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Nucleotide Recognition in Aqueous Media with Artificial Receptor Based on Porphyrin

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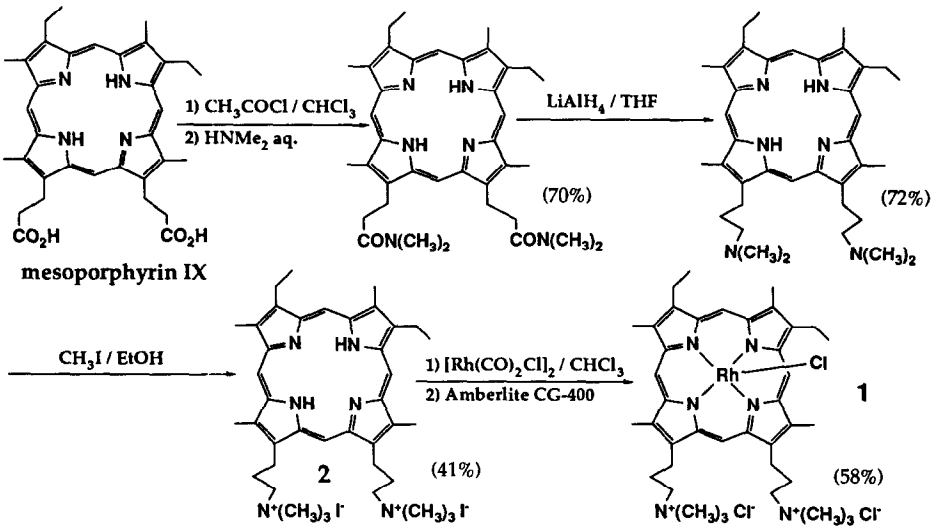
Key Words ; porphyrin, nucleotite, morecular recognition, Coulombic interaction

Abstract *; Molecular recognition of nucleotides using a Rh(III) complex of porphyrin bearing two ammonium moieties was investigated. The new receptor bound nucleotide monophosphate except UMP in an aqueous buffer solution with appreciable binding constants. The association constants showed significant pH dependencies, which suggested that present molecular recognition involved a Coulombic interaction in addition to coordination of nucleobases on the Rh atom.*

Molecular recognition of nucleosides and their related compounds is one of most intensively investigated subjects in the biomimetic chemistry.[1] In order to mimic nucleic acid recognition, various types of host-guest systems have been developed and some of these systems indeed exhibit remarkably specific recognition toward nucleobase and their related compounds via multiple hydrogen bonding. [2]

Recognition behavior of these artificial hosts has been sometimes investigated by using organic aprotic solvents. The nonpolar environment provided by these solvents around the host molecules makes the hydrogen bond interaction (and other polar interactions) most effective. In contrast, molecular recognition of relatively polar host molecules in an *aqueous solution* is expected to encounter serious difficulties because of existence of severe competitive interference by the solvent molecules. There may be two different tactics to overcome these difficulties, i.e., a) employment of molecular design which make it possible to encapsulate binding sites and isolate them from the solvent, b) introduction of a new recognition element which may be more effective and specific than the interfering interaction with the solvent molecules. Recently, we found that nucleobase coordinate on porphyrin metal complexes,[3] among which a rhodium complex showed extremely large association constants for nucleobases of 10^7 M^{-1} order in an organic solvent.[3a] Based on these results, we prepared a rhodium complex of a new water soluble porphyrin and examined it as the host designed according to the second tactics mentioned above. The results indicate that the porphyrin Rh(III) complex effectively recognizes nucleotides in an aqueous solution and the Coulombic interaction acts as a cooperative recognition element.

The water soluble porphyrin, **1**, used in this work is prepared from mesoporphyrin IX according to Scheme 1. After metalation of the porphyrin bearing diammonium groups, **2**, with $[Rh(CO)_2Cl]_2$,[4] the product was purified on Amberlite CG-500 ion exchange column and recrystallized from EtOH/hexane to afford the desired host porphyrin Rh(III) complex.[5] Both



Scheme 1. Preparation of new water soluble porphyrin (yields are given in parentheses)

of **1** and **2** are soluble in aqueous buffer solutions at wide range of pH (4 - 10). Interestingly, the electronic spectra of **2** in an aqueous solution showed a concentration dependency, i.e., a significant blue shift and broadening which indicate formation of dimeric form of **2** even at μM order of concentration.[6] The electronic spectra of **1**, however, exhibits no sign of such dimerization in an aqueous solution.

On addition of dAMP to the aqueous solution of **1**, the Soret band of **1** shows a significant red shift and decrease of the absorbance with clear isosbestic point as shown in Figure 1. The same behavior was also observed for other nucleotides (GMP and CMP) except UMP which have no basic (coordination) point in the nucleobase part. These results are quite similar to those observed previously for nucleobase recognition in an organic solvent[3a] and strongly indicate that nucleotides coordinate on the Rh atom to form 1 : 1 complexes in the present aqueous buffer solution.[7] Usual Benesi-Hildebrand analysis of the spectral change shown in Figure 1 gives the association constant between **1** and dAMP to be 860 M^{-1} at pH 7.10.[8] The most

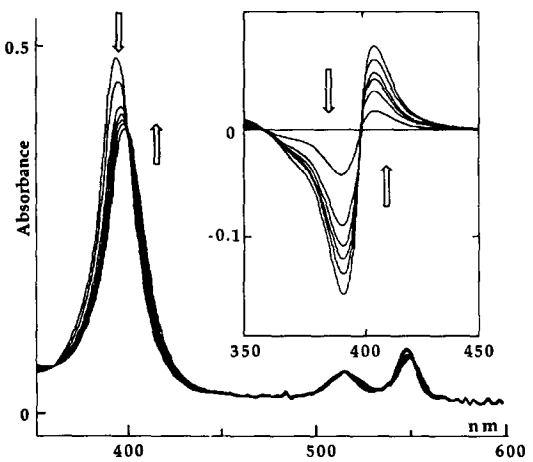


Figure 1. Spectral change of **1** upon addition of dAMP in a 13 mM phosphate buffer solution (pH 7.1) at 15 °C. The concentration of **1** was 5 μM and those of dAMP were 0, 0.5, 1.0, 1.5, 2.0, 3.9, and 8.3 mM, respectively.

The inset shows different specta of the Soret rgion obtained from the same data set. Clear isosbestic points are observed at 360 and 401nm .

interesting aspect of present nucleotide recognition is its pH dependence, which will suggest important information on intermolecular interactions operating in observed complexation. Thus, association constants between **1** and dAMP were determined in the range from pH 5 to pH 9. Association constants for ADP bearing diphosphate and adenine deoxyriboside (dA) having no phosphate were also measured for purposes of comparison. The results are summarized in Figure 2. It is clear that the association constant for dAMP shows a sigmoidal dependence of association constants upon pH of the solution, while those for ADP and dA are practically independent of pH. Assuming a following simplest model where monoanionic and dianionic forms of dAMP interact with **1** independently, a nonlinear least-squares

$$dAMP^- \overset{K_a}{\rightleftharpoons} dAMP^{2-} + H^+$$

$$\mathbf{1} + dAMP^- \overset{K_1}{\rightleftharpoons} \mathbf{1}\bullet dAMP^- \quad , \quad \mathbf{1} + dAMP^{2-} \overset{K_2}{\rightleftharpoons} \mathbf{1}\bullet dAMP^{2-}$$

analysis of data in Figure 2 results in $K_1 = 530 \pm 50\ M^{-1}$, $K_2 = 1030 \pm 50\ M^{-1}$ and $pK_a = 6.9 \pm 0.1$, respectively. It should be noted that the pK_a value of AMP thus obtained agrees reasonably with the known value ($pK_a = 6.67$ at 25 °C) for second proton dissociation of the phosphate group in dAMP obtained by an acid-base titration.[9] These results strongly indicate that the present dAMP recognition contains not only the coordination interaction but also the Coulombic interaction between the phosphate anion of dAMP and the ammonium cation of **1**.[10] The conclusion consists with the pH-independent behavior of the association between dA and **1**. Assuming that the increase of the association constant from K_1 to K_2 is due to the increase of the anionic charge in AMP, the free energy change for the Coulombic interaction between a single anion and cations in the present nucleotide recognition is roughly estimated to be -0.4 kcal/mol ($-RT \ln (K_2 / K_1)$). Furthermore, if the same order of the Coulombic interaction may operate in the complex of $dAMP^-$ with **1**, the free energy change for the coordination of the adenine group on Rh atom of **1** is also estimated to be -3.2 kcal/mol ($-RT \ln K_1 + 0.4$) which corresponds to 280 M^{-1} of the association constant. The estimated value for coordination shows fairly good agreement with the observed association constant for dA. Interestingly, the association constants of ADP are also practically independent of pH, which

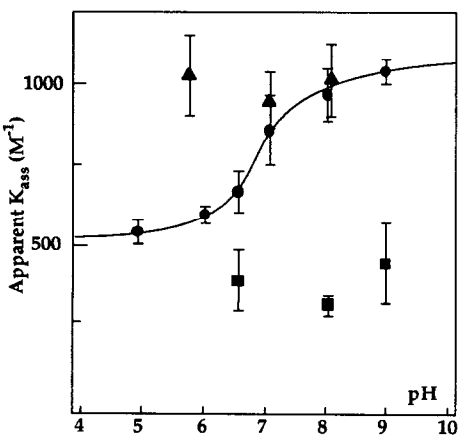


Figure 2. Plot of the K_{ass} between nucleoside derivatives and **1** versus pH in 13 mM phosphate buffer at 15 °C. ■ ; adenine deoxyriboside (dA), ● ; dAMP, ▲ ; ADP.
The solid line shows the theoretical pH dependence of K_{ass} obtained from the following equilibrium constants (see text) ; $pK_a = 6.9$, $K_1 = 530\ M^{-1}$ and $K_2 = 1030\ M^{-1}$.

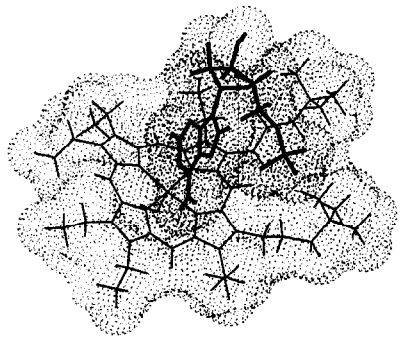


Figure 3. One of possible structures of the 1•dAMP optimized by use of Driding II force field (Molecular Simulation Inc.). Dots shows the solvent accessible surface.

suggests that the third additional anion on ADP (pK_{a_3}=7.20) [9] generating in the present pH range does not contribute to the present nucleotide recognition. These results indicate that the full recognition of the present system consists of coordination of the nucleobase on the Rh atom in **1** and the Coulombic interaction between two cations on **1** and two anion on AMP (Figure 3). Thus, the Coulombic interaction, though its absolute energy gain is not so large, may effectively assist the molecular recognition of nucleotides by the small receptor molecule in an aqueous solution, if the receptor has other strong recognition element such the coordination on the Rh atom in the present case.[11]

Reference and Note

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4) Aoyama, Y. ; Yoshida, T. ; Sakurai, T. ; Ogoshi, H., *Organometallics*, **1986**, *5* 168.

5) **2** ; ^{1}H NMR ($CDCl_3/CD_3OD$, 90 MHz) δ 9.95 (s, 4H), 4.3 - 3.8 (m, 16H), 3.45 (s, 12H), 2.95 (s, 18H), 1.65 (t, J=9Hz, 6H). *Anal.* Calcd. for $C_{40}H_{58}N_6I_2 \cdot H_2O$: C, 53.69; H, 6.76; N, 9.39. Found : C, 54.00; H, 6.86; N, 9.38. **1** ; ^{1}H NMR (DMSO-d_6, 90 MHz) δ 10.50 - 10.00 (m, 4H), 4.50 - 3.85 (m, 16H), 3.85 - 3.50 (m, 12H), 3.15 (s, 18H), 1.93 (t, J=8Hz, 6H). UV-VIS (MeOH) λ_{max} (log ε) 396 (5.00), 516 (3.98), 548 (4.22). FAB-MASS m/e 758 (M-2Cl), 723 (M-3Cl).

6) For example, see Hambright, P. In *Porphyrin and Metalloporphyrins* ; Smith, K. M., Ed.; Elsevier Scientific Publishing : New York, 1975; Chapter 6. The association constant for dimerization of **2** is estimated to be 1.3×10^6 M^{-1}.

7) The X-ray crystallographic data for the complex of OEP•Rh with 9-ethyladenine indicate coordination of the adenine moiety at the N1 position, see the doctoral thesis of H. Hatakeyama, Kyoto University, 1992.

8) The association constants of **1** with GMP and CMP are determined to be 560 ± 50 M^{-1} and 150 ± 30 M^{-1} at pH 8.22, respectively.

9) *Handbook of Biochemistry* , Sober, H. A., Ed., Chemical Rubber Co. ; Cleaveland, 1970.

10) Aoyama, Y. ; Nonaka, S. ; Motomura, T. ; Toi, H. ; Ogoshi, H., Chem Lett., **1991**, 1241.

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