Biophysical Chemistry 23 (1985) 91–104
Elsevier

BPC 01028

INDUCED FITTING BETWEEN A COMPLEX OF FOUR NUCLEOTIDES AND THE COGNATE AMINO ACID

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Received 5th February 1985
Accepted 10th July 1985

Key words: Genetic code; tRNA; Amino acid; Semi-empirical calculation; Conformation

The conformation of the hydrogen-bonded complex of a trinucleoside diphosphate (anticodon bases), a nucleic acid base (discriminator base), and an amino acid is investigated. This complex has been named C4N (complex of the four nucleotides) by one of the authors. Concerning the aminoacylation of tRNA and the genetic code, it has been proposed that C4N accepts the cognate protein amino acid by the lock-and-key relationship. The purpose of the calculation is to investigate the conformational and energetic properties of C4N from the energy minimum principle. The calculation is carried out by using the empirical potential functions. Glycine, glutamine, and valine are taken as typical cases. The formation energies are estimated. It is shown that some conformational changes are induced in the anticodon trinucleoside diphosphate by the binding of the discriminator base. Conformational changes of C4N and the amino acid are also induced by the binding of the amino acid to C4N.

1. Introduction

1.1. The C4N model

We present in this paper the calculational investigation of the conformations of the molecular complexes assumed in the 'C4N model'.

The C4N model is a molecular model for the genetic code and the amino acid recognition process of the tRNA proposed by one of the authors [1–3]. This model assumes that three anticodon bases and a discriminator base (the discriminator base is a base on the amino acid accepter stem and is located at the fourth position from the 3'-terminal of the tRNA [4]) can form a complex by using three hydrogen bonds, termed 'C4N' (from 'complex of the 4 nucleotides'). A pocket on C4N can accept the cognate protein amino acid by the lock-and-key relationship. Furthermore, in this model, the conformation of a tRNA is assumed to change globally from the well-known L- to the U-type structure, where the anticodon loop and the amino acid accepter stem can directly interact with each other to result in the formation of C4N. C4N could be the specific recognition site of the tRNA for the cognate amino acid. Various features of the genetic code can be explained in terms of the C4N model [1–3].

1.2. The global conformational change of tRNAs

The conformational change of tRNAs to the U-type structure is still a matter of controversy. X-ray analyses have shown that the three-dimensional structures of tRNAs are of the L type [5]. The two rods of the L are made from the double helices, and are connected by additional hydrogen bonds at the corner of the L. The tertiary hydrogen bonds at the corner of the L appear to keep the L-type structure firm and stable in the crystalline form.

Some studies, however, suggest that flexibility

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and global conformational change of tRNAs do occur. A laser light scattering experiment detected a change in the diffusion constant of a tRNA in a low salt solution, suggesting a major conformational change of the molecule to a compact form [6]. A small angle X-ray scattering experiment on a tRNA dimer showed a tRNA conformation with the acceptor arms folded toward the anticodon arms so as to match with the observed scattering curves in solution [7]. A singlet-singlet fluorescence transfer experiment on a tRNA showed that the discriminator base approached the anticodon bases when the magnesium concentration was low [8]. Calculation of the molecular dynamics of a tRNA demonstrated the intramolecular flexibility of tRNA [9]: the tRNA was shown to be surprisingly flexible, swivelling through angles as large as 30° and with an energy cost of only a few kcal/mol. Recently it has been shown that an alanyl-tRNA synthetase of *Bombyx mori,* which is definitely a monomer, can accept one $tRNA^{Ala}$ to result in the aminoacylation of alanine (M. Kawakami, manuscript in preparation). Only the presence of the U-type structure of $tRNA^{Ala}$ can explain the experimental result.

1.3. The hollow type and the basin type of the C4Ns

In the C4N model two types of C4Ns are proposed [2]. We term one the hollow type (previously called the convex type [2]) and the other the basin type (previously called the concave type [2]) in this paper. The difference between these two types is in the binding scheme of the discriminator base to the anticodon bases. The orientation of the discriminator base to the anticodon bases is inverted between the hollow and basin types. This inversion is shown in figs. 1–3 (figs. 1 and 2, basin type; fig. 3, hollow type).

In the case of the basin type of C4Ns, a basin is formed at the area surrounded by the discriminator base and the edges of the three anticodon bases. The cognate amino acid is bound to this basin. The specificity for an amino acid of the basin type of C4N is mainly due to the hydrogen bonds. The disposition of the hydrogen-bonding sites on the basin determines the amino acid and is dependent on the anticodon sequence and discriminator species.

In the case of the hollow type of C4Ns, a deep pocket is formed between the second and third anticodon bases. This pocket should be the recognition site of the cognate amino acid. The side chain of the amino acid intercalates into this pocket. The conformation of the side chain is determined by the size and shape of the pocket which are dependent on the anticodon sequence.

For the four large hydrophobic amino acids (isoleucine, leucine, phenylalanine, valine) with the second anticodon A and the discriminator A, the hollow type of C4N is used. The side chains of these amino acids have essentially polyhedral forms so as to match their pockets made of planar nucleic acid bases. The basin type of C4N can also be formed even in these four cases and they may be used for accepting other amino acids as in the mitochondrial cases of methionine-isoleucine or threonine-leucine[2], although the details are beyond the scope of this paper. Many important features of the genetic code and the amino acid recognition process of tRNAs can be explained clearly in terms of the C4N model.

1.4. Purpose of the calculation

Our aim in this paper is to determine the detailed conformation of the C4Ns and of the C4N-amino acid complexes. Although an investigation using the CPK molecular model can clarify the basic features of the C4N model, it is necessary to study the conformation by a more quantitative method in order to obtain more detailed information, for example, binding energy, atomic coordinates, detailed conformational features.

For the calculation, we chose glycine, valine and glutamine, of which the anticodons are 5′CpCpC3′, 5′CpUpG3′ and 5′CpApC3′, and discriminator bases U, G and A, respectively. Glycine was selected as the smallest, and thus most typical protein amino acid. Glutamine was chosen as being representative of the hydrophilic protein amino acids. Valine was selected as being typical of the large hydrophobic protein amino acids. As noted before, the C4N model assumes that the C4Ns for glycine and glutamine are of the basin type, and that the C4N for valine is of the hollow type. Our investigation follows the scheme of the C4N model

Table 1

Hydrogen bonds assumed in the C4N model

(A) Glycine and its C4N	(B) Glutamine and its C4N	(C) Valine and its C4N
(1) 1st anticodon C: N4-H [a] Discriminator U: O2	discriminator G: N2-H [c] 1st anticodon C: N_3	1st anticodon C: N4-H [b] discriminator A: N3
(2) Discriminator U: N3-H 2nd anticodon C: N3	discriminator G: N1-H 2nd anticodon U: O4	2nd anticodon A: N6-H [c] discriminator A: N1
(3) 3rd anticodon C: N4-H [b] Discriminator U: O4	3rd anticodon G: N1-H discriminator G: O6	discriminator A: N6-H [e] 3rd anticodon C: N3
(4) 3rd anticodon C: N4-H [a] Glycine: O'	3rd anticodon G: N2-H [c] glutamine: O'	discriminator A: N6-H [f] valine: O'
(5) Glycine: N'-H 2nd anticodon C: O2	glutamine: N'-H 2nd anticodon U: O2	valine: N'-H 3nd anticodon C: O2
(6)	glutamine: N_ϵ-H [d] 1st anticodon C: O2	
(7)	2nd anticodon U: N3-H glutamine; O_ϵ	

[a] This hydrogen is located at the N3 side. [b] This hydrogen is located at the C5 side. [c] This hydrogen is located at the N1 side. [d] This hydrogen is located at the O_ϵ side. [e] This hydrogen is located at the C5 side. [f] This hydrogen is located at the N1 side.

and its assumptions with respect to the hydrogen bonds are shown in table 1 [2].

The calculations were restricted to systems of three molecules; a trinucleoside diphosphate of the anticodon bases, a discriminator base, and an amino acid. We did not take account of the whole tRNA conformation.

The bond lengths and bond angles are fixed in our method except for slight variation of the ribose ring. All of the variables are the torsion angles, the pseudo-rotational parameters for puckering of ribose, and the positions and directions of the separated molecules. Empirical potential functions are used to calculate the total energy, and the complex conformation proposed in the C4N model is determined by the energy minimization method.

2. Method

The conformation of the anticodon trinucleoside diphosphate is expressed by 19 variables which contain the 13 torsion angles and 3 pairs of pseudo-rotational variables for puckering of the ribose ring. The discriminator base is considered to be a rigid planar molecule without internal conformational freedom. Six variables are used for expression of the position and orientation of the discriminator base. The amino acids (glycine, glutamine, valine) have two to five internal conformational degrees of freedom of the torsion angles. Including the six variables for the position and orientation, 8–11 variables are used for the amino acids. The total number of variables is 25 in the calculation for C4N and 33–36 in that of the C4N-amino acid complex. All terminal hydrogens (e.g., the amino group of an amino acid) are taken into account explicitly in the calculation except O2'-H of the riboses.

The parameters of the bond lengths and angles are taken from quantum mechanics and X-ray analyses [10–13]. The geometry of the furanose ring is expressed by the pseudo-rotation parameters P and τ_m [14]. In our method of calculation, the length of the valence bond between C1' and O1' is considered to vary freely.

The empirical potential functions are used to calculate the total energy. We considered six types of empirical potential functions; Lennard-Jones type 6–12 potential [15], electrostatic potential

[13,16], hydrogen-bond potential [15], implicit torsional potential [15,17], lone-pair repulsion potential between phosphate oxygen atoms [18], and implicit puckering potential.

Since the usual Lennard-Jones potential parameters give too large a base stacking energy and too small a contact distance, we corrected the Lennard-Jones parameters between the bases [19]. The dielectric constant is taken to be R_{ij}. The minimum energy of a hydrogen bond is set to be -3.0 kcal/mol. The hydrogen-bonding potential is the 'corrected sum' type described by Hopfinger [15], which has an angular dependence on both the donor and acceptor of the hydrogen bond. The assignments of the hydrogen bonds assumed in the C4N model are shown in table 1 [2]. We express the energy of the pseudo-rotation as a simple function of the parameters P and τ_m as follows:

$$E(\tau_m, P) = C(\cos(P - 90°))\tau_m^3(\tau_m - \tau_a) + 5.0 \text{ kcal/mol} \quad (1)$$

where τ_a is the minimum point of τ_m. Our setting of τ_a is 40°. $C(x)$ is given by the expression:

$$C(x) = C_3x^3 + C_2x^2 + C_1x + C_0 \quad (2)$$

The values of the coefficients C_3, C_2, C_1 and C_0 are determined sufficiently precisely that $E(\tau_m, P)$ should be a reasonable function as follows: The energy of the inversion of the puckering is 5 kcal/mol: the barrier between C3′ endo and C2′ endo is 2 kcal/mol: the minimum points of P are $P = 20$ and 160°: the energy barrier at O1′ exo is 4 kcal/mol.

The most probable conformation is determined by means of the energy minimum principle. The conformation of the A helix is selected for the initial value for the anticodon trinucleotide diphosphate. The initial values of the torsion angles of the amino acids are taken from the statistics obtained by the X-ray analysis [20]. The discriminator base is first located by rough estimation using computer graphics at an orientation from the anticodon trinucleoside diphosphate suitable for the formation of C4N. Next, rough minimization is performed using an artificial strong hydrogen-bond function. Finally, using the hydrogen-bond function with the natural physical meanings, the minimization is carried out by gradually setting the minimum energy of the hydrogen bond to be -3.0 kcal/mol. By these procedures, one of the probable conformations of C4N is obtained. In the calculation of the C4N-amino acid complex, the initial values of the orientation for the amino acids are also obtained by rough estimation using computer graphics, and similar procedures are carried out. Although this method of determination of the minimum does not guarantee that the obtained conformation is the global minimum, it is a reasonable method for establishing a local minimum.

The calculation considers the following three stages.

(1) The anticodon trinucleoside diphosphate, discriminator base and cognate amino acid are all separated.

(2) The anticodon trinucleoside diphosphate and discriminator base form C4N, but the amino acid is separated.

(3) The amino acid becomes attached to C4N. The total energies are compared between these three stages and the C4N formational and amino acid binding energies are calculated.

3. Results

Using our minimization procedure, we succeeded in determining the conformations which are stable as local minima and have the hydrogen bonds assumed in table 1. Therefore, the assumption in table 1 is at least reasonable and is not forbidden directly by steric hindrance, too large an electrostatic repulsion, or other kinds of excess energy loss, because if the assumption in table 1 was forbidden, then no stable local minimum could be found by the energy minimization. Figs. 1–3 show the complexes of the C4Ns and their cognate amino acids assumed in the C4N model. Tables 5–7 list the atomic coordinates of figs. 1–3. These are at stage 3 described in section 2. The complexes at the other stages were also obtained by our calculation. Tables 2–4 detail the energy structures contributed from various energy terms, compared among the three stages for each amino acid.

Tables 2 and 3 list the energy structures for glycine and glutamine, respectively, of which the

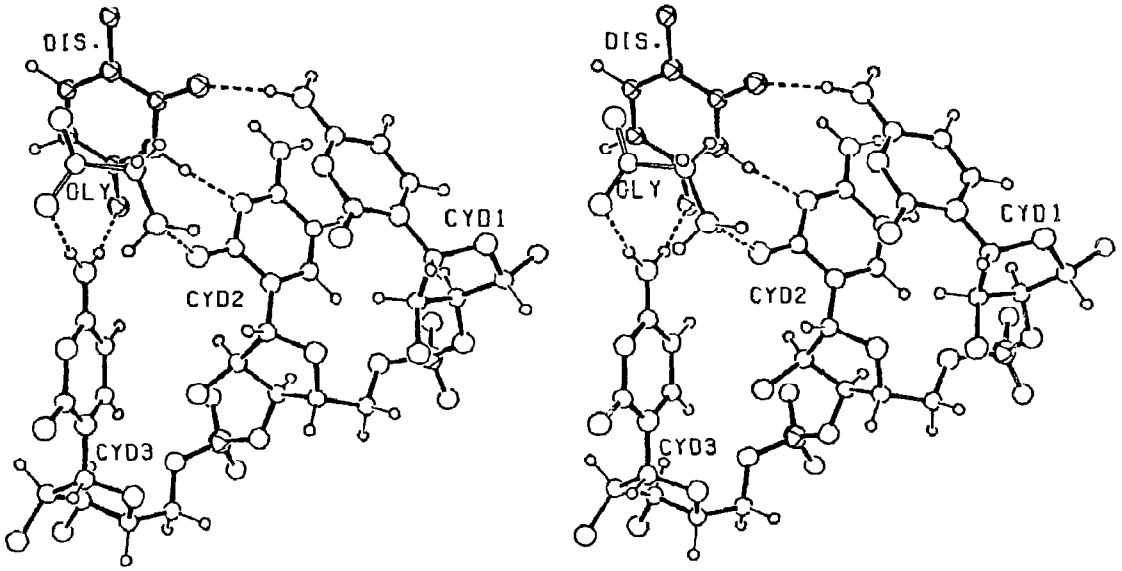

Fig. 1. Stereograph of a complex of C4N (anticodon trinucleoside diphosphate 5'CpCpC3', discriminator base U) and glycine. Dashed lines represent the hydrogen bonds.

C4Ns are assumed to be of the basin type. No significant change is found in the energy structures of the anticodon moiety between the first and second stages. In the case of valine in table 4, however, some changes are found in the energy structures of the anticodon moiety between the first and second stages. The stacking energy between the second and third anticodon bases

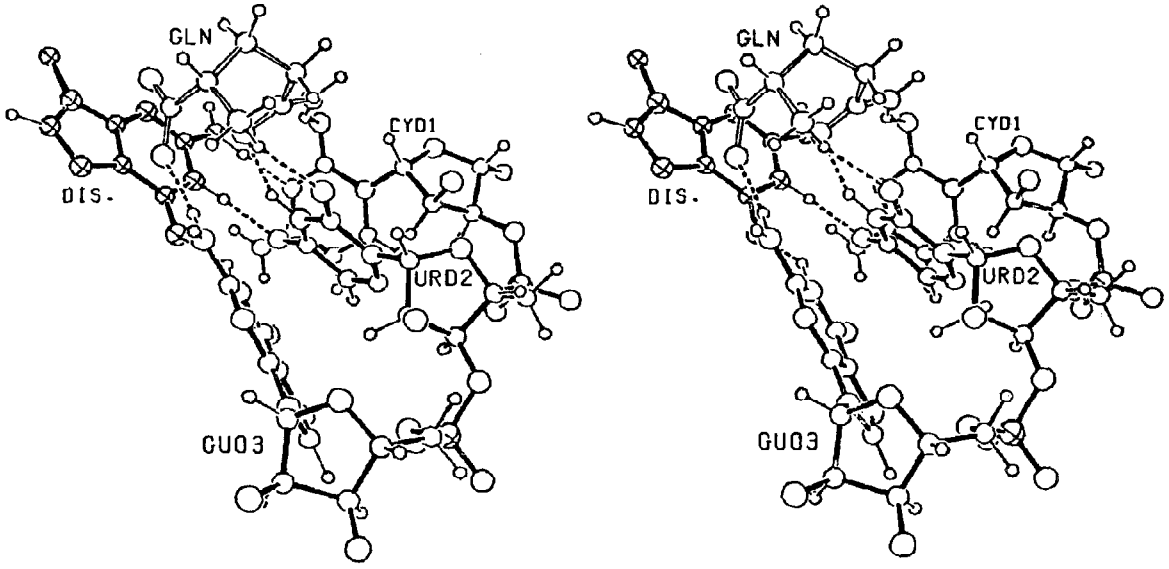

Fig. 2. Stereograph of a complex of C4N (anticodon trinucleoside diphosphate 5'CpUpG3', discriminator base G) and glutamine. Dashed lines represent the hydrogen bonds.

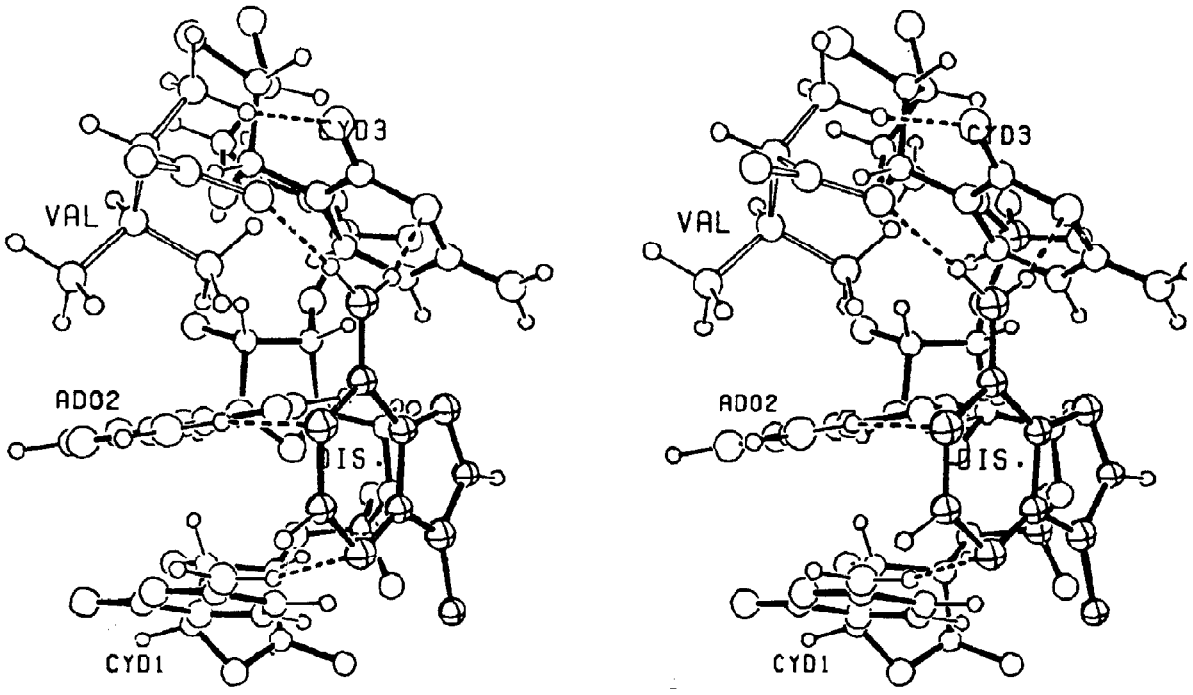

Fig. 3. Stereograph of a complex of C4N (anticodon trinucleoside diphosphate 5'CpApC3', discriminator base A) and valine. Dashed lines represent the hydrogen bonds.

Table 2

Energy contributions in the case of glycine (anticodon 5'-CpCpC3', discriminator base U, amino acid glycine, units in kcal/mol)

	1st stage	2nd stage	3rd stage
Total energy of system	−42.5	−54.0	−82.8
Anticodon part	−40.2	−37.9	−36.2
Torsional and puckering energy	4.7	6.2	6.7
Lone-pair repulsion energy	1.4	1.1	1.0
Stacking between 1st and 2nd bases	−5.3	−5.0	−4.9
Stacking between 2nd and 3rd bases	−3.7	−2.4	−2.1
Interaction between backbone and bases	−23.4	−23.0	−22.5
Interaction between phosphates	5.9	5.5	5.0
Interaction among riboses	−4.0	−3.6	−3.1
Interaction between phosphate and riboses	−15.7	−16.6	−16.6
Other energy contributions	−0.0	0.0	−0.2
Anticodon-discriminator interaction	-	−13.7	−13.9
Electrostatic and van der Waals	-	−5.4	−5.1
Hydrogen bonds	-	−8.3	−8.7
Amino acid part	−2.3	−2.3	−2.0
C4N-amino acid interaction	-	-	−30.6
Electrostatic and van der Waals	-	-	−25.5
Hydrogen bonds	-	-	−5.0

Table 3

Energy contributions in the case of glutamine (anticodon 5'CpUpG3', discriminator base G, amino acid glutamine, units in kcal/mol)

	1st stage	2nd stage	3rd stage
Total energy of system	−63.9	−77.7	−107.4
Anticodon part	−49.0	−43.0	−39.1
Torsional and puckering energy	7.7	7.9	9.2
Lone-pair repulsion energy	0.9	0.8	0.8
Stacking between 1st and 2nd bases	−4.1	−3.6	−4.5
Stacking between 2nd and 3rd bases	−7.0	−5.6	−5.4
Interaction between backbone and bases	−26.5	−25.9	−22.8
Interaction between phosphates	3.5	4.5	5.1
Interaction among riboses	−4.4	−0.5	−1.8
Interaction between phosphate and riboses	−16.9	−18.6	−18.7
Other energy contributions	−2.2	−2.0	−1.1
Anticodon-discriminator interaction	–	−19.8	−18.4
Electrostatic and van der Waals	–	−11.3	−10.3
Hydrogen bonds	–	−8.3	−8.2
Amino acid part	−14.8	−14.8	−6.6
C4N-amino acid interaction	–	–	−43.1
Electrostatic and van der Waals	–	–	−33.4
Hydrogen bonds	–	–	−8.7

changes from −8.3 to −2.5 kcal/mol. The interaction between the backbone and anticodon bases changes from −34.3 to −23.4 kcal/mol. These data show that the stacking between the second and third anticodons becomes weak and that the backbone becomes more stretched by formation of

Table 4

Energy contributions in the case of valine (anticodon 5'CpApC3', discriminator base A, amino acid valine, units in kcal/mol)

	1st stage	2nd stage	3rd stage
Total energy of system	−64.0	−72.7	−107.8
Anticodon part	−54.9	−40.9	−40.7
Torsional and puckering energy	9.0	7.8	8.0
Lone-pair repulsion energy	2.0	2.0	1.9
Stacking between 1st and 2nd bases	−6.6	−6.3	−6.3
Stacking between 2nd and 3rd bases	−8.3	−2.5	−2.4
Interaction between backbone and bases	−34.3	−23.4	−23.2
Interaction between phosphates	6.3	4.2	4.3
Interaction among riboses	−8.6	−9.2	−9.4
Interaction between phosphate and riboses	−13.0	−14.1	−14.6
Other energy contributions	−1.4	0.6	1.0
Anticodon-discriminator interaction	–	−22.7	−23.0
Electrostatic and van der Waals	–	−14.0	−14.2
Hydrogen bonds	–	−8.6	−8.8
Amino acid part	−9.1	−9.1	−7.4
C4N-amino acid interaction	–	–	−36.5
Electrostatic and van der Waals	–	–	−31.6
Hydrogen bonds	–	–	−4.9

Table 5

Coordinates of atoms in a complex of glycine and its C4N

First anticodon C										
	C5′	C4′	H4′	O1′	H3′	H2′	H1′	O2′	C2′	C1′
X	−0.91	−0.25	−0.72	1.18	−0.33	1.11	2.32	0.39	0.77	1.81
Y	5.55	5.46	6.21	5.68	3.30	2.99	5.49	4.57	4.01	4.86
Z	5.80	4.44	3.80	4.54	4.62	2.70	2.84	1.50	2.87	3.57
	C3′	O3′	N1	C2	N3	C4	C5	C6	O2	N4
X	−0.40	−1.66	2.79	3.96	4.82	4.62	3.43	2.55	4.21	5.53
Y	4.07	3.89	3.98	3.64	2.81	2.36	2.71	3.51	4.04	1.57
Z	3.85	3.20	4.24	3.56	4.26	5.45	6.15	5.51	2.41	6.02
	H5	H6	H4	H4						
X	3.24	1.63	6.36	5.37						
Y	2.34	3.80	1.33	1.22						
Z	7.16	6.02	5.53	6.94						
Phosphate										
	P	O	O							
X	−2.30	−3.64	−2.41							
Y	2.44	2.49	1.73							
Z	3.03	2.48	4.30							
Second anticodon C										
	O5′	H5′	H5′	C5′	C4′	H4′	O1′	H3′	H2′	H1′
X	−1.26	−1.29	−2.13	−1.26	0.0	0.05	1.17	−0.51	1.69	2.98
Y	1.75	3.14	1.61	2.06	1.52	1.91	1.90	−0.40	−1.27	1.20
Z	2.04	0.52	0.17	0.64	0.0	−1.02	0.76	0.88	0.54	0.13
	O2′	C2′	C1′	C3′	O3′	N1	C2	N3	C4	C5
X	2.03	1.49	2.11	0.0	−0.66	2.52	3.82	4.11	3.31	1.98
Y	−0.52	−0.34	0.86	0.0	−0.53	0.49	0.01	−0.32	−0.22	0.26
Z	−1.42	0.0	0.69	0.0	−1.15	2.05	2.26	3.57	4.57	4.37
	C6	O2	N4	H5	H6	H4	H4			
X	1.63	4.64	3.71	1.27	0.63	4.64	3.08			
Y	0.60	−0.10	−0.57	0.35	0.97	−0.91	−0.50			
Z	3.11	1.34	5.79	5.19	2.91	5.93	6.56			
Phosphate										
	P	O	O							
X	−0.83	−0.36	−2.22							
Y	−2.11	−2.86	−2.49							
Z	−1.35	−0.21	−1.55							
Third anticodon C										
	O5′	H5′	H5′	C5′	C4′	H4′	O1′	H3′	H2′	H1′
X	0.06	−1.20	−0.85	−0.43	0.70	0.28	1.74	1.46	3.55	3.48
Y	−2.36	−2.74	−1.02	−2.02	−2.07	−1.87	−1.11	−3.83	−3.76	−1.05
Z	−2.66	−4.25	−3.94	−3.96	−4.96	−5.94	−4.62	−3.92	−5.12	−5.71
	O2′	C2′	C1′	C3′	O3′	N1	C2	N3	C4	C5
X	2.82	2.79	3.00	1.40	0.73	3.85	5.14	5.87	5.46	4.15
Y	−3.12	−3.07	−1.66	−3.42	−4.39	−1.70	−1.16	−1.24	−1.76	−2.32
Z	−7.01	−5.48	−4.95	−4.93	−5.73	−3.75	−3.82	−2.65	−1.54	−1.46

Table 5 (continued)

Third anticodon C										
	C6	O2	N4	H5	H6	H4	H4			
X	3.39	5.60	6.26	3.77	2.38	7.18	5.94			
Y	−2.26	−0.65	−1.78	−2.76	−2.68	−1.39	−2.18			
Z	−2.58	−4.85	−0.48	−0.54	−2.56	−0.54	0.38			
Discriminator U										
	N1	C2	N3	C4	C5	C6	O2	H3	O4	H5
X	9.00	7.81	6.79	6.85	8.06	9.09	7.68	5.92	5.82	8.14
Y	−1.45	−0.71	−1.30	−2.43	−3.19	−2.67	0.39	−0.79	−2.79	−4.15
Z	4.11	4.06	3.34	2.73	2.77	3.46	4.62	3.28	2.13	2.27
	H6	C3′								
X	10.03	10.14								
Y	−3.22	−0.91								
Z	3.52	4.85								
Glycine										
	H′	H′	H′	N′	CA	HA	HA	C′	O′	O′
X	6.38	6.68	7.21	7.05	8.30	8.87	8.11	9.05	8.87	9.78
Y	0.38	1.99	0.87	1.05	0.93	1.72	0.91	−0.34	−0.76	−0.87
Z	0.93	0.68	−0.42	0.56	1.29	1.06	2.27	0.89	0.27	1.76

Table 6

Coordinates of atoms in a complex of glutamine and its C4N

First anticodon C										
	C5′	C4′	H4′	O1′	H3′	H2′	H1′	O2′	C2′	C1′
X	−1.54	−0.38	−0.11	0.74	−1.34	0.61	2.43	1.27	0.64	1.40
Y	5.02	4.54	5.35	4.15	2.59	1.63	3.33	3.34	2.70	3.05
Z	6.47	5.63	4.95	6.46	5.46	4.36	5.57	3.30	4.54	5.82
	C3′	O3′	N1	C2	N3	C4	C5	C6	O2	N4
X	−0.74	−1.49	1.42	2.47	2.41	1.48	0.40	0.41	3.40	1.53
Y	3.28	3.56	1.89	1.75	0.63	−0.26	−0.13	0.95	2.57	−1.30
Z	4.86	3.68	6.71	7.62	8.43	8.40	7.47	6.66	7.70	9.23
	H5	H6	H4	H4						
X	−0.40	−0.40	2.28	0.81						
Y	−0.87	1.08	−1.39	−2.00						
Z	7.42	5.94	9.87	9.20						
Phosphate										
	P	O	O							
X	−2.38	−3.50	−2.94							
Y	2.43	2.99	1.50							
Z	2.98	2.25	3.93							
Second anticodon U										
	O5′	H5′	H5′	C5′	C4′	H4′	O1′	H3′	H2′	H1′
X	−1.31	−1.30	−2.13	−1.27	0.0	0.08	1.16	−0.51	1.70	3.03
Y	1.75	3.14	1.61	2.06	1.52	1.91	1.90	−0.41	−1.31	1.11
Z	2.01	0.48	0.11	0.61	0.0	−1.02	0.79	0.88	0.44	0.45

Table 6 (continued)

	O2′	C2′	C1′	C3′	O3′	N1	C2	N3	C4	C5
X	2.08	1.49	2.06	0.0	−0.66	2.22	3.50	3.56	2.56	1.25
Y	−0.35	−0.33	0.80	0.0	−0.53	0.36	0.39	−0.05	−0.46	−0.50
Z	−1.41	0.0	0.84	0.0	−1.15	2.23	2.81	4.12	4.82	4.24

	C6	O2	H3	O4	H5	H6
X	1.14	4.50	4.45	2.81	0.39	0.15
Y	−0.08	0.78	−0.04	−0.82	−0.85	−0.10
Z	2.96	2.19	4.56	5.98	4.80	2.48

Phosphate

	P	O	O
X	−0.88	−1.15	−2.00
Y	−2.11	−2.76	−2.42
Z	−1.32	−0.05	−2.18

Third anticodon G

	O5′	H5′	H5′	C5′	C4′	H4′	O1′	H3′	H2′	H1′
X	0.52	−0.06	1.09	0.82	1.97	2.19	3.13	0.71	2.55	4.50
Y	−2.56	−2.54	−1.26	−2.31	−3.19	−2.97	−2.94	−4.78	−6.06	−4.19
Z	−1.95	−3.93	−3.45	−3.32	−3.76	−4.81	−2.94	−2.99	−2.22	−2.08

	O2′	C2′	C1′	C3′	O3′	N1	C2	N3	C4	C5
X	3.81	2.83	3.42	1.61	1.36	4.24	5.06	4.75	3.48	2.58
Y	−5.87	−5.26	−4.07	−4.65	−5.28	−2.58	−2.55	−2.97	−3.44	−3.52
Z	−3.91	−2.90	−2.18	−3.60	−4.86	2.57	1.50	0.29	0.25	1.29

Third anticodon G

	C6	N7	C8	N9	H1	N2	O6	H8	H2	H2
X	2.94	1.38	1.60	2.84	4.60	6.30	2.26	0.85	6.94	6.58
Y	−3.06	−4.06	−4.31	−3.95	−2.23	−2.04	−3.05	−4.75	−2.00	−1.71
Z	2.58	0.86	−0.42	−0.83	3.43	1.69	3.62	−1.09	0.92	2.59

Discriminator G

	N1	C2	N3	C4	C5	C6	N7	C8	N9	H2
X	5.48	6.05	7.33	8.01	7.52	6.15	8.53	9.62	9.35	4.50
Y	−1.02	−0.33	−0.36	−1.17	−1.90	−1.85	−2.61	−2.29	−1.43	−0.91
Z	6.85	7.87	8.18	7.34	6.28	5.97	5.66	6.36	7.38	6.72

	N2	O6	H8	H2	H2	C3′
X	5.24	5.54	10.61	5.61	4.26	10.29
Y	0.44	−2.44	−2.67	0.96	0.49	−0.86
Z	8.62	5.06	6.15	9.39	8.42	8.36

Glutamine

	H′	H′	H′	N′	CA	HA	CB	C′	O′	O′
X	6.54	7.48	7.80	7.48	8.36	9.30	8.06	8.25	7.67	8.75
Y	0.61	1.51	−0.10	0.75	1.08	1.08	2.47	0.02	−1.05	0.30
Z	2.35	1.33	1.55	2.00	3.11	2.77	3.66	4.21	3.91	5.32

	HB	HB	CG	HG	HG	CD	OE	NE	HE	HE
X	8.42	8.56	6.55	6.39	6.15	5.84	6.04	4.99	4.87	4.48
Y	2.54	3.22	2.75	3.82	2.46	1.97	0.78	2.70	3.67	2.27
Z	4.69	3.04	3.64	3.81	2.68	4.75	4.93	5.47	5.27	6.21

Table 7

Coordinates of atoms in a complex of valine and its C4N

First anticodon C										
	C5′	C4′	H4′	O1′	H3′	H2′	H1′	O2′	C2′	C1′
X	0.73	1.73	1.57	3.08	1.28	3.15	4.79	3.20	2.96	3.82
Y	6.66	6.17	6.73	6.33	4.14	3.22	5.33	4.67	4.29	5.11
Z	1.56	0.54	−0.38	1.02	1.20	−0.11	0.32	−1.65	−0.19	0.77
	C3′	O3′	N1	C2	N3	C4	C5	C6	O2	N4
X	1.56	0.56	4.05	5.27	5.40	4.50	3.25	3.07	6.18	4.74
Y	4.68	4.40	4.37	3.72	3.05	2.99	3.65	4.32	3.74	2.31
Z	0.30	−0.68	2.02	2.21	3.41	4.33	4.15	2.99	1.36	5.45
	H5	H6	H4	H4						
X	2.47	2.12	5.62	4.04						
Y	3.61	4.83	1.85	2.26						
Z	4.91	2.81	5.58	6.17						
Phosphate										
	P	O	O							
X	−0.99	−1.84	−1.32							
Y	4.55	4.29	5.89							
Z	−0.33	−1.48	0.12							
Second anticodon A										
	O5′	H5′	H5′	C5′	C4′	H4′	O1′	H3′	H2′	H1′
X	−1.17	−2.07	−1.62	−1.31	0.0	0.16	1.09	−0.50	1.73	2.86
Y	3.46	1.95	1.52	2.07	1.52	1.92	1.87	−0.41	−1.23	1.34
Z	0.82	−0.27	1.39	0.50	0.0	−1.00	0.89	0.87	0.54	0.07
	O2′	C2′	C1′	C3′	O3′	N1	C2	N3	C4	C5
X	2.05	1.50	2.07	0.0	−0.66	6.28	6.12	4.97	3.94	4.01
Y	−0.48	−0.31	0.92	0.0	−0.54	−0.07	0.30	0.53	0.36	−0.01
Z	−1.42	0.0	0.69	0.0	−1.15	3.28	1.99	1.40	2.27	3.59
	C6	N7	C8	N9	H2	N6	H8	H6	H6	
X	5.26	2.74	1.94	2.62	7.03	5.41	0.86	6.33	4.61	
Y	−0.26	−0.08	0.25	0.52	0.40	−0.62	0.30	−0.78	−0.73	
Z	4.20	4.15	3.14	1.99	1.39	5.47	3.22	5.83	6.06	
Phosphate										
	P	O	O							
X	−1.56	−2.74	−2.02							
Y	−1.86	−1.66	−2.32							
Z	−1.04	−0.23	−2.33							
Third anticodon C										
	O5′	H5′	H5′	C5′	C4′	H4′	O1′	H3′	H2′	H1′
X	−0.53	0.12	1.15	0.54	1.39	2.17	1.97	−0.46	0.73	2.78
Y	−2.88	−4.08	−2.68	−3.47	−4.33	−4.79	−3.54	−5.03	−6.06	−4.27
Z	−0.36	−1.91	−1.54	−1.11	−0.21	−0.82	0.86	0.70	2.56	2.62
	O2′	C2′	C1′	C3′	O3′	N1	C2	N3	C4	C5
X	2.51	1.35	1.83	0.55	0.42	0.85	0.58	−0.35	−0.96	−0.69
Y	−6.61	−5.64	−4.23	−5.39	−6.57	−3.55	−4.07	−3.36	−2.30	−1.76
Z	1.54	1.77	2.10	0.48	−0.31	2.96	4.23	4.96	4.58	3.28

Table 7 (continued)

Third anticodon C										
	C6	O2	N4	H5	H6	H4	H4			
X	0.21	1.14	−1.84	−1.20	0.44	−2.02	−2.31			
Y	−2.41	−5.09	−1.71	−0.86	−2.03	−2.08	−0.88			
Z	2.52	4.66	5.38	2.93	1.52	6.29	5.08			
Discriminator A										
	N1	C2	N3	C4	C5	C6	N7	C8	N9	H2
X	3.39	3.72	2.96	1.82	1.40	2.23	0.16	−0.15	0.82	4.67
Y	−0.72	0.54	1.61	1.27	0.02	−1.10	0.07	1.36	2.12	0.69
Z	7.49	7.85	7.64	7.02	6.62	6.85	6.01	6.06	6.65	8.35
	N6	H8	H6	H6	C3′					
X	1.91	−1.07	2.54	1.04	0.82					
Y	−2.35	1.78	−3.10	−2.53	3.57					
Z	6.50	5.66	6.70	6.04	6.88					
Valine										
	H′	H′	H′	N′	CA	HA	CB	C′	O′	O′
X	2.87	3.74	3.74	3.72	4.88	5.72	4.98	4.78	3.74	5.76
Y	−5.53	−6.09	−6.93	−6.01	−5.25	−5.76	−3.95	−5.02	−4.48	−5.40
Z	4.64	3.35	4.78	4.36	4.81	4.63	4.01	6.32	6.75	7.01
	HB	CG1	CG2	HG1	HG1	HG1	HG2	HG2	HG2	
X	4.92	3.84	6.32	3.75	2.91	4.06	6.43	6.35	7.14	
Y	−4.17	−3.00	−3.26	−2.22	−3.56	−2.55	−2.38	−2.96	−3.96	
Z	2.95	4.40	4.31	3.64	4.46	5.36	3.68	5.36	4.11	

the hollow-type C4N for valine. These conformational changes amount to a change in total energy of the anticodon moiety from −54.9 to −40.9 kcal/mol. This energy loss is compensated by the newly formed anticodon-discriminator interaction of −22.7 kcal/mol. The binding of the discriminator base induces a conformational change in the anticodon trinucleoside diphosphate. This kind of conformational change is not found in formation of the basin-type C4Ns for glycine and glutamine. In formation of the basin-type C4Ns, the discriminator base is bound to the anticodon trinucleoside diphosphate without causing a large conformational change.

This difference between the hollow and basin types is a somewhat inevitable result of the geometry of the hydrogen bonds assumed in table 1. Studies using the CPK molecular model have shown that the conformation of the two types of C4Ns should be different, although a less precise study using the CPK model left the problem unresolved [2]. Our calculation using empirical potentials shows clearly that the conformational change is induced by formation of the hollow-type C4N and that only a smaller conformational change is induced by formation of the basin-type C4Ns.

Comparison of the energy structures at the second and third stages in tables 2–4 does not show a conformational change in the C4N moiety (sum of the energy of the anticodon moiety and the anticodon-discriminator interaction energy) on binding of the amino acid to the C4N as large as that on formation of the hollow-type C4N. Only in the case of glutamine does the energy of the anticodon moiety change from −43.0 to −39.1 kcal, and that of the anticodon-discriminator interaction from −19.8 to −18.4 kcal/mol. The C4N model assumes one more hydrogen bond between glutamine and its C4N than for glycine and glutamine as shown in table 1. Naturally, the additional hydrogen bond should impose a stronger restriction on C4N for the binding of glutamine. However, this change, even in the case of glutamine, is quite smaller than the energy change of the anti-

codon moiety in formation of the hollow-type C4N for valine in table 4.

The study using the CPK molecular model has proposed that the two types of C4Ns provide favorable binding sites for their cognate amino acids [2]. The basin-type C4Ns have been described as binding the cognate amino acid mainly via hydrogen bonds. The hollow-type C4Ns have been explained to bind the cognate amino acid via hydrogen bonds between the peptidyl moiety of the amino acid and the bases of C4N and via hydrophobic interaction with the amino acid in the pocket between the second and third anticodon bases. Our calculation, using the assumption of table 1, confirms the results obtained by using the CPK model and shows that binding of the discriminator base to the anticodon trinucleoside diphosphate occurs without a large conformational change and provides a stable hydrogen bonding site for the amino acids. It also shows that the binding of the discriminator base causes the anticodon bases to open and make a pocket for the amino acid binding. The amino acids bind to their C4Ns without causing a large conformation change in the C4Ns. A further calculation is necessary for investigation of the flexibility of the C4Ns.

Tables 2–4 list the energy change of the amino acids on binding to the C4N. The energy of glycine changes from -2.3 to -2.0 kcal/mol, that of glutamine from -14.8 to -6.6 kcal/mol and that of valine from -9.1 to -7.4 kcal/mol. Amino acids are small molecules and these energy changes thus correspond to rather large conformational changes.

The binding energies of glycine, glutamine and valine to their C4Ns are -28.8, -29.7 and -35.1 kcal/mol, respectively. The energy of formation of the C4Ns for glycine, glutamine and valine are -11.5, -13.8 and -8.7 kcal/mol, respectively.

4. Conclusion

We determined through energy minimum calculations the conformation of the hydrogen-bonded base complexes assumed in the C4N model. Up to now, the conformation of the C4Ns had only been studied by means of the CPK molecular model. The calculation resulted in some important features of the C4N model being ascertained. Formation of the hollow-type C4N for valine induces a conformational change in the anticodon trinucleoside diphosphate. The conformation of the anticodon trinucleoside diphosphate becomes more stretched, and the second and third anticodon bases become unstacked. These features are favorable to intercalation by the side chain of valine. Formation of the basin-type C4Ns for glycine and glutamine does not induce such a large conformational change as that seen in the hollow-type C4N for valine. The conformations of the C4Ns are retained after binding of the amino acid. However, the binding induces a conformational change in the amino acid to enable the necessary hydrogen bonding for the recognition of the C4N. The formation energies of the C4Ns range from -8.7 to -13.8 kcal/mol and the binding energies of the amino acids to the C4Ns vary from -28.8 to -35.1 kcal/mol.

Acknowledgments

We are grateful to Mrs. M. Aida for her fruitful advice concerning the angular dependence of hydrogen bonding from the quantum-mechanical standpoint. We also thank Professor Kotani for his continuous encouragement during this work.

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