the local minimum. Hence a new method was proposed<sup>15</sup> for the evaluation of the conformational entropy of biomolecules with the covariance matrix by analyzing the potential energy surface. The element of the covariance matrix at the  $i$ th minimum is

$$\sigma_{kl}^{(i)} = \rho_{kl}^{(i)} \sigma_k^{(i)} \sigma_l^{(i)} \quad (5)$$

where  $\sigma_k^{(i)}$  and  $\sigma_l^{(i)}$  are standard deviations of the  $k$ th and  $l$ th torsion angles, respectively, and  $\rho_{kl}^{(i)}$  is the correlation coefficient between them. Each standard deviation for each torsion angle was obtained while the other torsion angles were fixed at the minimum value. The detailed procedure for the evaluation of the covariance matrix is appeared in ref 15.

## 2. Configurational Entropy of Bound Water Molecules.

The coordinates of a water molecule bound to the biomolecule can be expressed with six external variables in Figure 1. If it is assumed that the joint probability distribution function for the external variables of a water molecule follows the multivariate Gaussian distribution function, the configurational entropy of a water molecule is approximately given by

$$S_w = 1/2 nR + 1/2 R \ln[(2\pi)^n \sigma_w^*] \quad (6)$$

where  $n$  is the external degrees of freedom of a water molecule and  $\sigma_w^*$  is the determinant of the reduced covariance matrix of water molecule. To make the determinant of the covariance matrix be dimensionless quantity, a standard deviation of each external variable must be reduced approximately.

The potential barriers of the external variables can be approximately classified into the three cases: the very high, low, and intermediate barriers. For very high barriers, the motion eventually approximates to a harmonic oscillation of small amplitude. For very low barriers, the concept of the free rotation is adopted and the torsional motion with the intermediate potential barrier can be treated approximately as a hindered rotation. To reduce elements of the determinant for various potential barriers of the external variables, harmonic oscillator and hindered rotation approximations were used. Theoretical derivations of the two methods are minutely presented in ref 15.

### C. Model Compound

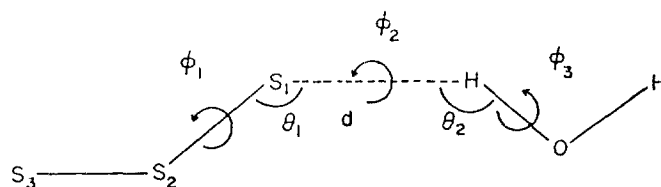


Figure 1. Geometrical parameters for water molecule.

We have considered the anticodon loop of yeast tRNA<sup>phe</sup> which consists of seven nucleotides, C<sub>m</sub>32, U33, G<sub>m</sub>34, A35, A36, Y37, and A38 in sequence from 5'-side (the geometrical definition of tRNA refers to ref 4). Especially, since Y-base is a very sensitive indicator of the structure of the anticodon loop and five bases on the 3'-side of the loop are effected by Y-base, we only selected four nucleotide, G<sub>m</sub>34, A35, A36, and Y37 in the anticodon loop, and each end phosphate of the nucleotide was terminated with methyl group. For the hydrated Y-base, Mg<sup>2+</sup> were placed along the nucleotide backbone like as in the native structure.

The atomic coordinates of the anticodon loop were obtained from the result of an extensive crystallographic refinement of the orthorhombic crystal structure.<sup>27</sup> The orientation of 2'-hydroxyl group of ribose corresponds to the position with a dihedral angle of 222° (see ref 10).

Since our attention was centered on the conformational change of Y-base and its aliphatic side chain in the anticodon loop due to the hydration and Mg<sup>2+</sup> binding, the computations are carried out on Y-base of anticodon. The hydration of anticodon loop was restricted in the region of Y-base and backbone of Y37 and A38. A magnesium ion is located in the upper part of anticodon loop, which is coordinated to one oxygen of phosphate 37. The other five oxygen atoms of the coordination are given by water molecules. The hydrogen bonding scheme of the water molecules and the magnesium coordination are in good agreement with results observed by Quigley *et al.*<sup>30,31</sup> The notations used in this work are illustrated in Figure 2.

### D. Determination of Conformational Energy and Entropy

The total conformational energy of the Y-base was calculated as the sum

$$E_{tot} = E_{intra} + E_{inter} \quad (7)$$

where  $E_{intra}$  is the internal conformational energy of Y-base and  $E_{inter}$  is the interaction energy between Y-base and anticodon. For the hydrated system, a hydrogen bond energy was additionally included in  $E_{inter}$ .

The torsion angles of the Y-base and its side chain were allowed to move during minimization by using a Quasi-Newton method developed by Fletcher<sup>32</sup> with a convergence criterion of 0.005 kcal/mole and with a step length of 2 degrees for all the torsion angles. The number of iterations was limited to 100 cycles. In order to generate the coordinates of the Y-base and its side chain, nine independent torsion angles are needed during the energy minimization. Crystallographic results of yeast tRNA<sup>phe</sup><sup>33</sup> were used as the preliminary geometries of minimization. The starting points for minimization of the total energy of each conformation were then determin-

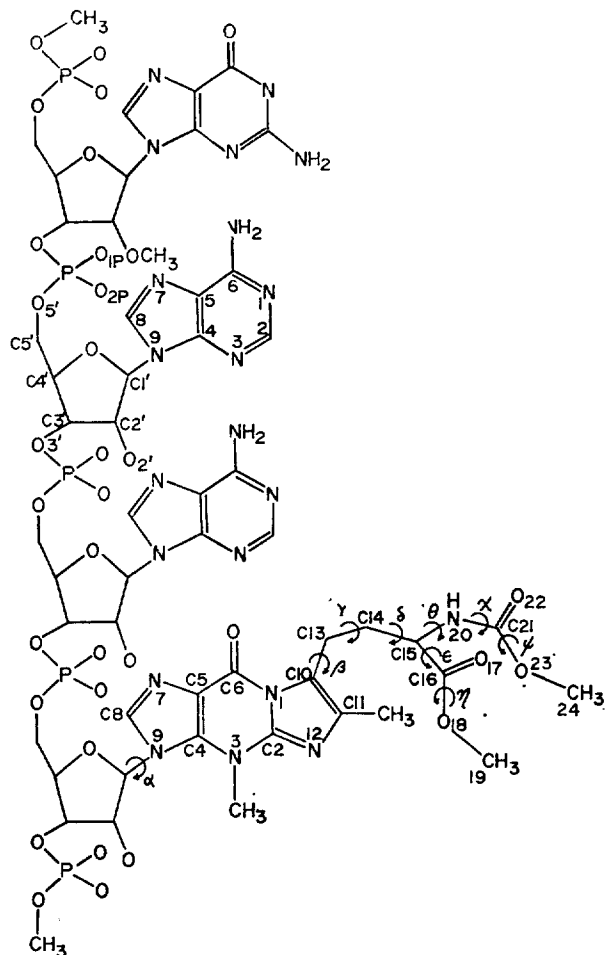


Figure 2. Nomenclature of model compound and definition of torsion angles.

ed by the refinement of those geometries varying each torsion angle of the backbone one after another.

For the hydrated system, the hydration schemes were obtained from the optimization of water molecules bound to the system, and then the conformations of the Y-base were minimized once more. This procedure was repeated until the difference of each torsion angles obtained from the previous and late iterations remains 1 degree. A counterion  $Mg^{2+}$  was also allowed to move during the minimization of total interaction energy. Each water molecule bound to the system was described by the six external variables  $d$ ,  $\theta_1$ ,  $\theta_2$ ,  $\phi_1$ ,  $\phi_2$  and  $\phi_3$  depicted in Figure 1. The optimization step lengths were 0.01 Å for distance and 2 degree for all the angles.

The total entropy change of the compound through the hydration was estimated as

$$\Delta S_{tot} = \Delta S_{sub} + \Delta S_w \quad (8)$$

where  $\Delta S_{sub}$  is the conformational entropy change of the substrate and  $\Delta S_w$  is the configurational entropy change of water molecules bound to the Y-base. It is assumed that  $\Delta S_w$  is the difference between the entropy of liquid water at room temperature and the configurational entropy of the optimized water molecules. Hence,  $\Delta S_w$  is represented as

$$\Delta S_w = S_{sw} - nS_w^0 \quad (9)$$

where  $S_{sw}$  is the sum of the configurational entropy  $S_w$  given by eq (6),  $n$  the number of bound water molecules, and  $S_w^0$

the entropy of liquid water at room temperature (16.72 e. u. at 298.15 K).<sup>34</sup>

## Results and Discussion

*The Optimized Geometry of Y-Base.* The optimized torsion angles for Y-base are listed in Table 1. The notation for torsion angles of Y-base is illustrated in Figure 2. Three kinds of torsion angles are compared with each other: the first column corresponds to the result of calculation with a dielectric constant  $\epsilon = 1.0$ , the second to those of the hydrated compound with a distance-dependent dielectric constant described previously, and the third to the X-ray crystallographic results of ref 31. It is clear that torsion angles of hydrated Y-base are more close to those of X-ray crystallographic results than those of nonhydrated one.

*Hydration of Y-Base.* To investigate the stability of Y-base due to the hydration and  $Mg^{2+}$  binding, it is necessary to calculate the internal conformational energy of Y-base itself as well as the configurational entropy of Y-base and water molecules bound to it. The conformation of Y-base was initially minimized using the data of crystallographic study in Table 1 without considering the hydration. For the free Y-base,  $Mg^{2+}$  was not considered in this calculation. However, the hydrated Y-base was optimized using the same initial geometry with the distance-dependent dielectric constant. Since the water molecules in the first hydration shell are mainly responsible for the hydration structure of solute,<sup>28,29</sup> the directly bound water molecules to Y-base and water molecules bound to phosphate of Y37 and A38 which indirectly contact to Y-base were considered in this calculation.

$Mg^{2+}$  has lost one water molecules from its inner hydration sphere and makes one direct contact to phosphate anion of Y37 and is allowed to move during the energy minimization. It is remarkable that three contacts made by  $Mg^{2+}$  in the anticodon site can be arranged in exactly the same manner with the information from X-ray analysis.<sup>30,31</sup> The optimum geometry of  $Mg^{2+}$  is listed in Table 2, the average distance between  $Mg^{2+}$  ion and water molecules coordinated to it is 1.88 Å, which is close to the average  $Mg^{2+}$  - oxygen bond length observed in the crystal.<sup>30</sup> The interaction energies between  $Mg^{2+}$  and its coordinated water molecules lie within -52.9 to -66.5 kcal/mole, to which the polarization energy contribute dominantly due to the large electric charge of  $Mg^{2+}$  and which is in good agreement with *ab initio* resul-

TABLE 1: Torsion Angles in Y-Base

|            | Free  | Hydrated | X-ray |
|------------|-------|----------|-------|
| $\alpha$   | 23.1  | 23.1     | 24.7  |
| $\beta$    | 328.0 | 335.0    | 305.4 |
| $\gamma$   | 193.8 | 222.8    | 245.9 |
| $\delta$   | 75.2  | 75.2     | 101.3 |
| $\epsilon$ | 238.7 | 233.7    | 200.7 |
| $\eta$     | 89.8  | 90.8     | 69.5  |
| $\theta$   | 325.6 | 331.6    | 301.7 |
| $\chi$     | 173.7 | 174.7    | 181.6 |
| $\phi$     | 150   | 150      | 152.1 |

TABLE 2: Optimum Position of  $Mg(H_2O)_5^{2+}$ 

| Atom             | Reference atoms     |                |                | d          | Optimum geometry <sup>a</sup> |                               |          |          |          | E (kcal/mole)     |              |
|------------------|---------------------|----------------|----------------|------------|-------------------------------|-------------------------------|----------|----------|----------|-------------------|--------------|
|                  | S <sub>1</sub>      | S <sub>2</sub> | S <sub>3</sub> |            | $\theta$                      | $\phi$                        |          |          |          |                   |              |
| Mg <sup>2+</sup> | O1(P37)             | P37            | O5'(P37)       | 1.80       | 130.                          | 238.                          |          |          |          | -354.04 kcal/mole |              |
| Water            | Reference atoms     |                |                |            | d                             | Optimum geometry <sup>a</sup> |          |          |          |                   | E(kcal/mole) |
|                  | S <sub>1</sub>      | S <sub>2</sub> | S <sub>3</sub> | $\theta_1$ |                               | $\theta_2$                    | $\phi_1$ | $\phi_2$ | $\phi_3$ |                   |              |
| W1               | Mg <sup>2+</sup> O1 | (P37)          | P37            | 2.56       | 169.                          | 35.8                          | 15.      | 163.     | 193.     | -52.85            |              |
| W2               | Mg <sup>2+</sup> O1 | (P37)          | P37            | 2.43       | 61.                           | 44.8                          | 84.      | 151.     | 165.     | -66.47            |              |
| W3               | Mg <sup>2+</sup> O1 | (P47)          | P37            | 2.61       | 103.                          | 30.8                          | 280.     | 5.       | 166.     | -59.77            |              |
| W4               | Mg <sup>2+</sup> O1 | (P37)          | P37            | 2.52       | 100.                          | 37.8                          | 30.      | 274.     | 154.     | -63.21            |              |
| W5               | Mg <sup>2+</sup> O1 | (P37)          | P37            | 2.53       | 101.                          | 38.8                          | 202.     | 316.     | 180.     | -64.05            |              |

<sup>a</sup> Units are Å and degrees for distance and angles, respectively.

TABLE 3: Optimum geometry, Interaction Energy, and Configurational Entropy of Water Molecules Bound to Y-Base<sup>a</sup>

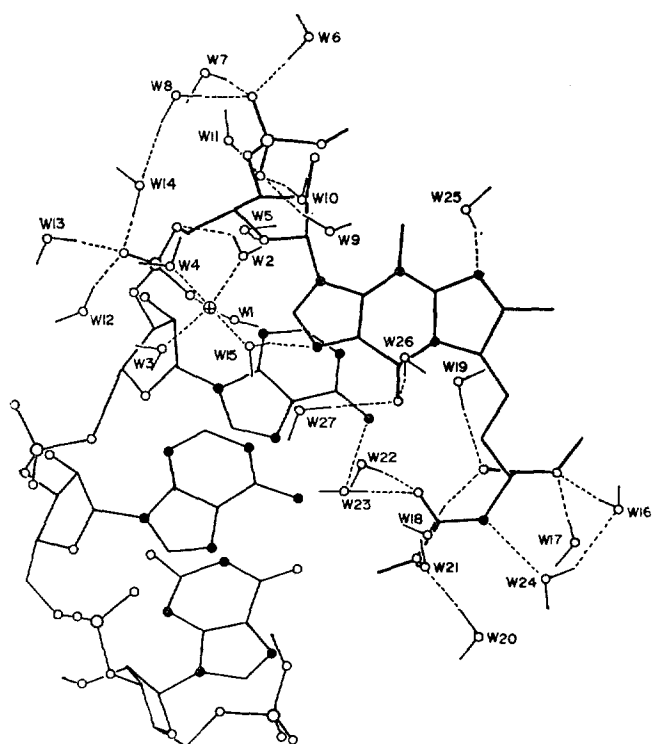
| Water molecules | Reference atoms |                |                | d    | $\theta_1$ | $\theta_2$ | $\psi_1$ | $\psi_2$ | $\psi_3$ | Optimum geometry |                |        |
|-----------------|-----------------|----------------|----------------|------|------------|------------|----------|----------|----------|------------------|----------------|--------|
|                 | S <sub>1</sub>  | S <sub>2</sub> | S <sub>3</sub> |      |            |            |          |          |          | E                | S <sub>w</sub> | F      |
| W1              | Mg              | O1(P37)        | P37            | 2.56 | 169.       | 35.8       | 15.      | 163.     | 193.     | -52.85           |                |        |
| W2              | Mg              | O1(P37)        | P37            | 2.43 | 61.        | 44.8       | 84.      | 151.     | 165.     | -66.47           |                |        |
| W3              | Mg              | O1(P37)        | P37            | 2.61 | 103.       | 30.8       | 280.     | 5.       | 166.     | -59.77           |                |        |
| W4              | Mg              | O1(P37)        | P37            | 2.52 | 100.       | 37.8       | 30.      | 274.     | 154.     | -63.21           |                |        |
| W5              | O1'(s37)        | C4'(s37)       | C3'(s37)       | 1.84 | 110.       | 178.       | 148.     | 86.      | 142.     | -12.62           |                |        |
| W6              | O2 (P38)        | P38            | O1 (P38)       | 1.58 | 113.       | 178.       | 226.     | 64.      | 178.     | -22.85           |                |        |
| W7              | O2 (P39)        | P38            | O1 (P38)       | 1.61 | 106.       | 185.       | 127.     | 132.     | 182.     | -19.43           |                |        |
| W8              | O2 (P38)        | P38            | O1 (P38)       | 1.52 | 115.       | 186.       | 40.      | 213.     | 180.     | -19.39           |                |        |
| W9              | O1 (P38)        | P38            | O2 (P38)       | 1.88 | 94.        | 186.       | 157.     | 58.      | 205.     | -16.70           |                |        |
| W10             | O1 (P38)        | P38            | O2 (P38)       | 1.68 | 124.       | 165.       | 86.      | 142.     | 156.     | -13.48           |                |        |
| W11             | O1 (P38)        | P38            | O2 (P38)       | 1.62 | 104.       | 172.       | 11.      | 176.     | 181.     | -18.98           |                |        |
| W12             | O2 (P37)        | P37            | O1 (P37)       | 1.63 | 121.       | 166.       | 59.      | 282.     | 171.     | -28.63           |                |        |
| W13             | O2 (P37)        | P37            | O1 (P37)       | 1.58 | 118.       | 182.       | 154.     | 98.      | 198.     | -16.63           |                |        |
| W14             | O2 (P37)        | P37            | O1(P37)        | 1.52 | 118.       | 171.       | 277.     | 49.      | 166.     | -17.02           |                |        |
| W15             | Mg              | O1(P37)        | P37            | 2.53 | 101.       | 38.8       | 202.     | 316.     | 180.     | -64.05           | 7.37           | -66.25 |
| W16             | O18(Y)          | C16(Y)         | C15(Y)         | 1.96 | 109.       | 175.       | 341.     | 60.      | 14.      | -16.62           | 12.15          | -20.24 |
| W17             | O18(Y)          | C16(Y)         | C25(Y)         | 1.93 | 106.       | 183.       | 236.     | 27.      | 0.       | -12.40           | 23.38          | -19.37 |
| W18             | O17(Y)          | C16(Y)         | C15(Y)         | 1.71 | 128.       | 168.       | 66.      | 359.     | 185.     | -22.53           | 13.17          | -26.46 |
| W19             | O17(Y)          | C16(Y)         | C25(Y)         | 1.80 | 113.       | 180.       | 260.     | 130.     | 179.     | -20.96           | 23.10          | -27.84 |
| W20             | O23(Y)          | C21(Y)         | N20(Y)         | 1.94 | 112.       | 188.       | 27.      | 155.     | 357.     | -18.68           | 14.18          | -22.90 |
| W21             | O23(Y)          | C21(Y)         | N20(Y)         | 1.91 | 107.       | 184.       | 281.     | 335.     | 356.     | -13.31           | 17.21          | -18.44 |
| W22             | O22(Y)          | C21(Y)         | N20(Y)         | 1.86 | 112.       | 186.       | 116.     | 236.     | 176.     | -8.66            | 12.35          | -12.34 |
| W23             | O22(Y)          | C21(Y)         | N20(Y)         | 1.70 | 130.       | 168.       | 221.     | 56.      | 207.     | -8.49            | 7.93           | -10.85 |
| W24             | H20(Y)          | N20(Y)         | C15(Y)         | 2.30 | 134.       | 35.        | 5.       | 174.     | 184.     | -17.82           | 12.57          | -21.57 |
| W25             | N12(Y)          | C2(Y)          | N3(Y)          | 2.00 | 95.        | 195.       | 290.     | 184.     | 355.     | -10.34           | 16.01          | -15.11 |
| W26             | O6(Y)           | C6(Y)          | C5(Y)          | 1.90 | 94.        | 166.       | 94.      | 350.     | 150.     | -13.75           | 14.79          | -18.16 |
| W27             | O6(Y)           | C6(Y)          | C5(Y)          | 2.21 | 101.       | 178.       | 10.      | 33.      | 180.     | -22.50           | 19.32          | -28.26 |

<sup>a</sup> The water molecules from W1 to W14 are bound to backbone of anticodon, those from W15 to W27 are bound to Y-base (refers to footnotes in Table 2); E, S<sub>w</sub>, and F are the interaction energy (kcal/mol), the configurational entropy (e.u.), and the free energy (kcal/mole) at room temperature, respectively.

ts.<sup>35</sup> One of the interesting features of hydration scheme is that there is a direct water-bridge formation between Mg<sup>2+</sup> and N7 of Y-base, which may contribute considerable to the conformational change of Y-base: W15 forms a bridge between Mg<sup>2+</sup> and N7 of Y-base with a binding energy of -64.1 kcal/mole and the configurational entropy of 7.4 e.u.

Five water molecules are bound to Mg<sup>2+</sup> directly coordinated to a phosphate anion of Y37. Ten water molecules are bound to the backbones of Y37 and A38 with the interaction

energy of -12.6 to -28.6 kcal/mole. Interaction energies of twelve water molecules bound to Y-base amount to -8.5 to -22.5 kcal/mole. The optimum geometry and binding energies of water molecules are summarized in Table 3 and the hydration scheme is shown in Figure 3. The interaction energies of water molecules bound to phosphate anions of Y37 and A38 lie within -16.6 to -28.6 kcal/mole. The interaction energy of W5 bound to sugar is -12.6 kcal/mole. These results are in good agreement with ref 13. The configurational



**Figure 3.** Structure of hydrated Y-base. For clarity, hydrogen atoms are not shown. Each atom is designated as follows: carbon, •, oxygen ○, nitrogen ●, phosphorus ○, and  $Mg^{+2}$  ⊕.

entropies of water molecules bound to Y-base lie within 7.9 to 23.4 e. u. and are shown in Table 3. It is found that W23 forms a bridge between amino group of A36 base and O22 of Y-base with a binding energy of  $-8.5$  kcal/mole. Since W23 bound to Y-base forms a hydrogen bond with the base of the anticodon and its binding energy is lower than the other water molecules, it may be easily excluded from anticodon in order to form specific hydrogen bonds with the bases of the corresponding codon of the messenger RNA during the protein synthesis. The free energies of water molecules bound to Y-base and  $Mg^{+2}$  are  $-10.9$  to  $-28.3$  and  $66.3$  kcal/mole, respectively.

#### The Stability of Y-Base due to the Hydration and $Mg^{+2}$ .

The interaction energies for the minimized Y-base in the free and hydrated states are summarized in Table 4. The internal conformational energy of Y-base in the free state is  $-3.4$  kcal/mole as compared to  $-2.0$  kcal/mole in the hydrated state and the loss of internal conformational energy through the hydration and  $Mg^{+2}$  binding is thus  $1.4$  kcal/mole. However, Y-base becomes stable due to hydrogen bond between Y-base and water molecules bound to it. The intermolecular interaction energies of the hydrated Y-base are shown in Table 5. The total energy of them is  $-177.7$  kcal/mole, in which the interaction energy between Y-base and water molecules bound to it is the major factor.  $Mg^{+2}$  bound to phosphate anion is contributed not to the stability of Y-base but to the stability of anticodon backbone. Since  $Mg^{+2}$  is indirectly in contact to Y-base by means of water molecules and stabilized the water molecules bound to Y-base, it gives an indirect effect on the conformation of Y-base.

**TABLE 4: Interaction Energies of Y-Base<sup>a</sup>**

| Components of interaction energies | Free Y-base | Hydrated Y-base |
|------------------------------------|-------------|-----------------|
| $E_{el}$                           | 5.56        | -24.76          |
| $E_{pot}$                          | -10.58      | -4.79           |
| $E_{nb}$                           | -0.36       | -4.37           |
| $E_{tor}$                          | 2.03        | 3.69            |
| $E_{hb}$                           |             | -285.08         |
| $E_{tot}$                          | -3.35       | -33.92          |

<sup>a</sup> Values in the 1st and 2nd columns correspond to interaction energies of intramolecular and intermolecular interactions.

**TABLE 5: Intermolecular Interaction Energies of Y-Base**

| Interaction type      | $E_{el}$ | $E_{pot}$ | $E_{nb}$ | $E_{hb}$ | $E_{tot}$ |
|-----------------------|----------|-----------|----------|----------|-----------|
| Anticodon             | -10.32   | -0.76     | -4.82    |          | -15.90    |
| $Mg^{2+}$             | 5.68     | 0.15      | -0.02    |          | 5.81      |
| Water(Y) <sup>a</sup> | 138.94   | -12.56    | -4.95    | -285.08  | -165.65   |
| Water(B) <sup>a</sup> | -3.63    | 0.91      | -1.27    |          | -3.99     |
| Total                 | 130.67   | -12.26    | -11.06   | -285.08  | -177.73   |

<sup>a</sup> Water(Y) means water molecules bound to Y-base, Water(B) means water molecules to backbone.

**TABLE 6: Intermolecular Interaction Energies of Water(Y)<sup>a</sup>**

| Interaction type      | $E_{el}$ | $E_{pot}$ | $E_{nb}$ | $E_{hb}$ | $E_{tot}$ |
|-----------------------|----------|-----------|----------|----------|-----------|
| Y-base                | 138.94   | -12.56    | -4.95    | -285.08  | -163.65   |
| $Mg^{2+}$             | -92.60   | -47.89    | 15.25    |          | -125.24   |
| Water(B) <sup>a</sup> | 14.81    | -2.73     | -0.59    |          | 11.49     |
| Anticodon             | 36.41    | -4.67     | -0.50    | -22.12   | 9.12      |
| Total                 | 97.55    | -67.85    | 9.22     | -307.20  | -268.28   |

<sup>a</sup> Water(Y) means water molecules bound to Y-base, water(B) means water molecules bound to backbone.

**TABLE 7: Interaction Energies of Y-base due to the Hydration**

| Free Y-base          | Hydrated Y-base       |
|----------------------|-----------------------|
| $E_{intra} = -3.35$  | $E_{intra} = -1.99$   |
| $E_{inter} = -33.92$ | $E_{inter} = -177.73$ |
| $E_{tot} = -37.27$   | $E_{tot} = -179.72$   |

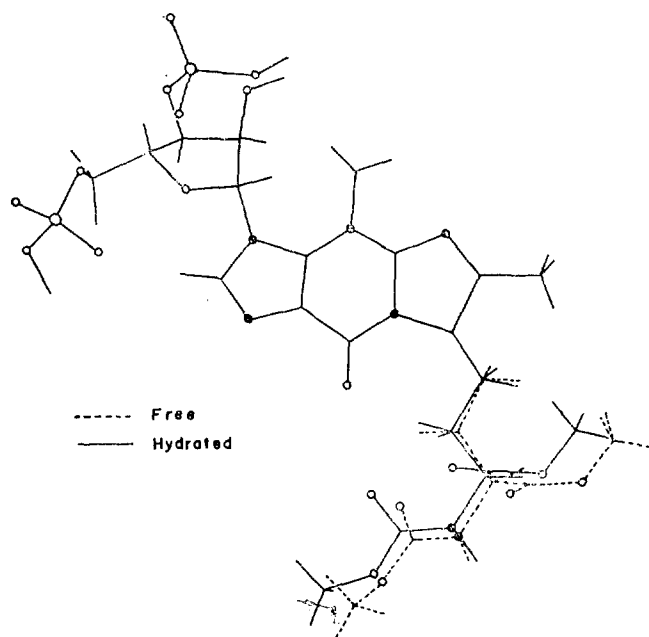
$$\Delta E = -142.44 \text{ kcal/mole}$$

$$\Delta S = -76.89 \text{ e.u.}^a$$

$$\Delta F = -119.52 \text{ kcal/mole}^b$$

<sup>a</sup> Obtained from eq(4). <sup>b</sup> Calculated at 298.15 K.

According to interaction type, the intermolecular interaction energies of water bound to Y-base are listed in Table 6. Interaction energy between  $Mg^{+2}$  and water molecules bound to Y-base is  $-125.2$  kcal/mole, and so  $Mg^{+2}$  contributes dominantly to the stability of water molecules bound to Y-base. Interaction energy between anticodon and water molecules bound to Y-base is  $9.1$  kcal/mole, whose result strongly supports the earlier conclusion that the anticodon loop of tRNA has a highly ordered structure with the Y-base well protected from the solvent.<sup>5</sup> Although the anticodon disturbs the hydration of Y-base, it may be neglected the influence of anticodon on the hydration of Y-base because of the high interaction energy between Y-base and water molecules bound to it. Since the interaction energy between the hydra-



**Figure 4.** Conformation of Y-base in the free and hydrated states.

ted Y-base and anticodon is not very large, Y-base may be reoriented inside the anticodon loop easily.

The configurational entropy change of Y-base through the hydration is  $-53.1$  e.u. Using the values in Table 3 and the experimental data,<sup>32</sup> the total entropy change of water molecules bound to Y-base at room temperature can be approximately calculated as  $-23.8$  e.u. The interaction energy changes for Y-base due to the hydration are summarized in Table 7. The total entropy change and the total free energy change through the hydration of Y-base are about  $-76.9$  e. u. and  $-119.5$  kcal/mole, respectively. The relative contribution of configurational entropy to free energy change amount to about 16%. It is clear that since the entropy change is mainly caused by the conformational entropy change of Y-base, we can not neglect the contribution of configurational entropy of conformer to the total free energy change of Y-base inside the narrow pocket of the anticodon loop. The decrease of the conformational entropy of Y-base is caused by the reduction of flexibility of Y-base by means of the hydration inside the pocket of the anticodon loop. The conformational changes for the free and hydration state of Y-base are shown in Figure 4.

### Conclusions

The hydration scheme of Y-base and  $Mg^{+2}$  binding is obtained from the conformational analysis of Y-base adjacent to anticodon by using an empirical potential function and are in good agreement with the results of X-ray crystallographic study. The interaction energies between Y-base and various environments show that the hydration effect is essential to the stability of Y-base, while  $Mg^{+2}$  is indirectly contributed to it. The change of configurational entropy is decreased due to the hydration, which is proved for the reduction of flexibility of Y-base inside the pocket of the anticodon

loop. Since we restrict our analysis to sites directly associated with Y-base, there is a limit to illustrate the function of Y-base of tRNA in a living cell. However, our results show that the environment effects including the hydration and cation binding are important in determining the conformation of Y-base in tRNA.

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## Isolation and Properties of $\beta$ -N-Acetyl-D-glucosaminidase B from Rat Uterus

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$\beta$ -N-Acetyl-D-glucosaminidase B was highly purified with the following sequence of steps; DEAE-cellulose, CM-cellulose, and Sephadex G-200 gel filtration chromatographies. The specific activity of the purified  $\beta$ -N-acetyl-D-glucosaminidase B was 2.2 units/mg protein with 12.9 % yield and 196.2 fold purity. The purified  $\beta$ -N-acetyl-D-glucosaminidase B showed single band on polyacrylamide gel electrophoresis. The final preparation of  $\beta$ -N-acetyl-D-glucosaminidase B was completely free from arylsulfatase and  $\beta$ -glucuronidase.  $\beta$ -N-Acetyl-D-glucosaminidase B had pH optimum of 4.5 in 0.5 M sodium citrate buffer. The molecular weight of  $\beta$ -N-acetyl-D-glucosaminidase B was 133,000 by Sephadex G-200 gel filtration. The  $K_m$  value of  $\beta$ -N-acetyl-D-glucosaminidase B using *p*-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide as substrate was 1.0 mM and  $V_{max}$  was 0.014  $\mu$  mole/min..  $\beta$ -N-Acetyl-D-glucosaminidase B was stable at 55°C for 70 minutes. The crude  $\beta$ -N-acetyl-D-glucosaminidase in 70 % ammonium sulfate retained 93 % activity after 7 months storage at -26°C. Bovine serum albumin, sodium chloride, and phosphate activated  $\beta$ -N-acetyl-D-glucosaminidase B. N-Acetyl-D-glucosamine,  $\alpha$ -methyl-D-mannoside, and acetate inhibited  $\beta$ -N-acetyl-D-glucosaminidase B.

### Introduction

$\beta$ -N-Acetyl-D-glucosaminidase is widely distributed in mammalian tissues and has been found in many reproductive organs<sup>1-4</sup>. Uterus and epididymis are known to be the richest source of  $\beta$ -N-acetyl-D-glucosaminidase<sup>5</sup>. This enzyme exists in two major forms,  $\beta$ -N-acetyl-D-glucosaminidase A and B<sup>6</sup>. Heating at 50°C destroys the activity of the acid form,  $\beta$ -N-acetyl-D-glucosaminidase A, while the basic form,  $\beta$ -N-acetyl-D-glucosaminidase B, is stable<sup>7</sup>.  $\beta$ -N-Acetyl-D-glucosaminidase B is considered to be a precursor of  $\beta$ -N-acetyl-D-glucosaminidase A<sup>8</sup>. Carrol and Robinson<sup>9</sup> have demonstrated that  $\beta$ -N-acetyl-D-glucosaminidase A and B are related to each other, despite differences in heat stability and electrophoretic mobility. The absence of both  $\beta$ -N-acetyl-D-glucosaminidase A and B activity in Sandhoff's disease suggests the existence of a structural and genetic relationship between two isozymes<sup>10</sup>.

It has been found that uterus has a high ability to develop sperm capacitation, a process by which sperm achieve the capacity to penetrate the ovum in the female reproductive tract<sup>11</sup>. Capacitation of sperm was investigated by injection of sperm into the uterus and the sperm found within cumulus after mating showed acrosomal changes characteris-

tic of capacitation<sup>12</sup>. Dukelow<sup>13</sup> suggested that capacitation was an enzymatic removal of seminal plasma membranes on sperm. The biochemical mechanism of sperm capacitation in the female reproductive organ is still undefined and the exact role of enzymes involved in that process is also uncertain.

In the present paper, we report the purification and some properties of  $\beta$ -N-acetyl-D-glucosaminidase B from rat uterus as an initial step of research in sperm capacitation.

### Materials and Methods

**Materials.** Rat (Sprague-Dowley, about 9 months old) was obtained from the Animal Breeding Center of Seoul National University. *p*-Nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide, DEAE-cellulose, *p*-nitrophenyl- $\beta$ -D-glucuronide, and *p*-nitrocatechol sulfate dipotassium salt were purchased from Sigma Chemical Co.. Sephadex G-200 was obtained from Pharmacia Fine Chemicals. CM-cellulose was obtained from Whatman Ltd.. All other reagents used were the highest purity available.

**Assays.**  $\beta$ -N-Acetyl-D-glucosaminidase activity was determined using *p*-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide as substrate by the method of Tarentino and Maley<sup>14</sup>. The assay mixture consists of 0.3 ml of 0.5 M sodium citrate buffer