Histidine Containing Porphyrins Directed for Two Metal Binding Sites

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Three types of histidine containing porphyrins different in the protection of imidazole N were synthesized by attaching four L-histidyl-glycyl moieties to α,α,α,α-*meso*-tetra(*o*-aminophenyl) porphyrin.

The peptide-porphyrins composed of the atropisomers of *meso*-tetra(*o*-aminophenyl)porphyrin (TAPP) and coordinative amino acids like histidine and cysteine are the ligands which potentially mimic the dinuclear inorganic sites of cytochrome c oxidases[1] and sulfite reductases,[2] respectively. The employment of amino acids as building blocks for the modification of TAPP isomers also contributes to the accumulation of the knowledge necessary for the construction of artificial metalloproteins based on TASP strategy.[3] However, there have been few reports on this combination.

Focusing our attention on the latter problem mentioned above, we examined here the syntheses of three α,α,α,α-TAPP peptides, **2a**, **2b**, and **2c** (Figure 1), which we designed by computer graphics.[4] They possess L-histidyl-glycyl (His-Gly) moieties as the picket-fences,[5] where glycine (Gly) functions as the conformationally flexible spacer between histidine (His) and TAPP. They are different in the protection of the imidazole group; tosyl (Tos)[6] at τ–N in **2a** and benzyloxymethyl (Bom)[7] at π–N in **2b** (Figure 1), therefore, different in the possible metal binding site of the imidazole. We preliminarily studied (1) the atropisomerism of the porphyrin derivatives upon coupling of the peptides and TAPP, (2) the effect of the protecting groups on coupling and deprotection, (3) stability of the peptide-porphyrins to light, and (4) the effect of flexible groups at the reaction point.

In the successfully performed experiment, Boc-Gly was first coupled with α,α,α,α-TAPP in CH_2Cl_2 using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (WSCI•HCl) as the coupling reagent. This afforded (Boc-Gly)$_4$-α,α,α,α-TAPP **1**.[8,9] After the deprotection of Boc group from **1** by trifluoroacetic acid (TFA)-thioanisole in CH_2Cl_2, Boc-His(Tos) and Boc-His(Bom) was introduced to (Gly)$_4$-α,α,α,α-TAPP with WSCI•HCl to give (Boc-His(Tos)-Gly)$_4$-α,α,α,α-TAPP **2a**[10] and (Boc-His(Bom)-Gly)$_4$-α,α,α,α-TAPP **2b**,[11] respectively. Then, the Tos group was cleaved from **2a** by 1-hydroxybenzotriazole (HOBT) in THF[6] to give (Boc-His-Gly)$_4$-α,α,α,α-TAPP **2c**.[12]

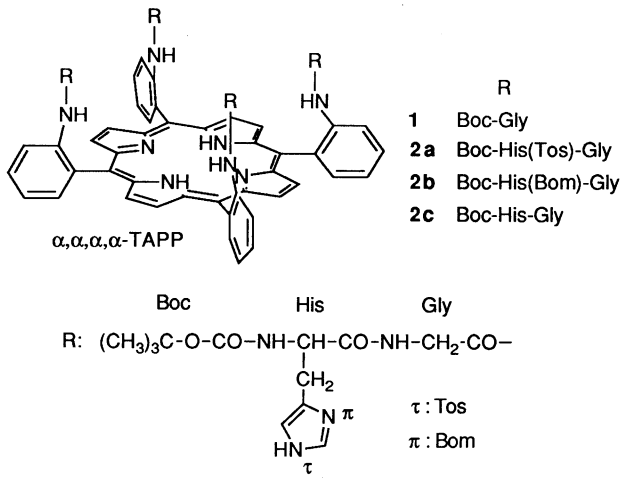

Figure 1. Schematic representation of histidine containing porphyrins.

Although TAPP and carboxylic acids are usually coupled *via* acid chlorides, this method is not applicable to Boc protected amino acids, because they are unstable for acid chloride synthesis. On the other hand, carbodiimide is an established condensation reagent in peptide synthesis for Boc protected amino acids. This method has scarcely been applied in success for the synthesis of TAPP peptides,[13] however, as is shown in this experiment, the method is useful for this purpose under reasonable condition. Hence, it is necessary to pay attention to the atropisomerism induced in the reaction. In our syntheses, all the products **1**, **2a**, **2b** and **2c** were identified to be α,α,α,α-atropisomer by ^{1}H NMR, namely, the β-pyrrole hydrogens of TAPP were all singlet suggestive of α,α,α,α-atropisomer (Figure 2). In general, tetraphenylporphyrins with bulky ortho substituents are stable to thermal atropisomerization at room temperature.[5,14] The other atropisomers except the highly unlikely α,β,α,β conformer do not give β-pyrrole singlets.

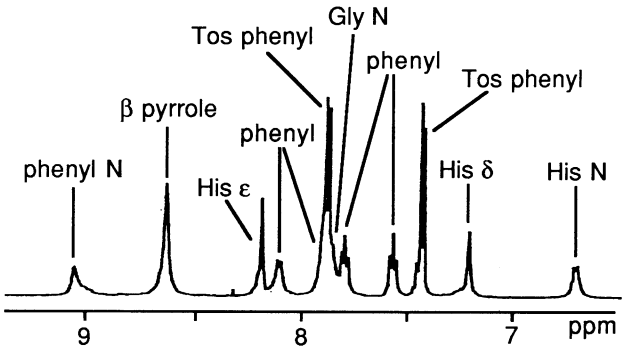

Figure 2. ^{1}H NMR of (Boc-His(Tos)-Gly)$_4$-TAPP **2a** in DMSO-d_6 at 500 MHz.

The protecting group of the imidazole of **2a**, Tos, was more easily cleaved than that of **2b**, Bom. The cleavage of Bom by trimethylsilyl trifluoromethanesulfonate (TMSOTf)-thioanisole/TFA did not give a pure product. This agrees with the result for single peptides by Fujii *et al.*,[15] who argued that Bom was not completely cleaved by this method. Therefore, in order to obtain the TAPP peptides with deprotected imidazole, the method *via* Boc-His(Tos) should be better.

It is noteworthy that **2a**, **2b**, and **2c** were sensitive to light under air in acetonitrile or methanol. The rate of porphyrin bleaching observed by UV-visible absorption spectra was **2c** >> **2a** ≈ **2b** in order. This order agrees with that of the basicity of the protected and deprotected imdazoles. This result suggests

that the bleaching is induced by the electron transfer from the donor (imidazole) to the acceptor (porphyrin). Otherwise, some indirect participation of imidazole sensitized by the porphyrin may be considered.[16] Figure 3 represents the decay of **2a** under fluorescent light (by HPLC).

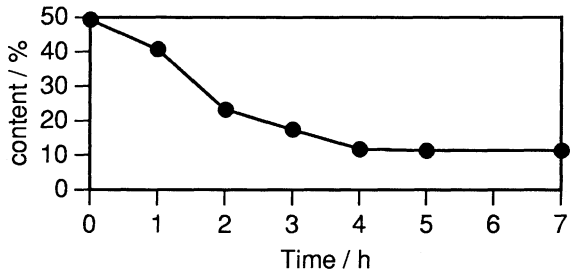

Figure 3. Time course of (Boc-His(Tos)-Gly)$_4$-TAPP **2a** content under room lighting.

The fragment condensation of α,α,α,α-TAPP with dipeptide Boc-His(Tos)-Gly was also attempted, but failed. Considering the success in the stepwise elongation mentioned above, this result suggests that the condensation is affected by the steric hindrance at the reaction point. Generally speaking, fragment condensation is favorable than stepwise elongation in peptide synthesis. Our result shows that stepwise elongation is required for the syntheses of TAPP peptides at least at the first stage of the coupling. This is important for the condensation of longer peptides with the amino groups of α,α,α,α-TAPP toward artificial proteins. The effective coupling of longer peptides with TAPP will be achieved by introducing a flexible spacer like glycine or alanyl-glycine by stepwise elongation prior to the coupling of large fragments.

Reference and Notes

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8 Boc (= t-butyloxycarbonyl) protection was used for N-terminus of amino acid. Every compound was purified on appropriate chromatography columns, and confirmed by ^{1}H NMR, FAB mass spectroscopy, and elemental analysis.

9 ^{1}H NMR (DMSO, 270 MHz, δ): 8.85 (4H, s, Ar NH), 8.70 (8H, s, pyrrole β), 8.14 (4H, d, Ar), 7.93 (4H, d, Ar), 7.85 (4H, t, Ar), 7.58 (4H, t, Ar), 6.46 (4H, s, Gly NH), 2.83 (8H, d, Gly α), 1.00 (36H, s, Boc), -2.76 (2H, s, pyrrole NH). Found: C, 62.50; H, 6.12; N, 12.06%. Calcd for $C_{72}H_{78}N_{12}O_{12}$•4.5H_2O: C, 62.46; H, 6.33; N, 12.14%. FAB-MS, (MH^+) Found 1303, Calcd 1303.

10 ^{1}H NMR (DMSO, 500 MHz, δ): 9.06 (4H, s, Ar NH), 8.64 (8H, s, pyrrole β), 8.18 (4H, s, His ε), 8.10 (4H, d, Ar), 7.89 (4H, Ar), 7.88 (8H, d, Tos Ar), 7.85 (4H, Gly NH), 7.79 (4H, t, Ar), 7.56 (4H, t, Ar), 7.43 (8H, d, Tos Ar), 7.21 (4H, s, His δ), 6.70 (4H, d, His NH), 4.02 (4H, s, His α), 2.98 (8H, dd, Gly α), 2.51 (8H, d, His β), 2.35 (12H, s, Tos CH_3), 1.16 (36H, s, Boc), -2.73 (2H, s, pyrrole NH). Found: C, 57.00; H, 5.56; N, 13.15%. Calcd for $C_{124}H_{130}N_{24}O_{24}S_4$•8$H_2O$: C, 57.00; H, 5.63; N, 12.87%. FAB-MS, (MH^+) Found 2468, Calcd 2468.

11 ^{1}H NMR (DMSO, 500 MHz, δ): 9.39 (4H, br s, Ar NH), 8.67 (8H, s, pyrrole β), 8.20 (4H, d, Ar), 7.88 (4H, s, Gly NH), 7.81 (4H, t, Ar), 7.77 (4H, d, Ar), 7.52 (4H, t, Ar), 7.47 (4H, s), 7.22 (20H, m, Bom Ar), 6.72 (4H, s), 6.18 (4H, br s), 5.10 (8H, br s, Bom CH_2), 4.25 (8H, s, Bom CH_2), 3.88 (4H, br s, His α), 3.24 (8H, d, Gly α), 2.40 (8H, br s, His β), 1.19 (36H, s, Boc), -2.91 (2H, s, pyrrole NH). Found: C, 62.88; H, 6.20; N, 13.65%. Calcd for $C_{128}H_{138}N_{24}O_{20}$•6$H_2O$: C, 62.99; H, 6.19; N, 13.78%. FAB-MS, (MH^+) Found 2332, Calcd 2332.

12 ^{1}H NMR (DMSO, 270 MHz, δ): 9.64 (4H, br s, Ar NH), 8.66 (8H, s, pyrrole β), 8.27 (4H, s, Ar), 7.94 (4H, s), 7.83 (4H, t, Ar), 7.77 (4H, d, Ar), 7.51 (4H, t, Ar), 7.20 (4H, br s), 6.51 (4H, br s), 6.00 (4H, br s), 3.67 (4H, br s, His α), 3.29 (8H, d, Gly α), 2.00 (8H, br s, His β), 1.18 (36H, s, Boc), -3.10 (2H, s, pyrrole NH). Found: C, 58.72; H, 6.13; N, 16.61%. Calcd for $C_{96}H_{106}N_{24}O_{16}$ •4H_2O•2AcOH: C, 58.75; H, 6.02; N, 16.45%. FAB-MS, (MH^+) Found 1852, Calcd 1852.

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