The Binding Nature between Chromophore and Apoprotein in the Photoreceptor of *Stentor coeruleus* Probed by Conformational Analysis

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To understand the nature of the linkage between chromophore and apoprotein in the photoreceptor of *Stentor coeruleus*, a conformational analysis has been carried out on the dipeptide amides linked to the chromophore hypericin using an empirical potential function. The conformational energies for the dipeptide amides of Glu (OHyp)-X-NHMe, where X = Leu, Phe, Asp, and Tyr, have been calculated to investigate the influence of peptide residues in stabilizing conformers. It was found that the increase of acidity of hypericin upon photoexcitation may be facilitated by the formation of intramolecular hydrogen bonds between hydroxyl groups of hypericin and carbonyl groups of peptide backbone, and that the stabilities of dipeptide amides do not significantly depend on peptide residues directly linked to chromophore.

Introduction

Stentor coeruleus exhibits photophobic and negative phototactic behaviour in response to red light.[1] These photoresponses are mediated by a photoreceptor pigment to perceive light stimuli and initiate a signal-generating process. It has been suggested that the general signal is in the form of a pH gradient across the celluar membrane as a result of proton release from the excited state of stentorin in the pigment granule.[2, 3]

Recently, the chromophore has been identified as a hypericin covalently linked to an apoprotein, on the basis of chromatographic and spectroscopic characterization.[2] The linkage of the chromophore to the protein in stentorin is readily cleaved by mild acid treatment, and a labile ester bond may be involved in the linkage of the chromophore through its hydroxyl group(s) to the apoprotein.[4] However, the nature and the location of the linkage are yet unknown. The amino acid compositions and the sequence of the apoprotein in stentorin have not also been thoroughly determined.

As a further step in characterizing the molecular mechanism of the proton release from hypericin in stentorin upon photoexcitation and in understanding the nature of the linkage between hypericin and apoprotein in stentorin, a study has been undertaken for the dipeptide amides covalently linked to the hypericin by calculating the conformational energy using an empirical potential function.

Methods and Definitions

Potential Function and Parameters. Conformational energy calculations were carried out with the empirical potential functions and energy parameters described by Kang and Jhon.[5-9] The total conformational energy E_{tot} is the sum of the electrostatic energy E_{el}, the polarization energy E_{pol}, the nonbonded energy E_{nb}, and the torsional energy E_{tor}. The hydrogen bond energy E_{hb}, an interaction energy between proton and proton-acceptor, is also included separately in the total energy. The total energy is given by the expression,

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

The value of the effective dielectric constant is estimated to range between 2 and 5 in nonpolar regions of protein interiors,[10] and a constant value 2 is used in this work. Details of the nature of potential energy functions and parameters used are well described in the previously noted references.

Model Compounds. As a model compound of the photoreceptor in stentorin, four dipeptide amides of Glu (OHyp)-X-NHMe (X = Leu, Phe, Asp, and Tyr) were chosen.* Although two possible structures have been proposed for the linkage of hypericin to the peptide in stentorin,[1] only the O_6'-ester-linked conformation was dealt in this paper because its conformation has more favorable stereochemistry than the O_4'-ester-linked one, because of having more hydroxyl groups of hypericin available to hydrogen bonds with carbonyl groups of peptide backbone. On the basis of experimental evidence,[1] glutamate was chosen as the first amino acid residue linked to hypericin in each dipeptide amide. For the second amino acid residues of dipeptides, two hydrophobic residues (*i.e.*, Leu and Phe) and two hydrophilic residues (*i.e.*, Asp and Tyr) were chosen, based upon the results of amino acid composition analysis of stentorin.[12] Each end of the dipeptides was terminated with methyl group. The structure and the notation for torsion angles of model compounds are illustrated in Figures 1 and 2. All bond lengths and bond angles were taken from the standard data[13] for hypericin and peptide residues.

Procedures of Computation. In order to generate the coordinates of hypericin-linked dipeptide amides of the type Glu (OHyp)-X-NHMe, 13 independent torsion angles are used. Since a search of the entire conformational space of dipeptide

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*Abbreviations: Glu(OHyp)-Leu-NHMe, O^6-Hypericinylglutamyl-N′-methylleucinamide; Glu(OHyp)-Phe-NHMe, O^6-Hypericinylglutamyl-N′-methylphenylalaninamide; Glu(OHyp)-Asp-NHMe, O^6-Hypericinylglutamyl-N′-methylaspartamide; Glu(OHyp)-Tyr-NHMe, O^6-Hypericinylglutamyl-N′-methyltyrosinamide; the nomenclature and conventions used here are those adopted by an IUPAC-IUB Commission.[11]

amides is not feasible, all combinations of each torsion angle minimum with low energy ($E_{tot}<-5$ kcal/mol) were used as starting conformations of dipeptide amides. At first, each torsion angle minimum was determined from the conformational energy contour maps calculated at 15° intervals of all torsion angles. Each torsion angles of backbone were then allowed to move one after another until the difference of each torsion angle between the previous and later iterations remains within 1°. The final torsion angles were optimized by using a quasi-Newton method developed by Fletcher[14] with a convergence criterion of 0.005 kcal/mol and with a step length of 2° for all the torsion angles. The number of iterations was limited to 100 cycles.

Results and Discussion

Minimum-energy Conformation. The optimized torsion angles of hypericin-linked dipeptide amides are listed in Table 1. The notation for torsion angles is illustrated in Figure 2. Two kinds of energy minimum conformations are obtained for each hypericin-linked dipeptide amide: (a) the hydrogen bonded (HB) conformation, in which 4′-hydroxyl group of hypericin forms a hydrogen bond with 2-carbonyl group of the second peptide residue, and there is an additional intra-residue hydrogen bond between 3-amido group and 1-carbonyl group of dipeptide backbone: (b) the extended (E) conformation, in which there is an intramolecular hydrogen bond between 7′-carbonyl group of hypericin and 1-amino group of glutamate. Conformational energies of hypericin-linked dipeptide amides are compared in Table 1.

By comparison of backbone torsion angles of each dipeptide amide, it is found that the influence of the second residue of the dipeptide on the overall conformation is not significant, and that the difference in conformational energies of the HB and E conformations is not large enough although there are somewhat structural differences in torsion angles χ_1, χ_2, χ_3, χ_5, ϕ_2, and ψ_2 of the two conformations. In the HB conformations compared to the E conformations, the large differences in torsion angles χ_2 and χ_5 may be ascribed to a hydrogen bond between 4′-hydroxyl group of hypericin and 2-carbonyl group

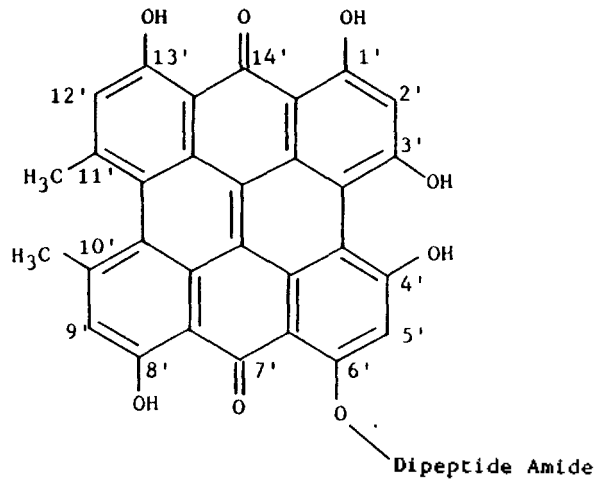

Figure 1. Structure of hypericin.

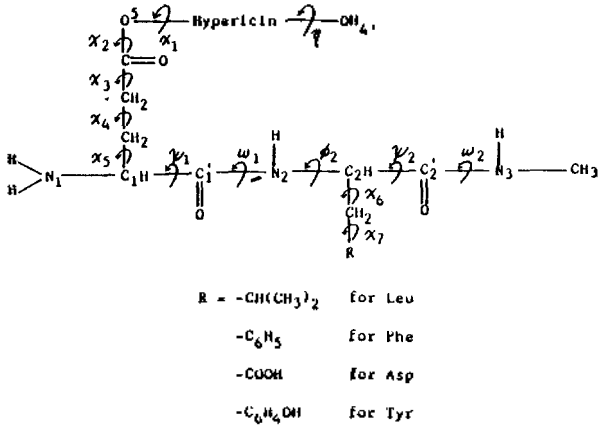

Figure 2. Structure and conformational definition of hypericin-linked dipeptide amide.

of the second peptide residue, and those in torsion angles ϕ_2 and ψ_2 may be due to an intra-residue hydrogen bond in dipeptide amide backbone.

Table 2 shows the contribution of components of conformation energy to the total interaction energy. In the HB conformation compared to the *E* conformation, the contribution of hydrogen bond to the total interaction is increasing. However,

TABLE 1: Backbone Torsion Angles and Conformational Energies of Hypericin-linked Dipeptide Amides[a]

Torsion Angle	Glu (OHyp)-Leu-NHMe		Glu (OHyp)-Phe-NHMe		Glu (OHyp)-Asp-NHMe		Glu (OHyp)-Tyr-NHMe	
	HB	E	HB	E	HB	E	HB	E
0	−85	−66	−84	−66	−84	−66	−84	−66
X_1	−56	−80	−57	−80	−57	−80	−57	−80
X_2	−126	−167	−125	−170	−126	−170	−125	−170
X_3	126	147	122	145	123	146	122	146
X_4	−63	−55	−62	−55	−62	−54	−62	−54
X_5	127	−179	127	−178	127	−178	128	−178
ψ_1	−153	−155	−150	−156	−150	−156	−149	−155
ω_1	177	180	179	180	178	180	177	180
ϕ_2	−76	−151	−76	−151	−77	−151	−76	−151
ψ_2	68	142	67	150	67	149	67	149
ω_2	174	180	175	180	175	180	175	180
X_6	−172	−177	−178	−177	−171	−178	−176	−175
X_7	73	68	67	83	84	88	65	89
Conformational Energy[b]	−26.3	−26.7	−28.1	−28.2	−29.1	−30.3	−27.2	−28.2

[a]HB and E denote the hydrogen bonded and extended conformations, respectively. [b]Interaction energies in kcal/mol.

TABLE 2: Components of Conformational Interaction Energies of Hypericin-linked Dipeptide Amides [a]

Components of Energy	Glu (OHyp)-Leu-NHMe		Glu (OHyp)-Phe-NHMe		Glu (OHyp)-Asp-NHMe		Glu (OHyp)-Tyr-NHMe	
	HB	E	HB	E	HB	E	HB	E
E_{el}	13.8	−1.1	14.7	−1.2	11.6	−4.7	15.3	−1.2
E_{pol}	−3.0	−2.3	−3.2	−2.3	−3.3	−2.3	−3.0	−2.3
E_{nb}	−6.0	−3.6	−7.7	−4.3	−6.0	−3.0	−7.7	−4.4
E_{tor}	10.5	1.2	10.0	0.9	10.1	0.9	10.1	0.9
E_{hb} [b]	−41.6	−20.9	−41.9	−21.3	−41.5	−21.2	−41.9	−21.2
E_{tot}	−26.3	−26.7	−28.1	−28.2	−29.1	−30.3	−27.2	−28.2

[a] Values of energies in kcal/mol. [b] Electrostatic, polarization, and nonbonded energies between proton and proton-acceptor are not separately calculated because these interaction energies are implicitly included in E_{hb}; in this work E_{hb} means the total interaction energy between proton and proton acceptor involved in hydrogen bond.

TABLE 3: Hydrogen-bonding Geometry and Energy of Hypericin-linked Dipeptide Amides[a]

Dipeptide Amide		Hydrogen-bonded Atoms	Hydrogen-bonding Distance	Hydrogen-bonding Energy[b]
Glu (OHyp)-Leu-NHMe	HB	H_4.....O_2= =C_2'	1.79	−24.2
	E	C_1'= =O_1.....H_3	1.87	−17.4
		C_7= =O_7.....H_1^2	1.84	−20.9
Glu (OHyp)-Phe-NHMe	HB	H_4.....O_2= =C_2'	1.78	−24.3
	E	C_1'= =O_1.....H_3	1.84	−17.6
		C_7= =O_7.....H_1^2	1.79	−21.3
Glu (OHyp)-Asp-NHMe	HB	H_4.....O_2= =C_2'	1.79	−24.1
	E	C_1'= =O_1.....H_3	1.88	−17.4
		C_7= =O_7.....H_1^2	1.81	−21.2
Glu (OHyp)-Tyr-NHMe	HB	H_4.....O_2= =C_2'	1.75	−24.4
	E	C_1'= =O_1.....H_3	1.86	−17.5
		C_7= =O_7.....H_1^2	1.81	−21.2

[a] Notations for atoms refer to Figures 1 and 2; distance, A; energy, kcal/mol. [b] In hydrogen-bonding energy, electrostatic, polarization, and nonbonded energies between proton and proton-acceptor are implicitly included; in this work hydrogen-bonding energy means the total interaction energy between proton and proton-acceptor involved in hydrogen bond.

the repulsive electrostatic energies between a nitrogen atom of amino group of glutamate and oxygen atoms of carbonyl groups of dipeptide amide backbone are more increased due to an intra-residue hydrogen bond of the backbone. Subsequently, the overall contribution of an intra-residue hydrogen bond to the total stabilization energy of the HB conformation is not significant. Nevertheless, this intra-residue hydrogen bond plays an important role in the formation of HB conformation. In addition, these intramolecular hydrogen bonds of the HB conformation bring the increase of torsional strain energies. In the E conformation, the unstability due to the repulsive energies between the atoms of peptide residue is decreased and the contribution of hydrogen bond to the total interaction energy is relatively significant. The freely extended conformation of dipeptide amide backbone brings the decrease of torsional strain.

However, it is believed that the HB conformation may be more favorable than the E conformation for the peptide with longer chain linked to hypericin due to increasing the number of hydrogen bonds between hydroxyl groups of hypericin and carbonyl groups of peptide backbone. Based upon this assumption, the $O_{4'}$-linked type of conformation was ruled out in this work. It is well known from spectroscopic and theoretical studies that the OH bond is weakened through the formation of hydrogen bond with proton-acceptor atom.[16] Thus, the intramolecular hydrogen bonds between carbonyl groups of peptide backbone and hydroxyl groups of hypericin may facilitate the proton release from the weakened hydroxyl bonds of hypericin upon photoexcitation, which is in good agreement with experimental results.[1]

The hydrogen-bonding geometries and energies of hypericin-linked dipeptide amides are shown in Table 3. The energy minimum conformations of model compounds are depicted in Figure 3.*

Effect of Amino Acid Residues on the Conformation of Hypericin-Dipeptide Amides. To investigate the effect of amino acid residues on the conformation of hypericin-dipeptide amides, we compared the conformational energies of Glu(OHyp)-NHMe, where X = Leu, Phe, Asp, and Tyr (shown in Table 1). From the results, it is known that there are no large differences in the conformational energies of the four hypericin-dipeptide amides, and that only the small structural differences are found in torsion anglex χ_6 and χ_7. This means that amino acid residues of dipeptide amides do not influence

*The minimum energy structures of hypericin-dipeptide amides were obtained from using APPLE II PC Graphics, and a BASIC listing of the program used for graphics is available from the authors upon request.

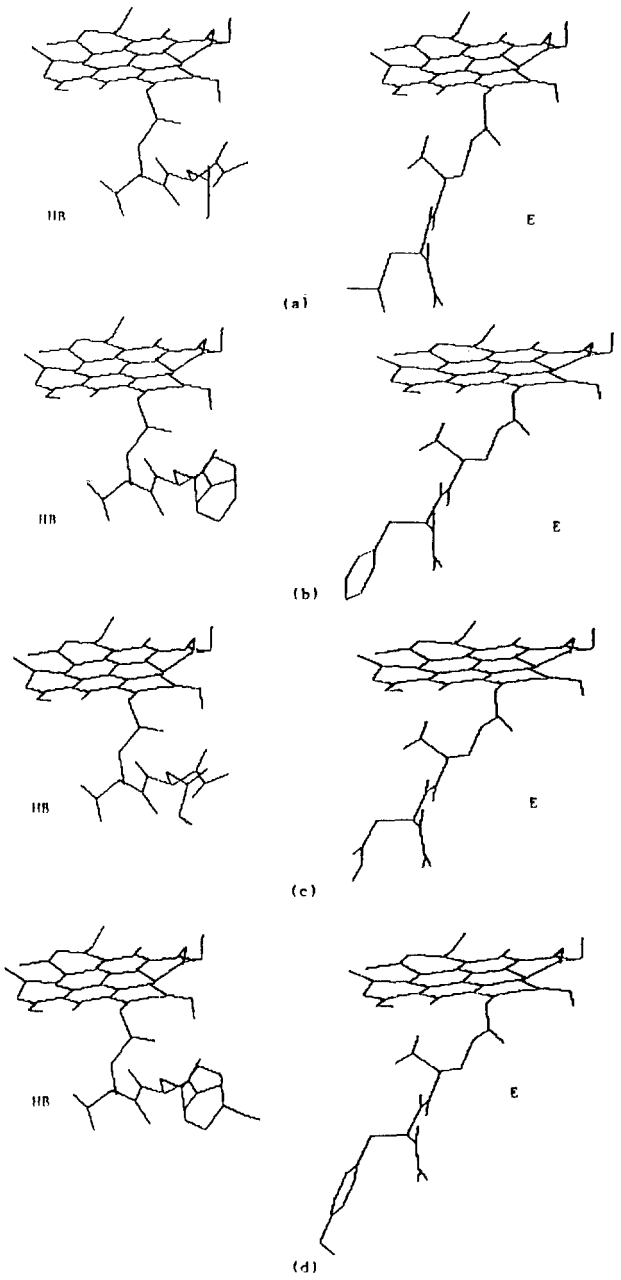

Figure 3. Minimum energy structures of hypericin-linked dipeptide amides: (a) Glu(OHyp)-Leu-NHMe; (b) Glu(OHyp)-Phe-NHMe; (c) Glu(OHyp)-As-NHMe; (d) Glu(OHyp)-Tyr-NHMe; for clarity, hydrogen atoms not involved in hydrogen bonds are not shown.

critically the conformations of the hypericin-dipeptide amides. However, we can not rule out effects of amino acid residues of polypeptide on the conformation of stentorin, which have not been studied yet. Analyzing hydrogen-bonding geometries and energies of dipeptide amides shown in Table 3, a similar conclusion can be obtained.

The stabilities of the HB and E conformers do not also significantly depend on the amino acid residues linked to chromophore. However, the conformers having hydrophilic amino acid residues are found to be somewhat more stable than those of having hydrophobic ones.

Conclusions

The results of conformational energy calculation using the empirical potential function of 4 hypericin-linked dipeptide amides lead to the following conclusions.

(1) The conformations of dipeptide amides are predominantly influenced by intramolecular hydrogen bonds between hydroxyl groups of hypericin and carbonyl groups of peptide backbone.

(2) The increase of acidity of hypericin upon photoexicitation may be facilitated by the formation of these intramolecular hydrogen bonds.

(3) Amino acid residues of dipeptide amides do not significantly influence the conformations of hypericin-linked dipeptide amides.

Although a further study on the evaluation of entropy changes due to the conformational changes of dipeptide amides and conformational energy changes due to environmental effects is needed, we obtained somewhat qualitative results on the binding nature between chromophore and peptide in the *Stentor* photoreceptor.

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