meso-Tetrakis(α,α,α,α-*o*-amidophenyl)porphinatoiron(II) Bearing a Proximal Histidyl Group at the β-Pyrrolic Position via an Acyl Bond: Synthesis and O_2 Coordination in Aqueous Media

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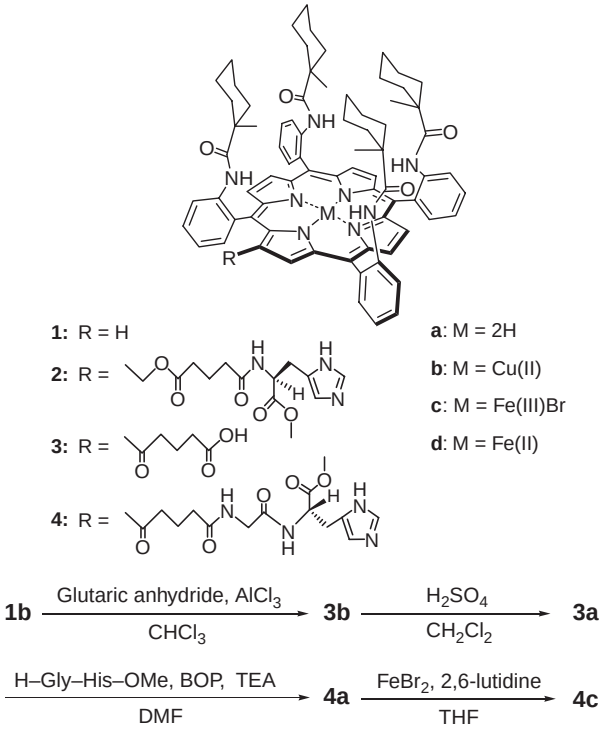
meso-Tetrakis(α,α,α,α-*o*-(1-methylcyclohexanamido)phenyl)porphinatoiron(III) bearing a proximal histidyl group at the β-pyrrolic position via an acyl bond (**4c**) has been synthesized. Human serum albumin (HSA) incorporating the ferrous complex (**4d**) formed a stable O_2 adduct under physiological conditions (pH 7.4, 37 °C). Although an electron-withdrawing acyl group is attached to the porphyrin periphery, the O_2-binding affinity of HSA-**4d** was slightly higher than that of a similar analogue with a histidyl–alkylene group (**2d**).

In the active centers of hemoproteins, a basic amino acid residue, axially coordinated to the prosthetic heme group, namely the proximal base, plays a crucial role in controlling their biological functions, for example, histidine in hemoglobin (Hb) and cysteine in cytochrome P450. To mimic the versatile performances of the hemoproteins, numerous porphyrin derivatives have been synthesized over the past decades.[1,2] The most important factor in the molecular design of these compounds is how to confer the proximal base into the porphyrin structure by a covalent bond.

We successfully introduced a histidyl–alkylene group to the β-pyrrolic position of *meso*-tetrakis(α,α,α,α-*o*-(1-methylcyclohexanamido)phenyl)porphine (**1a**) using the Vilsmeier reaction.[3] Human serum albumin (HSA) incorporating the ferrous complex (**2d**) can reversibly bind and release O_2 under physiological conditions (pH 7.4, 37 °C) in a fashion similar to Hb and myoglobin.[3b] The advantage of this strategy is to confer the proximal histidine to the porphyrin periphery in the last step of the synthesis.[3,4] However, the preparation processes are still labor-intensive: (1) formylation of the porphyrin, followed by (2) demetallation of copper, (3) reduction of –CHO to $–CH_2OH$, (4) connection with glutaric acid, and (5) binding of terminal histidine.[3,4] If the axial base can be introduced into the superstructured porphyrin in a few steps, it will lead to creating a new field in the hemoprotein model chemistry.

In this communication, we report for the first time, the one-step introduction of the 4-carboxybutanoyl group into the β-pyrrolic position of *meso*-tetrakis(α,α,α,α-*o*-(1-methylcyclohexanamido)phenyl)porphyrin, which is easily converted into the hisitidine-linked porphyrin (**4a**) by other two processes. The O_2-binding property of the HSA hybrid incorporating the ferrous complex (**4d**) was then investigated in aqueous media.

The copper(II) complex of the parent porphyrin (**1b**) was synthesized according to our previously reported procedure.[3] We have found that the 4-carboxybutanoyl group is introduced by the Friedel–Crafts reaction using glutaric anhydride and aluminium chloride ($AlCl_3$) (Scheme 1). The progress of the reaction was monitored by the red shift of the absorption maxima of the porphyrin and change in the R_f value during TLC. The brownish-red colored **3b** was purified by column chromatography and demetallated by H_2SO_4. The glycyl-*O*-methyl-L-histidine[5] was then coupled using benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP). Finally, an iron insertion was carried out using $FeBr_2$ and 2,6-lutidine in anhydrous THF. The analytical data of all compounds described here were satisfactorily obtained (see Supporting Information).[6] The bathochromic shifts (3–7 nm) observed in the UV–vis absorption spectrum of **4a** compared to **2a** were due to the electron-withdrawing acyl group at the β-pyrrolic position.[6]



Scheme 1. Synthesis route of **4c**.

The ferric porphyrin (**4c**) in toluene was converted into the ferrous complex (**4d**) by reduction in a heterogeneous two-phase system (toluene/aq. $Na_2S_2O_4$) under an argon atmosphere.[4] The UV–vis absorption spectrum of the orange solution showed the formation of a five-N-coordinate high-spin complex (λ_{max}: 440, 544, 564 nm).[3,4,7] Upon exposure to O_2 or CO, the spectral pattern immediately changed to those of the O_2 adduct complex (λ_{max}: 429, 551 nm) or carbonyl complex (λ_{max}: 429, 544 nm).

The aqueous solution of the HSA-**4d** hybrid [in phosphate-buffered saline (PBS) solution (pH 7.4), [HSA]/[**4d**] = 1/4

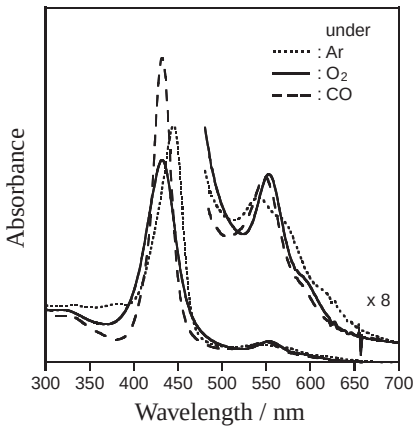


Figure 1. UV–vis absorption spectral changes of HSA-**4d** in PBS solution (pH 7.4) at 37 °C.

Table 1. O_2 binding parameters of HSA-porphinatoiron(II) in PBS solution (pH 7.4) at 25 °C[a]

Porphinatoiron(II)	$P_{1/2}$ /Torr	$k_{on}/\mu M^{-1}\,s^{-1}$		k_{off}/s^{-1}	
		fast	slow	fast	slow
2d[b]	1 (3)	54	8.8	89	14
4d	0.8 (2)	34	4.5	45	5.9

[a]The values in parenthesis are measured at 37 °C. [b]Ref 3b.

(mol/mol)] was prepared by a previously reported method.[3b] The UV–vis absorption spectrum of this aqueous solution under an argon atmosphere showed that **4d** formed a five-N-coordinate high-spin complex with an intramolecularly coordinated axial histidine (Figure 1). Upon exposure of HSA-**4d** to O_2, the absorption spectrum changed to that of the O_2 adduct complex. After reacting with the CO gas, a stable carbonyl complex was produced. The absorption maxima of HSA-**4d** showed 1–3 nm bathochromic shifts compared to those of the HSA-**2d** (Table S1).[3b,6]

The O_2-binding affinity of HSA-**4d** ($P_{1/2} = 0.8$ Torr) determined by the spectral changes at the different O_2 partial pressures was slightly higher than that of HSA-**2d** (Table 1). This is in significant contrast to the fact that the substitutions of two 3,8-vinyl groups of the imidazole-bound protoporphinatoiron(II) by acetyl groups decreased the O_2-binding affinity by 1/4–1/6 due to the reduction of the electron density in the porphyrin plane.[8,9] Our result suggested that (1) the reduced basicity of the porphyrin core by the introduction of one acyl group did not influence the O_2-binding equilibrium very much, and (2) that there is another structural factor that increases the O_2-binding affinity of the porphyrin.

To determine the association and dissociation rate constants for O_2 (k_{on}, k_{off}) to HSA-**4d**, the laser flash photolysis experiments were carried out.[3b,10,11] The absorption decay accompanying the O_2 recombination was composed of two phases of first-order kinetics, producing the fast and slow rebinding constants [k_{on}(fast) and k_{on}(slow)]. The k_{on}(fast) value was 7.6-fold higher than k_{on}(slow), and the molar concentration ratio of the two reactions was 3:1. The O_2 association to **4d** in the protein scaffold might be influenced by the microenvironment around the coordination site. This behavior was similarly observed in HSA-**2d**.[3b] The characteristics of the O_2 binding to **4d** was kinetically the low k_{off} values (approximately 1/2) compared to **2d**.

The structures of the ferrous complexes were then simulated.[12] It is remarkable that the porphyrin plane of **2d** in the five-coordinate high-spin complex was significantly domed compared to that of **4d** (Figure S1).[6] On the other hand, their O_2 adduct complexes showed similar structures having the flat porphyrin macrocycles. The difference in the five-coordinate species could be caused by the spacer moiety between the histidine and porphyrin. The rigid (histidyl-glycyl)carbonylbutanoyl group of **4d** presumably produces a favorable geometry to fix the proximal imidazole at the central iron(II) of the porphyrin, which could result in the relatively low dissociation rate constant of O_2.

In conclusion, we could successfully introduce the proximal histidyl group at the β-pyrrolic position of the *meso*-(tetrakis-*o*-amidophenyl)porphine via an acyl bond in two steps. The O_2 binding affinity was slightly higher than that of the imidazolyl–alkylene analogue, which might be due to the rigid structure of the spacer moiety between the histidine and porphyrin ring. This strategy would be useful to confer the proximal base to the superstructured porphyrin without any change in the activity, which allows us to create a new class of model heme compounds.

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