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A Selective Optode Membrane for Histidine Based on Fluorescence Enhancement of Meso−Meso-Linked Porphyrin Dimer

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A plasticized polymer film, poly(vinyl chloride) (PVC) incorporated with a specific porphyrin dimer, is shown to exhibit significant and analytical usefulness for optical response toward histidine. The porphyrin dimer containing a free-base porphyrin and a covalently linked metalloporphyrin is shown to be weakly fluorescent as a result of the photoinduced intramolecular electron transfer from the inner free-base porphyrin in singlet excited state to a low-spin cobalt(II). The fluorescence enhancement of the membrane by histidine is based on favorable extraction of histidine into the bulk organic membrane and complexation with the inner metallopophyrin moiety and inhibiting the electron transfer process. With the optode membrane described, histidine in a sample solution from 0.0045 to 1.53 mM can be determined. The calibration curve of the optode membrane for histidine shows a good correlation with the mathematically derived formalism and, thus, confirms the theoretically expected behavior. The sensor presented exhibits high selectivity toward histidine over several amino acids and common inorganic anions. The optical selectivity coefficients obtained for histidine over other biologically relevant amino acids and anions are shown to meet the selectivity requirements for the monitoring concentration levels of histidine in biological samples. The selective characteristic of the sensor has been discussed in the view of the coordination chemistry of metalloporphyrin.

The direct and selective analysis of minute quantities of biologically important compounds is particularly demanding, since it requires the specific recognition of particular element in the presence of closely related species. The recent rapid advance in genetic engineering and biotechnology and the need for nutritional assessment necessitate the development of a method for determination of trace concentrations of amino acids. Detection of amino acids in physiological fluids in the diagnosis of certain metabolic disorders is one of the important applications of such analysis.[1−4] Many analytical methods for amino acids have been developed so far. The most widely used techniques for quantification of amino acids are liquid chromatographic methods.[5,6] Unfortunately, they are not suitable for monitoring the analytes on-line. Recently, an electrode for histidine that is based on PVC membrane of Mn(TPP)Cl[7] was proposed; however, it is easily affected by the fluctuation in electric potential or electric field. There is still a significant need for the genesis of a new optical chemical sensor (optode) for amino acids, because the optode can be miniaturized, can be manufactured at low cost, and are quite safe. Analytical detection using an optical chemical sensor can be performed by connecting a chemically sensitive layer with a transducer device. The sensing material determines the main features of the device, such as sensitivity, selectivity, and dynamic parameters. So far, a few optical chemical sensors for direct determination of amino acids have been reported by Fabrizzi et al.,[8] Preuschoff et al.,[9,10] and Wolfbeis et al.[11]

The major challenge for the application of optical chemical sensors for the detection of amino acids is the lack of photochemically sensing materials that fulfill the requirements of recognition of the analytes and expression of the relation to the recognition by optical signals. One of the most important recognition elements in optical sensors involves the specific metal−ligand interactions.[12] Such interactions have been used in the development of anion-selective optical sensors based on different ionophores and chromophores.[13−15] Among them, metalloporphyrins are attractive condidates in light of their roles in biological systems. The specific interaction between metalloporphyrins and biological compounds makes the biological functions of them important. A naturally occurring porphyrin is vitamin B12, which contains the cobalt(II) cation.[16] Synthetic metalloporphyrins have

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attracted attention in relation to chemical or biological molecular recognition;[17] however, they were mainly confined to those of electrochemical[18−20] or absorption measurements.[21,22] To the best of our knowledge, the only transition metal coordination compound of this kind that has been employed as a fluorescent sensor is a porphyrin zinc derivative.[23] On the other hand, molecular fluorescence spectroscopy is one of the most important spectroscopic techniques, since it can can have an enormously lower limit of detection and more intense response signals than absorbance spectroscopy. Furthermore, the fluorescent sensors provide a means to significantly reduce the required membrane volume while retaining the same level of the signal-to-noise ratio.

There are numerous mechanisms by which fluorescence signal transduction may be affected. One of the most frequently used systems to vary fluorescence intensity is the photoinduced electron transfer (PET) mechanism.[24−27] Typical PET sensors are designed by following a two-component approach, tha is, by covalently linking a receptor subunit displaying selective affinity toward the envisaged substrate to a fluorescent fragment. The concept of PET sensor has been exploited by de Silva, Shinkai, and others for spectroscopic sensing of H^+,[25,28] metal cations,[29,30] anions,[26,31] and neutral analytes.[27,32]

The success of the above outlined modular approach prompted us to design a two-component fluorescent sensor for amino acids. In a previous report, it was demonstrated that porphyrin cobalt(II) displays a selective affinity toward histidine via a strong metal ion−nitrogen ligation reaction.[33] In the present work, we make the first attempt to design and use a porphyrin-dimer-based fluorescent sensor for selective histidine detection that does not require any chemical pretreatment of the analyte. The design of the approach consists of *meso*-tetraphenylporphyrin cobalt(II) (CoTPP) as a histidine-bonding site and a covalent attachment of *meso*-tetraphenylporphyrin(TPP) as a fluorescent site, where the cobalt(II)−nitrogen ligation reaction is responsible for the enhancement of the fluorescence emission of the fluorescent site. The sensor was constructed by immobilizing the sensing material in a plasticized PVC membrane, and the resulting optode membrane was characterized in terms of sensitivity, selectivity, and the principle of operation.

EXPERIMENTAL SECTION

Reagents. Bis(2-ethylhexyl) sebacate (BOS), high relative molecular mass poly(vinyl chloride) (PVC), and tetrahydrofuran (THF) were purchased from Fluka (Switzerland) and used for membrane preparation. Other chemicals were of analytical reagent grade and were used without further purification. Buffer solution (pH 8.0) was prepared by dissolving 0.1 mol of Tris-(hydroxymethyl)amino methane (Tris) in 1000 mL of water and then adjusting the pH with 0.1 M HCl. All solutions were prepared with twice-distilled water.

A stock solution of 0.2 M histidine [2-amino-3-(4-imidazolyl)-propionic acid] was prepared by dissolving L-histidine in water. Solutions of 0.2 M interferences were prepared by dissolving the appropriate amount of each compound in water. Working solutions were prepared by successive dilution of the stock solutions with water. All of the working solutions were buffered at pH 8.0 using Tris/HCl buffer solution.

Synthesis of the Porphyrin Dimer (4) (Figure 1). *5-(4-Hydroxyphenyl)-10,15,20-triphenylporphyrin (**1**)*.[34] Benzaldehyde (708 mg, 66.7 mmol) and 4-hydroxybenzaldehyde (482 mg, 33.4 mmol) were dissolved in 250 mL of propionic acid. The mixture was brought to reflux, and 6.92 mL of pyrrole (100 mmol) was added within 15 min. The resulting black solution was refluxed for 30 min, then cooled to room temperature. After the addition of 100 mL of methanol, the resulting solution was allowed to stand for 24 h. Filtration through a coarse sintered glass funnel, followed by methanol washes and drying at 120 °C, gave 960 mg of blue crystals. This mixture of porphyrins was dissolved in 5 mL of chloroform and loaded onto a 4 × 40 cm column of ∼100−200-mesh silica gel then eluted with 10:1 chloroform/ethanol(v/v). The first band was *meso*-tetraphenylporphyrin, and the second was the desired monohydroxyphenylporphyrin **1**. ^{1}H NMR(400 MHz, $CDCl_3$, relative to TMS): δ ppm 8.84−9.01 (d, 8H, β pyrrole), 8.212 (d, 6H, *ortho*-H of triphenyl), 7.768 (m, 9H, *meta*/*para*-H of triphenyl), 8.08 (d, 2H, *ortho*-H of hydroxyphenyl), 7.217 (m, 2H, *meta*-H of hydroxyphenyl), −2.784 (s, 2H, pyrrole NH). IR (KBr, cm^{-1}): 3423 (ν_{OH}), 3313 (ν_{NH}), 1595 ($\nu_{C=C}$), 1258 (ν_{C-O}).

*5-(4-Bromohexyloxyphenyl)-10,15,20-triphenylporphyrin (**2**)*.[35] To a solution of 700 mg of 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin (**1**) in 80 mL of dry dimethylformamide (DMF), 1.2 g of 1,6-dibromohexane and 1.0 g of K_2CO_3 were added. The mixture was refluxed 6 h, and the tarry solution was allowed to cool and stand for 24 h. A dark solid was collected by filtration, washed successively with methanol and water, and dried. The resulting product was purified by flash column chromatography (silica gel, ∼100−200 mesh, elute, chloroform). After the removal of the solvent on a rotary evaporator, a red solid was obtained in 47% yield. ^{1}H NMR(400 MHz, $CDCl_3$, relative to TMS): δ ppm 8.860−9.014 (d, 8H, β pyrrole), 8.202 (d, 6H, *ortho*-H of triphenyl), 8.101 (d, 2H, *ortho*-H of bromohexyloxyphenyl), 7.774 (m, 9H, *meta*/*para*-H of triphenyl), 7.26 (m, 2H, *meta*-H of bromohexyloxyphenyl), 4.259 (m, 2H, OCH_2), 1.253−3.743 (m, 10H, CH_2), −2.805 (s,

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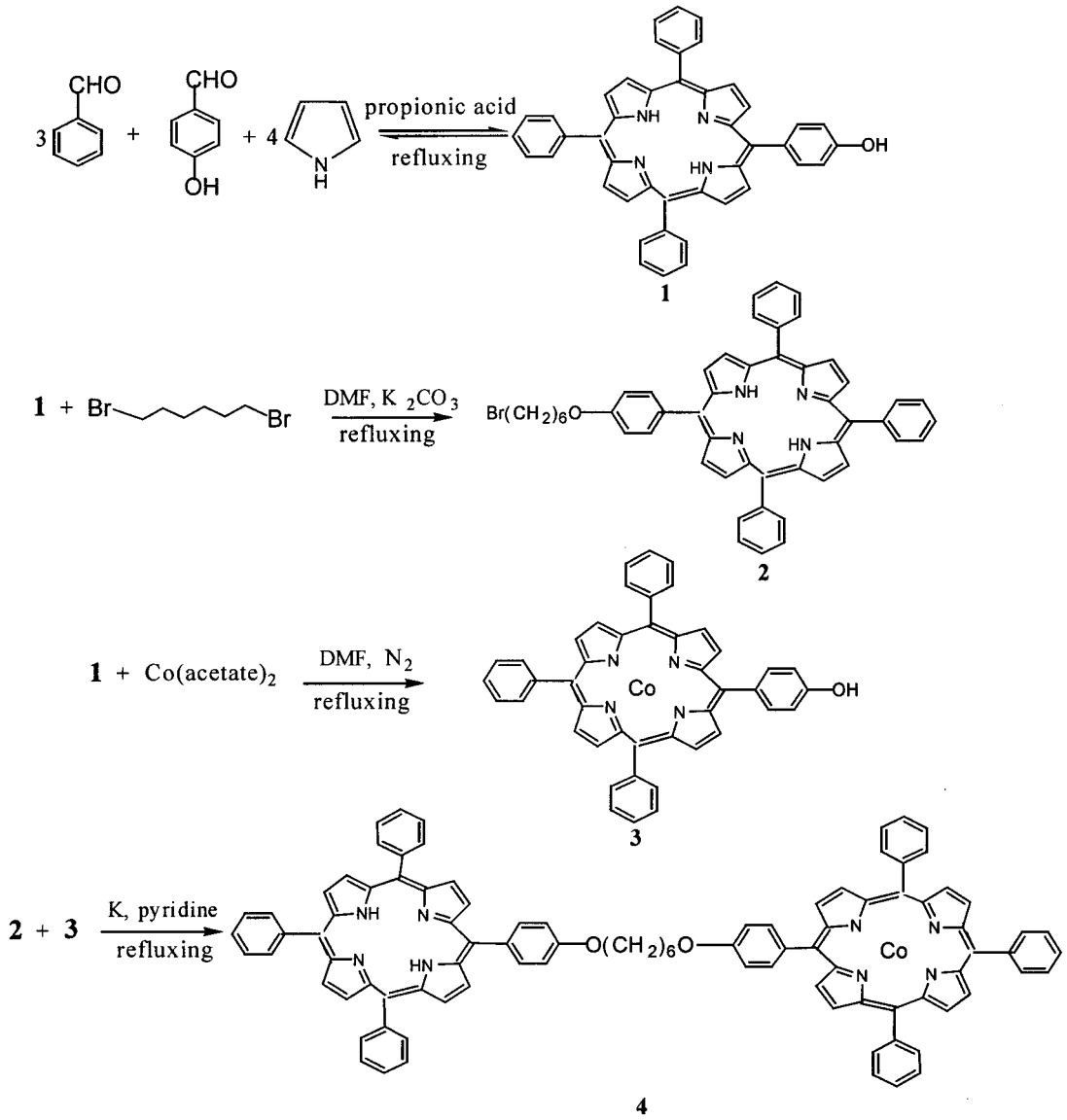

Figure 1. Synthesis of the porphyrin dimer.

2H, pyrrole NH). IR (KBr, cm^{-1}): 3383 (ν_{N-H}), 2858 (ν_{C-H}), 1595 ($\nu_{C=C}$), 1243 (ν_{C-O-C}).

*5-(4-Hydroxyphenyl)-10,15,20-triphenylporphyrin Cobalt(II) (**3**).* The Co(II) complex of porphyrin **1** was obtained according to a procedure reported by Buchler.[36] Under N_2, dissolving 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin and $Co(acetate)_2$ in DMF, the solution was refluxed 2 h. After the addition of an appropriate amount of water, the tarry solution was allowed to cool and stand for 24 h. The product was purified by column chromatography as described for complex **2**, but only the center of the reddish purple band was collected, and no attempt was made to exclude oxygen from the purification procedure. After the chromatographed sample was crystallized from chloroform and the crystals were heated to 120 °C for 30 min, a red product containing no solvent was obtained in 68% yield. Calcd for $CoC_{48}H_{28}N_4O_4$: C, 78.36; H, 3.81; N, 7.62. Found: C, 79.01; H, 3.74; N, 7.46. A mass spectrum of a pure product showed <1% impurity of a molecular weight (784 g/mol) higher than CoTPP (735 g/mol), and the mass spectrum showed no evidence of the presence of free porphyrin. The UV−vis spectra bands of the product had reached constant values at 530 nm (1.660×10^4), 416 nm (2.58×10^5), ~331 nm (1.03×10^4) and 264 nm (2.70×10^4). IR (KBr, cm^{-1}): 3432 (ν_{O-H}), 3020 (ν_{C-H}), 1597 ($\nu_{C=C}$), 1259 (ν_{C-O-C}).

*Porphyrin Dimer (**4**).* A 60-mL amount of dry pyridine was added to 370 mg of crude **3** and 20 mg of potassium metal. The resulting solution was refluxed with stirring for 2 h and then cooled to room temperature. After removing the excess potassium, 430 mg of crude **2** was added, and the reflux was continued for 6 h. Then 30 mL of pyridine was removed under reduced pressure on a rotary evaporator, and 50 mL of water was added to the remaining liquid. The tarry solution was allowed to stand for 24 h. A dark solid was collected by filtration, washed with five 50-mL portions of methanol and a large amount of water, and dried. The residue was purified by chromatography on a column packed with 40 g of ~100−200 mesh silica gel in chloroform. Elution with

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8% ethanol in chloroform (v/v) removed a fast-moving minor band of unreacted starting materials, then 3% ethanol in chloroform (v/v) was used to remove the product. Removal of solvent on a rotary evaporator afforded 491 mg (60%) of porphyrin dimer **4**. ^{1}H NMR (400 MHz, $CDCl_3$, relative to TMS): δ ppm 15.839 (s, 8H β pyrrole of the inner metalloporphyrin moiety), 12.98 (s, 8H, *ortho*-H of all phenyl groups of the inner metalloporphyrin moiety), 9.870 (s, 9H, *meta*/*para*-H of triphenyl of the inner metalloporphyrin moiety), 9.641 (s, 2H, *meta*-H of hexyloxyphenyl of the inner metalloporphyrin moiety), 8.834−9.042 (s, 8H, β pyrrole of the inner free-base porphyrin), 8.208 (d, 6H, *ortho*-H of triphenyl of the inner free-base porphyrin), 8.108 (d, 2H, *ortho*-H of hexyloxyphenyl of the inner free-base porphyrin), 7.749 (m, 9H, *meta*/*para*-H of triphenyl of the inner free-base porphyrin), 7.241 (d, 2H, *meta*-H of hexyloxyphenyl of the inner free-base porphyrin), 4.252 (m, 2H, C−H of hexyl group tail), 2.379 (m, 2H, C−H of hexyl group tail), ∼1.248−1.984 (m, 8H, C−H of hexyl group), −2.786 (s, 2H, NH of the inner free-base porphyrin moiety). IR (KBr, cm^{-1}): 3317 (ν_{N-H}), 2922 (ν_{C-H}), 2851 (ν_{C-H}), 1597 ($\nu_{C=C}$), 1259 (ν_{C-O-C}).

Preparation of Sensing Membrane. The sensing membrane M1 was prepared by using a spin-coating technique. A membrane cocktail was obtained by dissolving 2.5 mg of porphyrin dimer **4**, 80 mg of PVC, and 160 mg of BOS in 2 mL of freshly distilled THF. An aliquot of 0.2 mL of this solution was applied to the surface of a circular 35-mm diameter quartz plate that was mounted on a rotating (rotating frequency 600 rpm) aluminum alloy rod under a THF-saturated atmosphere.[37] After a spinning time of only 5 s, a membrane of ∼4 μm thickness was obtained on the quartz plate. In a similar manner, membrane M2 containing 1.5 mg of TPP as sensing material was prepared from the described membrane cocktail.

Measurement Setup. The quartz plate with sensing membrane was first fitted onto the front side of a measuring cell described elsewhere[37] that was suitable for fluorescence detection. A 35-mm-diameter black PVC plate without a sensing membrane was then fitted onto the other side to complete the cell. The cell was introduced in a Hitachi spectrophotometer (model F-2500) at the appropriate position[38] to measure the fluorescence intensity. About 3.4 mL of sample solution was introduced using a syringe, and the fluorescence emission spectra of the optode membrane were recorded in the range between 560 and 760 nm with an excitation wavelength of 423 nm. All fluorescence measurements were taken under batch conditions. Before measurements, all sensing membranes were conditioned in Tris/HCl plain buffer solution until the fluorescence intensity was stabilized. The limiting fluorescence intensities F_0 and F_1 were determined with the sensing membrane in contact with Tris/HCl buffer solution and 3.0 mM histidine solution, respectively.

For the selectivity measurements, a separate solution method (SSM)[39] was used throughout by using solutions of corresponding amino acids or anions in Tris/HCl buffer solution. The selectivity coefficients were evaluated by comparing the response function at $\alpha = 0.36$, which corresponds to 0.12 mM histidine, the normal level in human serum.[40] The response to histidine was used as the standard.

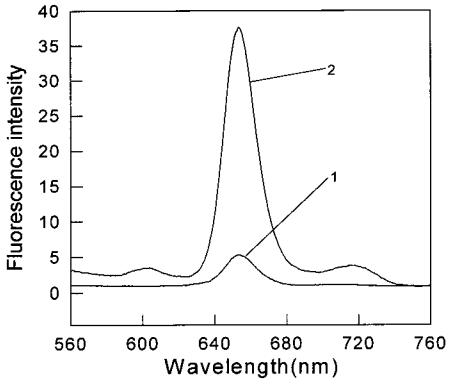

Figure 2. Fluorescence emission spectra of the porphyrin dimer (1) and TPP (2) in CH_2Cl_2 solution (porphyrin concentration 0.002 mM; excitation wavelength, 423 nm).

RESULTS AND DISCUSSION

Optical Properties of the Porphyrin Dimer. The optical properties of a porphyrin compound can be related to its molecular skeleton. The nucleus of porphyrin consists of four pyrrole rings linked by a methine bridge: the corresponding macrocycle is fully conjugated, containing an 18-electron aromatic π-system and planar. The extended aromatic system is responsible for the high molar absorbance (4.33×10^5 cm^{-1} M^{-1} at 419 nm in dodecane[41]) and, finally, for the intense fluorescence (fluorescence quantum yield $\varphi = 0.13$)[42] of the porphyrin. From an optical point of view, the porphyrin molecules are characterized by the presence of a pronounced absorption band (the Soret band) in the blue region of the visible spectra and the large Stockes shift ($\Delta\lambda > 200$ nm).[43] Meanwhile, TPP is also a highly hydrophobic reagent and retains almost the same optical properties after being immobilized in a plasticized PVC membrane.[38]

When TPP is covalently linked with a metalloporphyrin moiety, which is insulated by at least one methylene group, fluorescence quenching by the intramolecular metal center is, of course, a well-known phenomenon.[44] Figure 2 shows the fluorescence emission spectra of the porphyrin dimer **4** and TPP in CH_2Cl_2 (0.002 mM). The excitation wavelength is 423 nm, near the Soret band. Since the absorbances of these two compounds are similar to each other at this wavelength, one can directly compare their fluorescence intensities. It can be seen from Figure 2 that the fluorescence intensity of the porphyrin dimer (at 657 nm) is only 16% of that of TPP, indicating that the inner metalloporphyrin efficiently quenches the fluorescence of the inner free-base porphyrin moiety. This result has been ascribed to the photoinduced intramolecular electron transfer from the inner free-base porphyrin in the singlet excited state to a low-spin cobalt(II) (Figure 3a).[45] For such a derivative, electron transfer is the primary de-excitation path for

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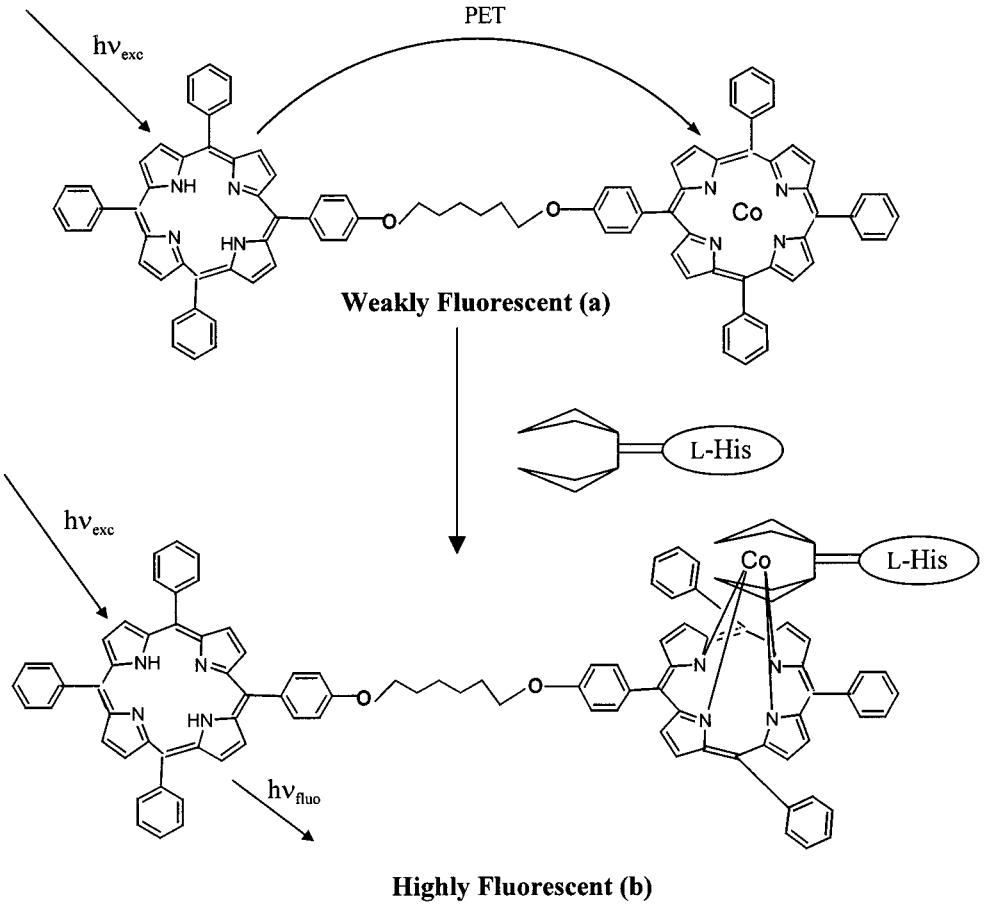

Figure 3. Basic photoinduced electron-transfer process in a porphyrin dimer system: (a) in a free state and (b) guest-species-bound.

the excited state, and the electron-transfer rates depend on the donor−acceptor angle and distance.[46] In the classic Marcus theory for nonadiabatic electron transfer, the rate of electron transfer, K_{eT}, is given by[47]

$$K_{eT} = (\pi/\hbar^2\lambda k_B T)^{1/2}|V|^2 \exp[-(\Delta G^\circ + \lambda)^2/4\lambda k_B T] \quad (1)$$

where $\hbar$ is Planck's constant divided by 2π, λ is the reorganization energy, k_B is the Boltzmann constant, T is the temperature, and V is the coupling matrix element.

The fluorescence quenching of the porphyrin dimer can be suppressed upon complexation with an ion or molecule and the inhibiting of the electron transfer (Figure 3b). Fluorescence then can become the dominant decay pathway for the excited free-base porphyrin. There are also other mechanisms for inhibiting electron-transfer, such as conformational changes,[24] micropolarity modulations,[48] or hydrogen bonding.[49] The "guest" species induces an increase in ionization/oxidation.

Optode Membrane Response and Principle of Operation. The optode membrane M1 was essentially weakly fluorescent where exposed to plain buffer of pH 8.0. When the membrane was in contact with the aqueous buffer solution containing histidine, an increase in fluorescence intensity was observed. Figure 4 shows the fluorescence emission spectra of the optode membrane M1 in the presence of histidine. As expected from the original design, complexation of the porphyrin dimer with histidine increases the fluorescence intensity of the membrane at two bands, 657 and 719 nm, which correspond to the TPP emission. The degree of fluorescence enhancement is related to histidine concentration. At a histidine concentration of about 20 mM, the fluorescence intensity of the optode membrane gradually leveled off. Higher histidine concentrations resulted in no appreciable

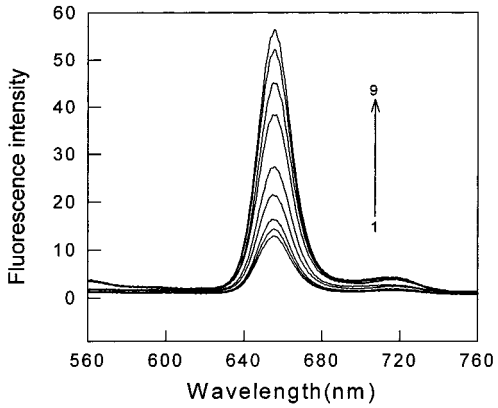

Figure 4. Fluorescence emission spectra of the optode membrane M1 in the presence of different concentrations of histidine at pH 8.0: 1, 0; 2, 0.004; 3, 0.008; 4, 0.02; 5, 0.04; 6, 0.08; 7, 0.2 ; 8, 0.4; and 9, 2.0 mM (excitation wavelength, 423 nm).

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changes. The fluorescence spectra of optode membrane M2 were scarcely changed by exposing it to histidine solution, indicating that the spectral changes are due to the interaction of the inner metalloporphyrin and histidine.

The fluorescence enhancement of the optode membrane M1 by histidine could be attributed to the extraction of histidine from aqueous solution into the membrane phase and the ligation interaction of histidine with porphyrin cobalt(II). If the complex equilibrium between histidine in the aqueous sample solution (aq) and porphyrin dimer in the plasticized PVC membrane phase (mem) will form an m−n complex, the overall equilibrium can be represented as

$$m\mathrm{A(aq)} + n\mathrm{B(mem)} \overset{K}{\rightleftharpoons} \mathrm{A}m\mathrm{B}n\mathrm{(mem)} \qquad (2)$$

where A represents histidine and B represents the porphyrin dimer. If the difference between the activities and concentrations is neglected for simplification, the corresponding equilibrium constant, K, can be expressed by the law of mass action,

$$K = k_{\mathrm{d}} \times \beta = \frac{[\mathrm{A}_m\mathrm{B}_n](\mathrm{mem})}{[\mathrm{A}]^m(\mathrm{aq})[\mathrm{B}]^n(\mathrm{mem})} \qquad (3)$$

where k_{d} and β are the distribution coefficient and complex formation constant; and $[\mathrm{A}_m\mathrm{B}_n]$, [A], and [B] are the concentrations of the respective species.

For the description of the response behavior of this optically sensing membrane, it is useful to introduce the degree of reaction, α, defined as the ratio between the free porphyrin dimer concentration, [B], to the total concentration of the porphyrin dimer, C_{B}. α can be expressed as

$$\alpha = \frac{[\mathrm{B}]}{C_{\mathrm{B}}} = \frac{F_1 - F}{F_1 - F_0} \qquad (4)$$

where F is the fluorescence intensity of the sensing membrane actually measured at a defined histidine concentration, F_0 is the fluorescence intensity of the optode membrane in the plain buffer solution, and F_1 is the fluorescence intensity when the porphyrin dimer is completely complexed by histidine. α is shown to be dependent on the concentration of histidine in the aqueous sample solution, [A], as follows.

$$\frac{\alpha^n}{1-\alpha} = \frac{1}{\mathrm{n}K{C_{\mathrm{B}}}^{n-1}[\mathrm{A}]^m} \qquad (5)$$

The relationship between α and [A], as expressed by eq 5, is the basis of the quantitative determination of histidine concentration in aqueous solution by using this optode membrane. The experimental data were fitted to eq 5 by changing the ratio of m to n and adjusting the overall equilibrium constant, K. Figure 5 shows the fitted curves that represent the experimental data for histidine. The curve referring to the 1:1 complex ratio and $K = 1.5 \times 10^4$ is the best one fitted to the experimental data (Figure 5, solid line, 3). The best-fitting curve can serve as the calibration curve for the determination of histidine.

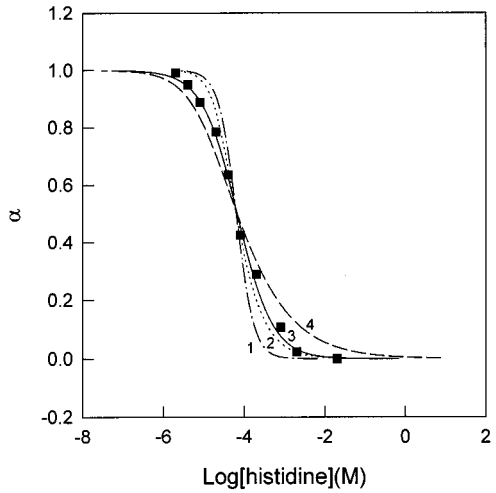

Figure 5. Response parameter values (α) at 423 nm/657 nm as a function of the logarithm of histidine concentrations at pH 8.0. Theoretical response of histidine as predicated by eq 5. The experimental data were fitted to the equation with the different complex ratios and equilibrium constant: (1) n:m = 1:2, $k = 2.5 \times 10^8$; (2) n:m = 2:2, $k = 2.2 \times 10^9$; (3) n:m = 1:1, $k = 1.5 \times 10^4$ (best fit); (4) n:m = 2:1, $k = 1.1 \times 10^5$ (■, experimentally observed data points).

Characteristics of the Optode Membrane M1. The response behavior for a given optical sensor depends significantly on the membrane composition. Several membrane compositions were investigated by selecting appropriate plasticizers and varying the ratio of PVC, plasticizer, and the sensing material, porphyrin dimer. A sensing membrane made of different plasticizers, such as bis(2-ethylhexyl) sebacate(BOS), dibutyl phthalate, tricresyl phosphate, and didecyl phthalate were prepared. Membranes consisting of BOS show the best response to histidine. At each porphyrin dimer concentration, maximum sensitivity was obtained with a BOS/PVC weight ratio of about 2:1. On the other hand, the concentration of the sensing material incorporated within the membrane influenced the magnitude of the sensor response and its sensitivity. The fluorescence intensity of the optode membrane increased with an increasing amount of the porphyrin dimer in the membrane; however, more sensing material did not produce a larger fluorescence signal change ($F/F_0 - 1$), where F_0 and F are the fluorescence intensities of the optode membrane in the absence and presence of histidine. Taking the sensitivity and the fluorescence intensity value of the bulk membrane into account, the optimal porphyrin dimer immobilizing was 2.5 mg, 1.03 wt % relative to the total amount of the membrane cocktail.

The fluorescence intensity of the optode membrane M1 in plain buffer solution and that of the sensing membrane when exposed to histidine-containing solution was influenced by the acidity of the medium. Although a decrease in response was observed at pH <6.0 or pH >10.0, at neutral or weak-base medium, the sensor response to histidine was nearly independent of pH. The pH dependence of the fluorescence intensity change ($F/F_0 - 1$) of the sensing system for 0.08 mM histidine is shown in Figure 6. The response reached a maximum value and remained a constant between pH 7.0 and 9.0. In this working pH range, histidine exists mainly in the neutral form, which is subjected to being extracted into the organic film phase, forming the complex with the metalloporphyrin, and causing fluorescence enhancement of the

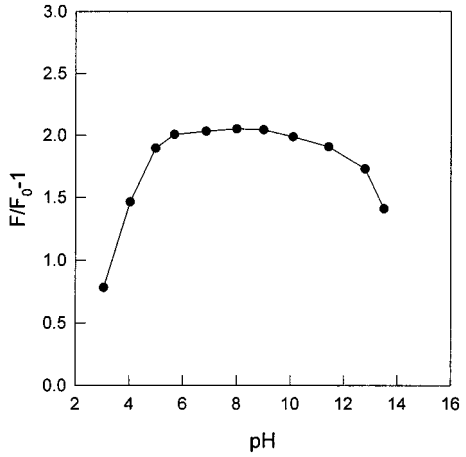

Figure 6. Fluorescence response of the optode membrane M1 at 423 nm/657 nm as a function of pH (histidine concentration ,0.08 mM).

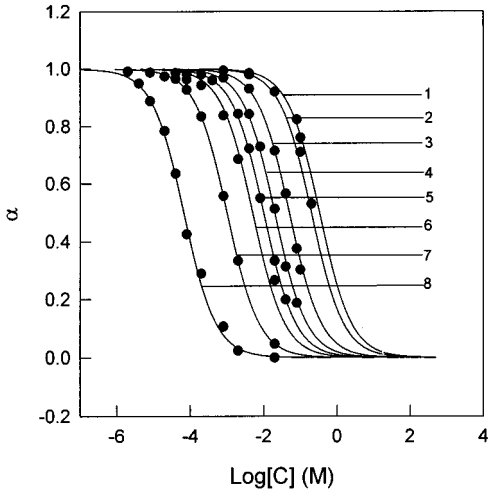

Figure 7. Response function of the optode membrane on exposure to aqueous amino acids measured at pH 8.0 and at a wavelength of 423 nm/657 nm: 1, methionine; 2, glycine; 3, tyrosine; 4, isoleucine; 5, tryptophan; 6, lysine; 7, arginine; 8, histidine. The solid lines were calculated according to eq 5.

sensing membrane. The observed decreasing response at higher pH values could be due to the formation of a metalloporphyrin−OH complex.[50] Lower pH leads to protonation and release of histidine. In subsequent experiments, a pH 8.0 Tris/HCl buffer solution was used as an ideal experimental condition.

With the optimum conditions, the optode membrane M1 exhibited the highest sensitivity to histidine in the ∼0.0045−1.53 mM range. The limit of detection, which was determined by alternately exposing the optode membrane to plain buffer and low concentration of histidine (5 times each) and calculating the histidine concentration corresponding to signal changes that are 6 times the standard deviation of the plain buffer signal, was found to be 0.002 mM. The reproducibility and reversibility of the optode membrane were evaluated by repetitively exposing it to 0.08 mM histidine solution and Tris/HCl buffer solution (pH 8.0). The mean fluorescence intensity values with their confidence intervals were found to be 38.33 ± 0.64 (n = 5, 0.08 mM histidine) and 12.67 ± 0.38(n = 5, Tris/HCl buffer solution). The response time of the sensor depends on the thickness of the membrane, the flow rate of the sample solution, and the change of the concentration of histidine. When the thickness of the membrane reaches the order of millimeters, the response time may reach 5 h. To prepare very thin membranes, the spin-on technique was used, which allows the production of very thin, homogeneous and reproducible PVC-based membranes. The forward response time (going from lower to higher histidine concentrations), t_{95}(time needed for 95% of the total signal change to occur) was within 6 min, whereas the time for the reverse response was in the range of 2−4 min over the entire concentration range. Additionally, the fluorescence intensity of the optode membrane M1 at $\lambda_{ex/em}$ = 423/657 nm reduced by about 8% during the determination of the sample solution over three weeks. The decrease in the fluorescence intensity of the membrane might be due to the leaching and the photodecomposition of the membrane components when it was excited by stronger incident lights.

Probably the most important characteristic feature of a sensor is its response to the species to be measured over that to other species present in the solution. To define the response of the membrane to other amino acids, the sensing membrane was subjected to different concentrations of histidine, arginine, lysine, tryptophan, isoleucine, tyrosine, glycine, and methionine separately for a contact time of 6 min. In Figure 7, the membrane responses, α, at pH 8.0 are plotted as functions of the concentration logarithms of histidine and other amino acids. The curve-fitting for the experimental points was calculated from eq 5 assuming a 1:1 complex ratio. The amino acid optical selectivity coefficients, log K^{opt} values (logarithm of the relative selectivities between the interfering amino acids (or anions) obtained by calculating the horizontal distance between the amino acids response curves or anions response curves (not given) at the point of α = 0.36)[39] are summarized in Table 1. Table 3 also shows the recoveries of histidine in the mixture of some potential interfering agents in human serum (see Table 2). As shown in Figure 7 and Tables 1 and 3, the porphyrin-dimer-based histidine-sensitive optode membrane shows excellent selectivity over the several amino acids and common anions. The selectivity of the present sensor for amino acids is histidine ≫ arginine > lysine > tryptophan > isoleucine > tyrosine > glycine > methionine. The order of selectivity of some the common anions is as follows: histidine ≫ SCN^- > salicylate > I^- > ClO_4^- > NO_2^- > Cl^- > Br^- > $H_2PO_4^-$ > SO_4^{2-}. This selectivity patterns deviates from the Hofmeister selectivity pattern that is based solely on lipo-

Table 1. Selectivity Coefficients (log K^{opt}) of the Optode Membrane M1 for Amino Acids and Common Ions in Comparison to Histidine at pH 8.0

interfering agent	log K^{opt}	interfering agent	log K^{opt}
arginine	−1.38	SCN^-	−1.43
lysine	−1.93	sailcylate	−2.44
tryptophan	−2.35	I^-	−2.65
isoleucine	−2.62	ClO_4^-	−3.06
tyrosine	−3.04	NO_2^-	−3.13
glycine	−3.52	Br^-	−3.34
methionine	−3.72	Cl^-	−3.39
		$H_2PO_4^-$	<−3.70
		SO_4^{2-}	<−3.90

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Table 2. Compositions of Simulated Serum[a]

compd	concn (mM)	compd	concn (mM)
tyrosine	0.08	glycine	0.14
arginine	0.22	aspartic acid	0.09
lysine	0.25	phenylalanine	0.15
histidine	0.12	alanine	0.45
isoleucine	0.11	$NaHCO_3$	15
methionine	0.032	NaCl	100
tryptophan	0.07		

[a] All amino acids are L or DL isomers.

Table 3. Recovery Studies of Histidine from Synthetic Serum Samples

	concn, mM		
sample	added	found, mean[a] ± SD[b]	recovery, (%)
1	0.02	0.016 ± 0.0006	97.2
2	0.10	0.103 ± 0.0031	103.4
3	0.20	0.198 ± 0.0082	99.1
4	0.50	0.483 ± 0.01	96.5
5	0.80	0.843 ± 0.011	105.4

[a] Mean of three determinations. [b] Standard deviation.

philicty. Furthermore, this pattern indicates the existence of a selective interaction between the metalloporphyrin and some specific anions which is consistent with the data reported previously.[18] As can be seen, the selectivity coefficients obtained with the porphyrin-dimer-based histidine optode systems fulfill the selectivity requirements for the histidine assay in physiological fluids. The origin of the fluorescence response of the porphyrin dimer toward histidine is likely due to direct and relatively strong interaction of the imidazole moiety of histidine as an axial ligand of cobalt(II).[51] Since the π-symmetry orbits of the low-spin Co(II) (d^7) ion are completely filled, this would suggest that the five-membered imidazole rings are better π acceptors from Co(II) than are aliphatic amines and pyridines of other amino acids,[33] which act to remove much of the steric interaction with the hydrogen bonds of the adjacent carbons.

Interaction Mechanism of Metalloporphyrin with Histidine. Although it has been supposed that there is an interaction between metalloporphyrin and amino acids, the interaction mechanism has not been studied in depth so far. The change of the UV−vis spectra of the porphyrin dimer in the visible region when in contact with a histidine-containing solution might shed some light on the interaction mechanism of metalloporphyrin with amino acids. Normal metalloporphyrin electronic absorption spectra show three prominent bands in the visible region. The most intense is the B, or Soret, band, which usually occurs near 419 nm for organic media of metallo-TPP derivatives. The other two intense bands are the Q and β bands, which are usually found between 500 and 600 nm in the same solvent.[52]

The interaction of the porphyrin dimer with histidine was investigated at room temperature by measuring the UV−vis spectra of a series of solutions in which the concentration of histidine was varied. The visible peak at 417 nm decreased by

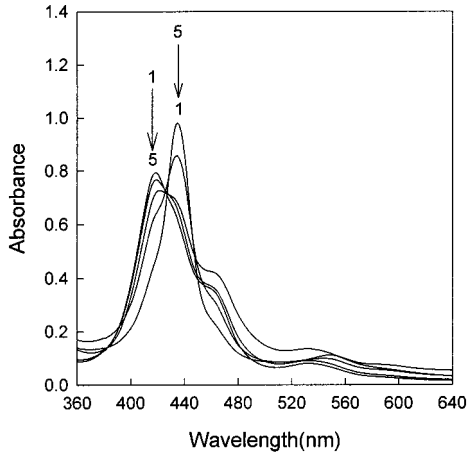

Figure 8. Effect of histidine concentration on the absorption of the porphyrin dimer at pH 8.0: (1) 0.0, (2) 0.08, (3) 0.2, (4) 0.4, (5) 0.8 mM.

the extinction coefficient and shifts slightly to longer wavelength (431 nm) as the concentrations of histidine were increased, as shown in Figure 8. This is characteristic for the histidine coordination with the central metal.[53] The Soret band reflects the valence state of the central metal. The coordination of histidine with the central cobalt(II) results in the delocalization of the cobalt(II) d orbitals, which causes the red-shift of the Soret band. Figure 8 also shows that the numbers of the absorption peaks do not change after porphyrin cobalt(II) coordinated with histidine. This indicates that after histidine is coordinated with the central metal, the symmetry group of the metallopropyrin−histidine complex agrees with the C_{2v} symmetry group of the metalloporphyrin,[54] and one histidine is coordinated with one central cobalt atom in the process of ligation interaction.

Determination of Synthetic Biological Samples. The high degree of histidine selectivity of the optode membrane makes it potentially useful for monitoring concentration levels of histidine in biological samples. To investigate the possibility of applying the sensor for the quantization of histidine, recovery studies were conducted using a synthetic serum containing ~0.02−0.8 mM histidine. The composition of the synthetic serum is listed in Table 2. The concentration of each component of the serum was chosen to match its normal level reported in human serum.[40] Acceptable recoveries were obtained, as shown in Table 3. The results suggest that application of the constituents of the synthetic serum sample do not interfere in any way with the detection of histidine. Therefore, it is feasible to apply the proposed sensor to quantitatively determine the concentration of histidine in serum samples.

CONCLUSION

We have reported here for the first time the synthesis and application of a new bisporphyrin-based fluorescence sensor for histidine. The design of the approach consists of *meso*-tetraphenylporphyrin cobalt(II) as a molecular probe and a covalent attachment of *meso*-tetraphenylporphyrin as a fluorescent reporter. The response mechanism of the approach is based on intramolecular photoinduced electron transfer and the coordination action

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between the central metal and amino acids. Under certain conditions, the coordination ability determines the selectivity sequence of amino acids to be sensed. The dye has been described that can be applied in optical sensor membrane to determine histidine in aqueous solution. The recognition process is based on favorable extraction of histidine into the bulk organic membrane and complexation with the inner metallopophyrin moiety and inhibiting the electron transfer process, which leads to increased fluorescence of the membrane. The optode membrane is easy to prepare and is selective to histidine over several amino acids and common anions. Another unique feature of the proposed sensor for histidine is that it responds directly to histidine itself rather than indirectly monitoring the products from derivatization procedures or enzymatic reactions.

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