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N-confused porphyrin possessing glucamine-appendants: Aggregation and acid/base properties in aqueous media

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ABSTRACT

A water-soluble derivative of N-confused porphyrin (NCP: 5,10,15,20-tetraaryl-2-aza-21-carbaporphyrin) was synthesized by introducing glucamine groups at the *para*-position of *meso*-aryl groups. The tetraglucamine-appended NCP (**TG-NCP**) exists as monocation in aqueous solution containing 6 mM sodium dodecyl sulfate (SDS) but exists as freebase to form aggregates in pure water. These properties are distinct from those of corresponding regular porphyrin, which exists as freebase in the micellar solution and practically insoluble in water.

Water-soluble porphyrins possessing hydrophilic substituents have been widely studied because of their promising properties for biological and medicinal application as well as environmental analyses. Some water-soluble porphyrins have been employed as photosensitizers for photodynamic therapy (PDT),[1] a core component in hybrid molecules for boron neutron capture therapy,[2] ion sensors in water[3] and so on. More recently, porphyrin analogues including expanded porphyrins and porphyrin isomers have also attracted much attention owing to their unique properties unseen in regular porphyrin.[4]

N-confused porphyrin (NCP) is one of the porphyrin isomers[5] that has been considered as a promising candidate for a PDT sensitizer because some NCP derivatives have shown to generate singlet oxygen efficiently in organic media.[6] NCP has also been expected to apply to biosensors since it can bind various anions including biologically important phosphate and chloride.[7] To investigate interactions between NCP and biomolecules in aqueous media, water-soluble NCP derivatives are necessary but such molecules have been scarcely known.[8] Herein, we report a synthesis of water-soluble NCP derivative possessing neutral polyol moieties at the *meso*-aryl groups. Its aggregation behavior as well as acid/base properties in aqueous solutions are also described.

A water-soluble NCP and corresponding derivative of regular porphyrin were synthesized as shown in Scheme 1. NCP **1a** and regular porphyrin **1b**, both of which bear methoxycarbonyl groups at the *para*-position of *meso* phenyl groups, were prepared according to the literature procedure.[9] Tetracarboxylic acid **1b** and **2b** were obtained by the KOH catalyzed hydrolysis of **1a** and **1b** in H_2O/CH_3OH. An NCP derivative bearing four glucamine units (**TG-NCP**) and corresponding porphyrin (**TG-P**)[10] were synthesized by a coupling of **1b** and **2b** with 1-amino-1-deoxy-D-sorbitol (D-glucamine) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBT), respectively.[10,11] **1b** and **TG-NCP** were characterized by spectroscopically, using ^{1}H NMR and MALDI-TOF mass.[12] IR spectrum of **TG-NCP** (as KBr pellet) exhibits a characteristic stretch of amide C=O at 1629.6 cm^{-1}.

UV–vis absorption spectra of 25 μM **TG-NCP** in ultrapure water show the Soret band at 429 nm (Fig. 1A, [SDS] = 0 mM), which is significantly blue-shifted and broadened compared to that of 5,10,15,20-tetraphenyl N-confused porphyrin (**NCTPP**), in which the Soret band is observed at 438 or 442 nm in CH_2Cl_2 or DMF, respectively.[13] Thus, such blue-shift of the Soret band of **TG-NCP** in water strongly suggests the formation of self-aggregates in water.

In contrast to **TG-NCP**, **TG-P** was found insoluble in ultrapure water.[10] **TG-P** became slightly soluble in the water containing 0.2 mM sodium dodecyl sulfate (SDS) (Fig. 1B). The Soret band is observed at 400 nm, which is also significantly blue-shifted and broadened compared to that of tetraphenylporphyrin (**TPP**) in organic solvent (λ_{max} = 419 nm in benzene).[14]

To examine whether the aggregates are formed in water with **TG-NCP** and **TG-P**, SDS was added to their aqueous solutions. The

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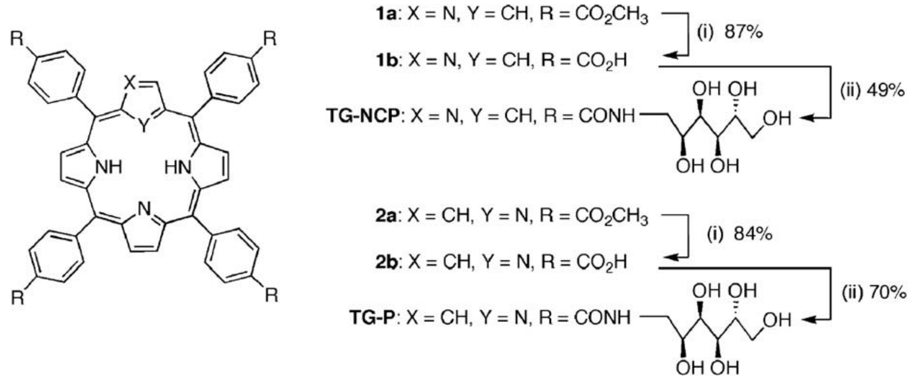


Scheme 1. Structures and synthesis of glucamine-appended N-confused porphyrin (**TG-NCP**) and regular porphyrin (**TG-P**). Reagents and conditions: (i) 20% aq KOH/CH_3OH (v/v = 1/4), ambient temp. (ii) EDC (10 equiv), HOBT (10 equiv), D-glucamine (20 equiv) in DMF/H_2O (v/v = 6/1), 50 °C.

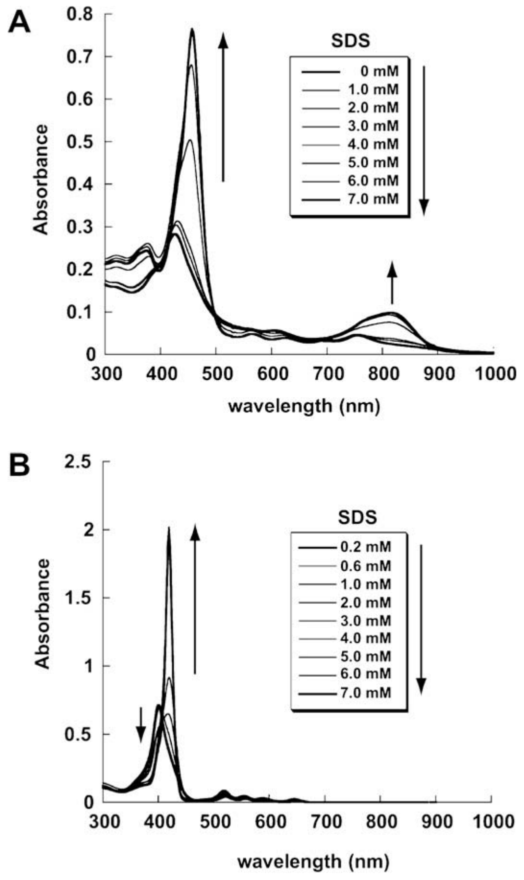


Figure 1. UV–vis absorption spectral changes of **TG-NCP** (A) and **TG-P** (B) in ultrapure water by the addition of various amounts of SDS. The pH of solutions with 0, 1, 3, or 7 mM SDS was 7.3, 6.3, 6.0, or 5.7, respectively.

changes in the absorption spectra of **TG-NCP** or **TG-P** were saturated when the concentrations of SDS were reached to 6 or 7 mM, respectively, which values are slightly smaller than the critical micelar concentration of SDS in water (8.5 mM). Along with the addition of SDS, both the absorption spectra became sharper and red-shifted (Fig. 1). This change strongly suggests that both **TG-NCP** and **TG-P** form H-type aggregates in neutral water.[15]

In the presence of 6 mM SDS, the absorption spectrum of **TG-NCP** exhibits the Soret band at 457 nm (Fig. 1A), which value is close to that of **NCTPP** monocation showing the λ_{max} at 451 nm in CH_2Cl_2.[5a] Therefore, **TG-NCP** can exist dominantly as monocation in neutral water (Table 1). On the other hand, **TG-P** with 7 mM SDS shows the Soret band at 419 nm, which is identical to that of **TPP** in benzene (419 nm).[14] These data indicate that **TG-P** exists as freebase in neutral pH consistent with the previous reports,[8,10] and is more acidic than **TG-NCP**.

To elucidate the acid/base properties of **TG-NCP** under the monomeric and aggregated conditions, quantitatively, pH titrations were carried out in the presence/absence of 6 mM SDS. In presence of SDS, titration profile reveals four regions which showed distinct spectral changes (Fig. 2): (i) from pH 12.6 to 10.5, intensity of the Soret band decreases modestly without a shift of λ_{max} (446 nm) (Fig. S1A); (ii) from pH 10.5 to 7.8, intensity of the Soret band increases, coinciding with a red-shift of the λ_{max} by a 11 nm (446–457 nm). Q-type bands also show spectral changes which give an increase of a broad Q-type band at 817 nm (Fig. 2A); (iii) from pH 7.8 to 4.2, intensity of the Soret band decreases slightly without a shift of λ_{max} (458 nm) (Fig. S1B); (iv) from pH 4.2 to 0.83, intensity of the Soret band increases with a modest red-shift (458–463 nm) accompanied by an emergence of two new Q-type bands at 660 and 804 nm (Fig. 2B). Spectral features of **TG-NCP** at pH 11 (Fig. S1A), pH 7.8–4.2 (Fig. S1B), and pH 0.83 (Fig. 2B) are closely similar to those of the freebase, monocation, and dication of monomeric **NCTPP** in organic solvent.[5a,13] Thus, the dominant species of **TG-NCP** in 6 mM SDS solution at pH 11, 7.8–4.2, and 0.83 can be assigned as freebase, monocation, and dication, respectively (Table 1). The equilibrium constants between freebase and monocation (pK_3) and between monocation and dication (pK_4) were estimated to be 9.3 and 3.0, respectively. These values are comparable to those of **NCTPP** measured in the aqueous micellar SDS solution, in which pK_3 and pK_4 were determined to be 8.4 and 3.3.[5a]

In the absence of SDS, **TG-NCP** shows a modest spectral change between pH 13.3 and 7.7 with the Soret band around 430 nm (Fig. 3A). In the pH range from 6.8 to 2.2, however, a drastic increase in the intensity of the Soret band accompanying red-shift of the λ_{max} is observed (Fig. 3B). In the pH range from 2.2 to 0.83,

Table 1
Absorption maxima (λ_{max}) of **TG-NCP** in different forms observed in aqueous solution.

TG-NCP	Soret band λ_{max} (nm)	Q-type bands λ_{max} (nm)
Freebase (monomer)[a]	446	546, 593, 666, 730
Monocation (monomer)[b]	457	817
Dication (monomer)[c]	463	660, 804
Freebase (aggregates)[d]	431	605, 753

[a] From Figure S1A.
[b] From Figure S1B.
[c] From Figure 2B (at pH 0.83).
[d] From Figure 3A (at pH 13.3).

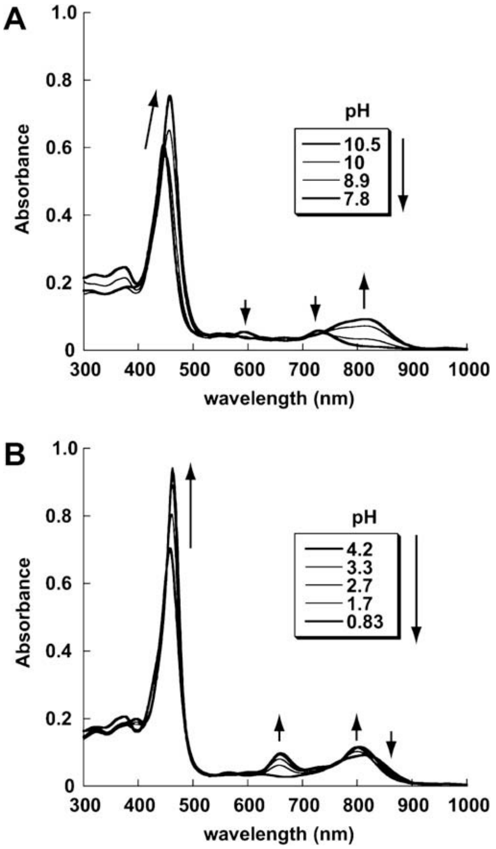


Figure 2. pH-dependent spectral changes of **TG-NCP** in ultrapure water containing 6 mM SDS. Absorption spectra during a titration between pH 10.5 and 7.8 (A), and between pH 4.2 and 0.8 (B). Titrations were performed with aqueous HCl or NaOH.

both the Soret and Q-like bands decrease without a shift of λ_{max} values (Fig. 3B). Comparison with the spectra in the presence of 6 mM SDS indicates that the spectral changes of **TG-NCP** observed in acidic pH without SDS would represent a disassembly process of aggregated freebases to monomeric dications. Increase of the absorption around pH 2.2 suggests that this process may involve an intermediate whose nature remains to be elucidated. This assignment is supported by the fact that the Soret band of **TG-NCP** under acidic conditions (pH <4) shows λ_{max} at 462 nm that is almost identical to that of **TG-NCP** with 6 mM SDS (463 nm) and that of **NCTPP** dication in CH_2Cl_2 (461 nm). At pH 0.83, the spectrum of **TG-NCP** is virtually unchanged by the addition of SDS (Fig. S2), indicating that **TG-NCP** dication exists as a monomeric form even without SDS. It is also worth to note that the pH titration experiments without SDS (Fig. 3) afford no strong evidence for accumulation of **TG-NCP** monocation although it is dominant species between pH 4 and 8 in the solution containing 6 mM SDS (Fig. 2A and S1B). Spectral changes suggest that pK value for the transition between aggregated freebases and monomeric dications (pK_{agg}) is around 4 (Fig. 3B). These data indicate that acid/base properties of NCP macrocycle are strongly affected by its aggregation (Scheme 2).

In summary, a water-soluble derivative of N-confused tetraarylporphyrin bearing four glucamine units and corresponding regular porphyrin have been synthesized. At neutral pH, glucamine-appended NCP (**TG-NCP**) exists as monocation in 6 mM SDS solution whereas it forms aggregates without protonation in pure water. These characteristic properties of **TG-NCP** as well as its water-solubility would originate from the *confused pyrrole* in the porphyrin core, while the corresponding regular porphyrin (**TG-P**) is practically insoluble in neutral water without SDS. In living cells, linear and cyclic polyols are known to play various roles.[16] Based on the findings that mono- and oligo-saccharides often act as specific markers or targeting-tags for proteins, organelle, and cells,[15] a number of porphyrin–carbohydrate conjugate molecules have been designed and investigated for pharmaceutical and diagnostic application.[17] This work therefore would allow NCP to register as a new player in these fields.

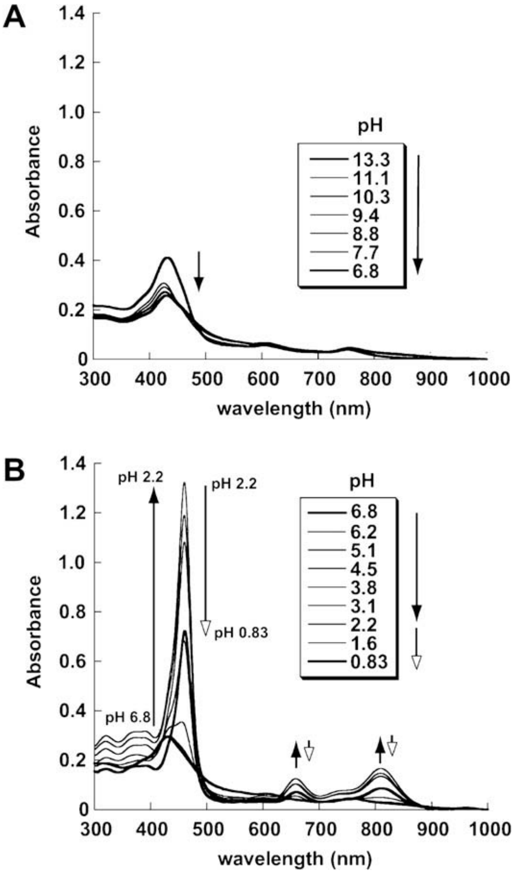
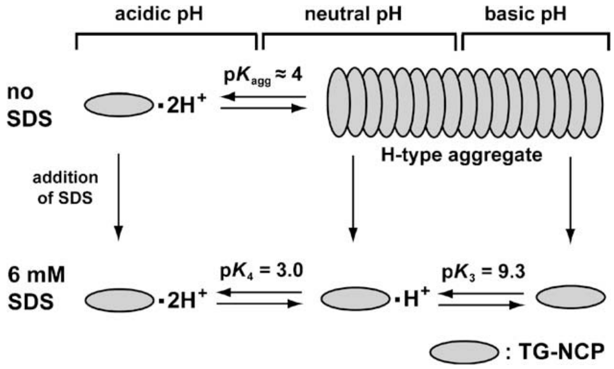


Figure 3. pH-dependent spectral changes of **TG-NCP** in ultrapure water without SDS. Absorption spectra during a titration between pH 13.3 and 6.8 (A), and between pH 6.3 and 0.83 (B). Titrations were performed with aqueous HCl or NaOH.



Scheme 2. A plausible dynamics of aggregation/disaggregation and acid/base behaviors of **TG-NCP** in aqueous solution. Spectral changes of **TG-NCP** by the addition of SDS at pH 13.3 are shown in Figure S3.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.079.

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12. Compound **1b**: **1a** (150 mg, 0.18 mmol) was dissolved in methanol (50 mL) and cooled to 0 °C and 20% aq KOH (12.5 mL) was added. The reaction mixture was allowed to warm to ambient temperature. After stirring for 48 h, the reaction mixture was acidified with trifluoroacetic acid (TFA) until precipitation began to form. After removal of the solvent, the residue was extracted with ethyl acetate. The organic layer was dried and evaporated and the residue was recrystallized with methanol/ethyl acetate to yield **1b** as a green solid. Yield; 122 mg (87%). ^{1}H NMR (CD$_3$OD): δ −2.57 (s, 1H), 8.21–8.32 (m, 8H), 8.37–8.50 (m, 9H), 8.54–8.61 (m, 4H), 8.65 (d, 1H, J = 4.8 Hz), 8.76 (d, 1H, J = 4.8 Hz); MS (MALDI, positive) 790.71 (M$^+$). **TG-NCP**: **1b** (28 mg, 72 μmol) and EDC (70 mg, 360 μmol) were dissolved in 20 mL DMF. After adding HOBT (47 mg, 360 μmol), reaction was stirring for 30 min. A solution of D-glucamine (130 mg, 0.7 mmol) was prepared in a 2:1 mixture of DMF and water, and added to the reaction mixture dropwise. The reaction was stirred for 48 h at ambient temperature. After the solvent was evaporated from the reaction mixture, the residue was dissolved in 0.1 M aq HCl, and the acidic solution was neutralized with aq NaOH. The precipitates were filtered, washed with water, and dried under vacuum to yield the product as a greenish blue solid. Yield; 25 mg (49%). ^{1}H NMR (DMSO-d_6 + TFA): δ −1.91 (br, 1H), 3.37–3.54 (m, 8H), 3.58 (br s, 8H), 3.64 (br, 4H), 3.68 (br, 4H), 3.78 (br, 4H), 3.95 (br, 4H), 8.28 (br, 4H), 8.43 (br, 8H), 8.52–8.68 (m, 12H), 8.75 (br, 1H), 8.83 (br, 1H), 8.92 (br, 1H), [Note: Due to the aggregation, each proton signals show some broadening. In addition, the presence of several conformers or magnetically inequivalent species makes the spectrum complicated to assign the signal explicitly]; MS (MALDI, positive) 1441.99 (M$^+$).
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