Aminoporphyrinic Acid as a New Template for Polypeptide Design

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The α,α,α,α-atropisomer of 5,10,15,20-tetrakis(2-amino-5-methoxycarbonylphenyl)porphyrin, methyl ester of an aminoporphyrinic acid, is efficiently prepared and combined with amino acids as the building block for an artificial membrane protein.

It would be interesting in polypeptide design to synthesize an α-helical peptide–porphyrin conjugate[1] as a membrane protein model. By using α,α,α,α-atropisomeric 5,10,15,20-tetrakis(2-carboxyphenyl)porphyrin, we have successfully prepared a four α-helix bundle structure on the porphyrin ring.[2] However, in the course of the further design and synthesis of artificial membrane proteins containing a porphyrin moiety such as illustrated in Fig. 1, atropisomeric and doubly substituted 5,10,15,20-tetraphenylporphyrins (TPP) would be highly desirable. A TPP bearing both amino and carboxy groups could be incorporated into peptide architecture. Thus, we attempted to prepare 5,10,15,20-tetrakis(2-amino-5-methoxycarbonylphenyl)porphyrin (TAMCPP), methyl ester of an aminoporphyrinic acid (TACPP) (Fig. 2).

The atropisomeric mixture of 5,10,15,20-tetrakis(2-methoxycarbonyl-5-nitrophenyl)porphyrin (TMCNPP) was prepared according to the literature[3,4] starting with the corresponding substituted benzaldehyde and pyrrole.† Purification by passing through a silica gel column gave TMCNPP in 37% yield. The atropisomeric mixture of TMCNPP was reacted with $SnCl_2 \cdot 2H_2O$ in 12 mol dm^{-3} HCl–acetic acid 1 : 1, v/v) at room temperature for 2 h to reduce the nitro group.[5] The methyl ester of TACPP was extracted with $CHCl_3$ and purified by silica gel chromatography [$CHCl_3$–MeCN, 9 : 1 v/v]; the yield in this step was 60%. The atropisomeric mixtures of TMCNPP and TAMCPP were partially separated by silica gel chromatography [benzene–MeCN (9 : 1, v/v) and $CHCl_3$–MeCN (9 : 1, v/v), respectively] and completely by analytical reversed-phase HPLC [μBondasphere C18 (3.9 × 150 mm), MeCN–EtOH–H_2O 30 : 40 : 30 and 30 : 35 : 35, v/v/v, respectively].‡

To find the best conditions to enrich the useful atropisomers, the atropisomerism of them in toluene was investigated at 80 °C by using the purified α,α,α,α-TMCNPP and α,α,α,α-TAMCPP. Fig. 3 shows the unusual abundance of α,β,α,β-isomer (43%) of TMCNPP in toluene, though the statistical existence of the isomer is expected as 12.5% (α,α,α,α : α,β,α,β : α,α,β,β : α,α,α,β = 1 : 1 : 2 : 4). The observed first-order rate constant and the activation free energy for isomerization to α,α,α,β-isomer at this temperature (k = 2.9 × 10^{-4} s^{-1} and $\Delta G^{\ddagger}$ = 111 kJ mol^{-1}, respectively) indicate that one of these atropisomers is stable enough in the further reaction at room temperature. These facts suggest that TMCNPP should be treated in hot toluene and then the nitro groups reduced at lower temperature[5] when the α,β,α,β-

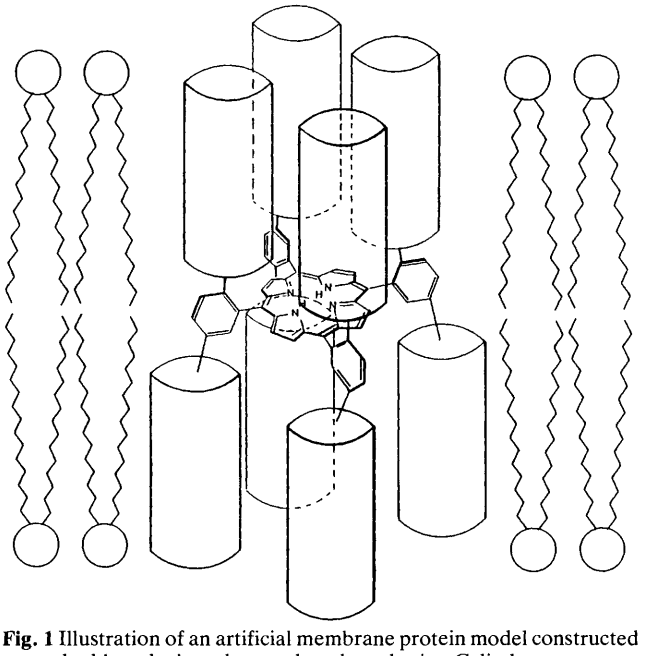
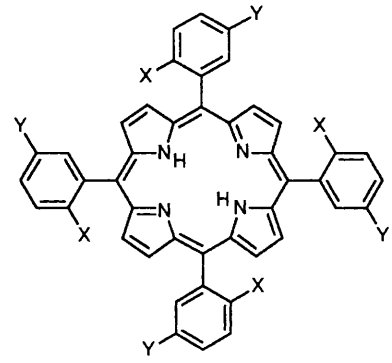


Fig. 1 Illustration of an artificial membrane protein model constructed on a doubly substituted tetraphenylporphyrin. Cylinders represent amphiphilic α-helix peptides.



X	Y	
NH_2	CO_2H	TACPP
NO_2	CO_2Me	TMCNPP
NH_2	CO_2Me	TAMCPP
Boc-Gly-NH	CO-L-Ala-OBzl	$(Boc\text{-}Gly)_4$-TACPP-$(L\text{-}Ala\text{-}OBzl)_4$

Fig. 2 Structure of the aminoporphyrinic acid and related compounds

† To prepare TMCNPP, methyl 3-formyl-4-nitrobenzoate (1.0 × 10^{-2} mol dm^{-3}) and pyrrole (1.0 × 10^{-2} mol dm^{-3}) in CH_2Cl_2 were reacted in the presence of $BF_3 \cdot OEt_2$ as catalyst (1.0 × 10^{-3} mol dm^{-3}) at room temp. for 20 h. Then, chloroanil (tetrachloro-*p*-benzoquinone) (3.0 equiv.) was added to the reaction mixture, which was stirred at room temp. for 24 h. The reaction mixture was purified by silica gel column chromatography.

‡ These separated atropisomers were identified by 400 MHz ^1H NMR measurements and silica gel thin layer and column chromatographies. TLC (R_f): TMCNPP [benzene–MeCN (9 : 1, v/v)], 0.49 (α,β,α,β), 0.41 (α,α,β,β + α,α,α,β), 0.29 (α,α,α,α); TAMCPP [$CHCl_3$–MeCN 9 : 1, v/v], 0.40 (α,β,α,β + α,α,β,β), 0.31 (α,α,α,β), 0.21 (α,α,α,α). Retention time (min) on HPLC: TMCNPP, 9.1 (α,α,β,β), 10.3 (α,α,α,β), 11.1 (α,α,α,α), 12.4 (α,β,α,β); TAMCPP, 6.7 (α,α,α,β), 9.7 (α,α,α,α), 11.0 (α,β,α,β), 12.6 (α,α,β,β). Field-desorption mass spectrum (FD-MS): *m/z* 1026 (M^+ for TMCNPP), 906 (M^+ for TAMCPP). Absorption spectrum in CH_2Cl_2: λ_{max}/nm (ε); α,α,α,α-TMCNPP; 422 (229000), 518 (19900), 552 (5800), 594 (6500), 651 (1800); α,α,α,α-TAMCPP: 419 (331000), 514 (261000), 543 (8100), 590 (7800), 652 (18600). ^1H NMR spectrum (δ): TMCNPP; −2.57 (NH, 2H), 3.96 (Me, 12H), 8.61 (CH, pyrrole, 8H), 8.51, 8.64, 8.77 (phenyl, 12H); TAMCPP; −2.70 (NH, 2H), 3.88 (CH_3, 12H), 8.88 (CH, pyrrole, 8H), 7.10, 8.32, 8.58 (phenyl, 12H). Details of the physicochemical properties of these porphyrins will be reported elsewhere.

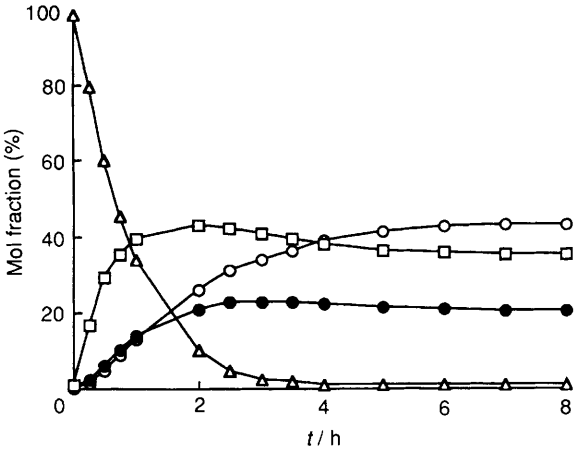


Fig. 3 Thermal isomerization of TMCNPP at 80 °C in toluene; (○) α,β,α,β, (□) α,α,α,β, (●) α,α,β,β, (△) α,α,α,α. Mol fractions were determined by HPLC analysis.

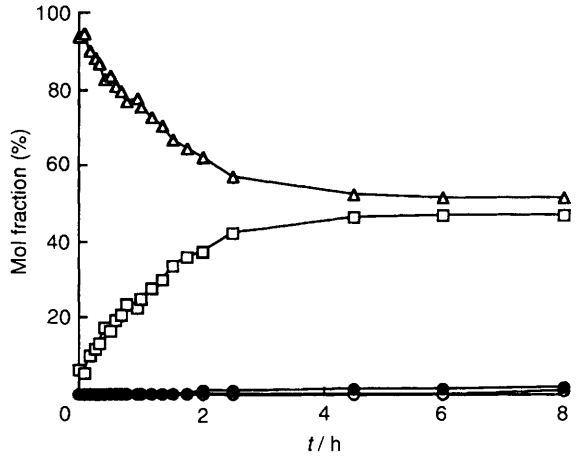


Fig. 4 Thermal isomerization of TAMCPP at 80 °C in toluene; (○) α,β,α,β, (□) α,α,α,β, (●) α,α,β,β, (△) α,α,α,α. Mol fractions were determined by HPLC analysis.

isomer is required. On the other hand, we observed that α,α,α,α-TAMCPP is abundant in thermal atropisomerization in toluene (48%) ($k = 6.3 \times 10^{-5}$ s^{-1} and $\Delta G^{\ddagger} = 115$ kJ mol^{-1} for isomerization to α,α,α,β-isomer) (Fig. 4). It should be noted that the reduction reaction mixture for TAMCPP already contained the α,α,α,α-isomer in as high a yield as 40% (α,β,α,β 9%; α,α,β,β 15%; α,α,α,β 36%). After the isolation of the most polar α,α,α,α-isomer with silica gel column, the mixture of the fore-eluted other three isomers was collected and treated in toluene at 80 °C to enrich the α,α,α,α,-isomer. The same ratio shown in Fig. 4 was observed again in HPLC analysis. Further enrichment of the α,α,α,α-isomer was not attained by treatment with silica gel.[6]

Though the reason for the unusual abundance of particular atropisomers in the thermal treatment mixtures has not been clarified, the discovery of the enrichment condition for α,α,α,α-isomer is quite beneficial for the utilization of this isomer in polypeptide design as a functional template.[2,7] For the demonstration of its endurance in peptide synthesis, Boc-Gly-OH (Boc = *tert*-butoxycarbonyl) was coupled with α,α,α,α-TAMCPP by the symmetrical anhydride method.[8] The product (Boc-Gly)$_4$-α,α,α,α-TAMCPP was then hydrolysed with NaOH to remove the carboxy protection. Further condensation reaction with H-L-Ala-OBzl (Bzl = benzyl)[8] gave (Boc-Gly)$_4$-α,α,α,α-TACPP-(L-Ala-OBzl)$_4$, which was deprotected with trifluoracetic acid to afford (H-Gly)$_4$-α,α,α,α-TACPP-(L-Ala-OBzl)$_4$ [(FD-MS, *m/z* 1755 (M^+)], a valuable starting unit for membrane proteins containing a porphyrin ring (Fig. 1). Atropisomerization was not observed during the synthesis. The hybrid peptide showed induced circular dichroism due to the L-Ala residues at the Soret band of the porphyrin ring ([θ] = −10 000 deg cm^2 dmol^{-1} at 422 nm).[8]

In conclusion, the most useful α,α,α,α-isomer of TAMCPP was synthesized in relatively high yield; this atropisomer could be employed in peptide synthesis. The aminoporphyrinic acid, polypeptide bifunctional and atropisomeric porphyrin, may find application in design as the template for artificial proteins.

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