Lipophilic Peptide as Novel Building Material of Molecular Sensor

Toshiharu Kuboyama, Beena Mathew, Shigeo Nakamura, and Makoto Takagi*
Department of Chemical Systems and Engineering, Graduate School of Engineering, Kyushu University, Higashi-ku, Fukuoka 812-8581

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Lipophilic peptide derivatives **1** - **3** proved to complex with amines in chloroform in 1:1 ratio allowing distinct color change. Peptide **3** as compared with **2** indicated strikingly greater binding affinity, suggesting the importance of subtle modulation in hydrogen-bonded secondary structure of peptide chains in non-polar solvent.

Molecular recognition plays vital roles in biological systems such as signal transduction by receptor, substrate recognition by enzyme, antigen capture by antibody, and so on. The process is aided by three-dimensionally controlled hydrogen bonding. A great number of chemists are recently involved in constructing the mimic systems through preparation of supermolecules starting from crown ethers, calixarenes, etc. up to recent molecular clefts.[1,2]

On the other hand, a combinatorial chemistry has been highlighted over the last decade.[3] The approach incorporates the concept of biological evolution, taking advantage of selecting optimum compounds from a library, a large pool of possible candidate compounds. Since the library is usually prepared by combining structural units like amino or nucleic acids, elaborate molecular design is not necessary in contrast to conventional supramolecular approach.

We have focused on amino acid as structural element in constructing host compounds for molecular sensing. Lipophilic peptide derivatives were prepared as model which were equipped with two functional sites: (1) recognition site which is constructed by amino acids, (2) reporting site designed to convert the binding between host and guest into optical signal. A long alkyl chain was incorporated into peptide to provide solubility or affinity to nonpolar organic media. The lipophilic property is desirable for most applications of sensory molecule such as ion selective electrode and optical fiber.

Compounds **1** - **3**[4] in Figure 1 were prepared by coupling N-terminally deprotected amines with 5-nitro-2-coumaranone.[5] Mixed anhydride method[6,7] was adopted in solution for peptide bond synthesis.

The complexation behavior of peptides **1** - **3** with amines was evaluated spectrophotometrically. The chloroform solution (3 cm^3) containing 8.3×10^{-6} M peptide was stirred in an optical cuvette at 25 °C. The solution was titrated with concentrated amine in chloroform, and the spectrum was measured after each addition of the amine solution. The amines used in this study were *n*-propylamine, *n*-butylamine, *n*-hexylamine, 1,3-diaminopropane, 1,4-diaminobutane, 1,6-diaminohexane, *N*,*N*-dimethyl-1,3-diaminopropane, *N*,*N*'-dimethyl-1,3-diaminopropane, *N*,*N*,*N*',*N*'-tetramethyl-1,3-diaminopropane, spermidine, and spermine.

The peptides **1** - **3** on amine addition revealed spectral change derived from proton dissociation from *p*-nitrophenol in aqueous solution on raising pH. Peptides **2** and **3** were especially featured

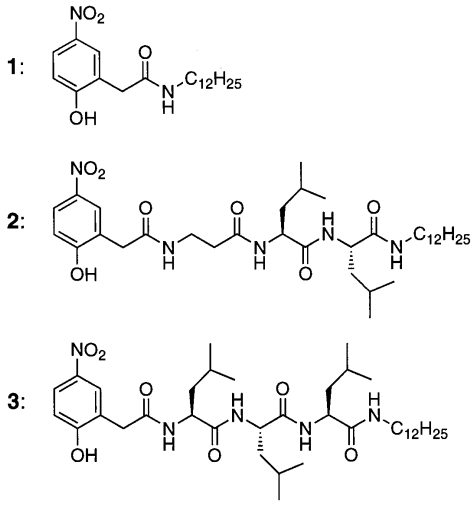

Figure 1. Structure of control and lipophilic peptides **1** - **3**.

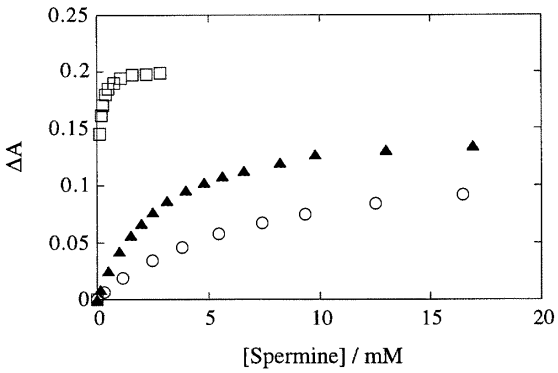

Figure 2. Absorbance change as a function of spermine concentration at a maximum absorption wavelength of complex.
Temperature 25 °C; 8.3×10^{-6} M **1** (○), **2** (▲), and **3** (□) in chloroform. The absorption maxima of free hosts were at 406.8 nm, 415.4 nm, and 412.6 nm, and isobestic points appeared at 348.0 nm, 350.2 nm, and 350.2 nm for **1**, **2**, and **3**, respectively.

with a well-defined isosbestic point in a wide range of peptide to amine ratio in solution, indicating the formation of single species, e.g., 1:1 complex. This suggestion was also in accord with a preliminary ^{1}H-NMR study on **3**-spermine system in choloroform (data not shown). The absorbance trace for complex formation between peptide host **1** - **3** and spermine are shown in Figure 2.

When 1:1 complex is formed between peptidic dye (HL) and amine, the following formulation can be derived for spectrophotometric data analysis.

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$$\mathrm{HL} + \mathrm{Amine} \overset{K_{ass}}{\rightleftarrows} \mathrm{Complex} \quad (1)$$

$$\Delta A = \frac{\Delta A_{\infty} K_{ass}[\mathrm{Amine}]}{1 + K_{ass}[\mathrm{Amine}]} \quad (2)$$

where K_{ass} is the association constant, ΔA is the apparent absorbance change, and ΔA_{∞} stands for the difference between initial and saturated absorbance. The value of K_{ass} was determined from the plot of ΔA against [amine] (free amine concentration) using a non-linear least-square program. The calculation resulted in excellent fit to eq (2), supporting the validity of only 1:1 complex formation. The constants obtained are summarized in Table 1.

Table 1. Association constants (K_{ass}) of lipophilic peptides **1** - **3** for various amines in chloroform

Amines	K_{ass} (M^{-1}) [a]		
	1	**2**	**3**
n-Propylamine	4.6	6.2	440
n-Butylamine	8.1	8.6	460
n-Hexyllamine	1.0	8.2	550
1,3-Diaminopropane	17	38	2400
1,4-Diaminobutane	16	63	4400
1,6-Diaminohexane	4.3	13	880
N,*N*-Dimethyl-1,3-diaminopropane	15	26	2100
N,*N*′-Dimethyl-1,3-diaminopropane	75	140	6100
N,*N*,*N*′,*N*′-Tetramethyl-1,3-diaminopropane	7.3	4.4	92
Spermidine	76	230	13000
Spermine	140	400	20000

[a] All K_{ass} values were calculated within $R^2 > 0.98$ (R: correlation coefficient).

Several unique features of the present host-guest complexation are found from Table 1. (i) Association constants of **2** (with four amide functional groups) with amines are higher than that of **1** (one amide group), but the difference is only several times at the greatest. On the other hand, the binding abilities of **3** (four amide groups) are strikingly greater than **2** by 20–80 times. This surprising difference between **2** and **3** was caused by only a single substitution of amino acid between β-alanine and leucine. (ii) Association constants tend to be larger as the number of amino acid residues in the host increases. (iii) Association constants become greater as the number of amino residues in the guest amine increases. This is exemplified by roughly 100 times increase on going from *n*-propylamine to spermine (tetramine). (iv) Primary and secondary amines are more preferable than tertiary amines as guest molecule. Thus, the K_{ass} value of **3** to *N*,*N*′-dimethyl-1,3-diaminopropane exceeds 6000 M^{-1}, while that of *N*,*N*,*N*′,*N*′-tetramethyl-1,3-diaminopropane is only 78 M^{-1}. Triethyamine, one of the simplest tertiary amines, interacted with **1**–**3** only negligibly as compared to those lower monoamines in the table (data not shown). (v) Inspecting 1,3-diaminopropane, 1,4-diaminobutane, and 1,6-diaminohexane as guest, there seems to exist an optimum methylene chain length connecting the two amino residues in order for them to be effectively complexed.

All the unique molecular recognition features mentioned above are most likely associated with hydrogen bonding interactions involving peptide residues and operating intramolecularly (before complexation) and intermolecularly (after complexation). In the present study on model sensory dyes, the structure of reporter group was kept the same as *p*-nitrophenylmethyl residue so that its interaction with guest amines should stay unaltered, i.e., *p*-nitrophenylmethyl group, as a monobasic proton-donating group, was supposed to interact stoichiometrically with a single amino group in the guest amine molecules. However, the increase in the number of (primary and secondary) amino groups in the guest molecule steadily strengthened the interaction. Moreover, a seemingly unimportant change in single amino acid in tripeptide from β-alanine to leucine caused a striking change in host-guest binding affinity. All these emphasize the vital role of a peptide chain that works as a supramolecular entity in recognizing the structure of guest molecule.

Unfortunately, no systematic study has been made to date on the secondary structures of peptides (the conformation of peptide chain) in nonpolar organic medium. Obviously a great deal of study is needed to understand the experimental findings made here. On the other hand, from technological point of view, we consider that the present preliminary observation is important enough to encourage the future research development leading to a new family of molecular hosts and the associated molecular sensors. It is because that the technique is based on simple peptide chemistry, and that it should allow the use of combinatorial approach in quite a straightforward manner.

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Reference and Notes

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