# Polymer-bound Protoheme–Mono-*N*-[3-(imidazol-1-yl)propyl]amide and –Mono-*N*-[5-(2-methylimidazol-1-yl)pentyl]amide

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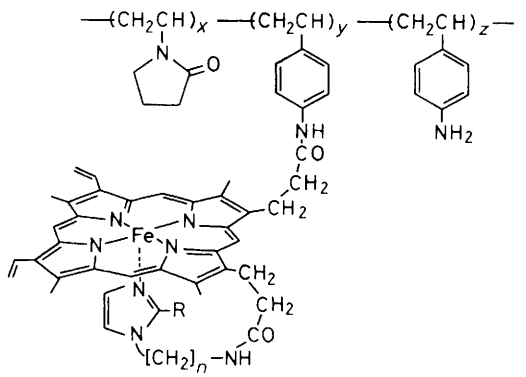
Poly(1-vinyl-2-pyrrolidone)-bound protoheme–mono-*N*-[3-(imidazol-1-yl)propyl]amide and –mono-*N*-[5-(2-methylimidazol-1-yl)pentyl]amide form oxygen adducts with a life-time of *ca.* 1 h in an aqueous medium cooled to −30 °C, but non-bound protoheme analogues do not form under the same conditions.

Much effort has been made to mimic natural oxygen carriers like hemoglobin by using modified synthetic iron-porphyrin complexes.[1–5] In one approach, a series of heme derivatives with covalently bound imidazole-ligands have been synthesized and well studied by Traylor.[3,6] The mesoheme–mono-*N*-[3-(imidazol-1-yl)propyl]amide monomethyl ester derivative is typical and forms an oxygen adduct in aprotic, organic solvents but not in aqueous media.† We have recently found that heme [iron(II)-protoporphyrin IX] bound to poly(1-vinyl-2-methylimidazole) (PMI) forms an oxygen adduct even in aqueous ethylene glycol solution at −30 °C.[7] We suggested that the reason for oxygen being bound in aqueous solution was that PMI forms a complex with pentaco-ordinate heme that has an extra large stability constant; this heme complex is situated in the hydrophobic environment of the polymer. In the present communication we report the synthesis and preliminary oxygen-binding study of poly(1-vinyl-2-pyrrolidone)-bound iron(II)-protoporphyrin IX–mono-*N*-[3-(imidazol-1-yl)propyl]amide (**1**) and –mono-*N*-[5-(2-methylimidazol-1-yl)pentyl]amide (**2**).

Poly(1-vinyl-2-pyrrolidone-co-4-aminostyrene) was allowed to react with iron(III)-protoporphyrin IX–mono-*N*-[3-(imidazol-1-yl)propyl]amide chloride or –mono-*N*-[5-(2-methylimidazol-1-yl)pentyl]amide chloride[8] in the presence of ethyl chloroformate.‡ The bound heme content was $x = 99$, $y = 0.75$, $z = 0.25$ mol % for (**1**) and $x = 99$, $y = 0.78$, $z =$ 0.22 mol % for (**2**); the molecular weight of the polymer-bound heme was $3.65 \times 10^4$ for both (**1**) and (**2**). This indicates that one polymer molecule contains *ca.* one heme unit. These polymers were soluble in water up to *ca.* 5 wt %. The heme solution was prepared by reducing the iron(III) derivative with sodium dithionite ($[Na_2S_2O_4]/[Fe^{III}] = 5$) under $N_2$ and the aqueous medium used was a mixture of pH 7.48 phosphate buffer and ethylene glycol (vol. ratio = 1/1).



(1) R = H, *n* = 3

(2) R = Me, *n* = 5

The u.v. and visible spectra of (**1**) and (**2**) were characterized by absorptions at 428, 530, 560 nm and 433, 557 nm, respectively which were assigned to a hexaco-ordinate complex, whose sixth co-ordination site was occupied by a solvent molecule, and the pentaco-ordinate complex, respectively. When these aqueous solutions were cooled to −30 °C and exposed to oxygen, (**1**) and (**2**) showed absorption spectra (410, 542, 572 nm and 412, 547, 577 nm) which were assigned

† Bayer and Holtzbach reported reversible oxygenation of histidylheme bound to poly(ethylene oxide) in an aqueous medium at room temperature (*Angew. Chem.*, 1977, **89**, 120), but the poly(ethylene oxide)-bound heme synthesized by us showed only a simple irreversible oxidation under our measurement conditions.

‡ The control experiment showed that non-covalently-bound heme was completely separated from the polymer by the purification procedure used.

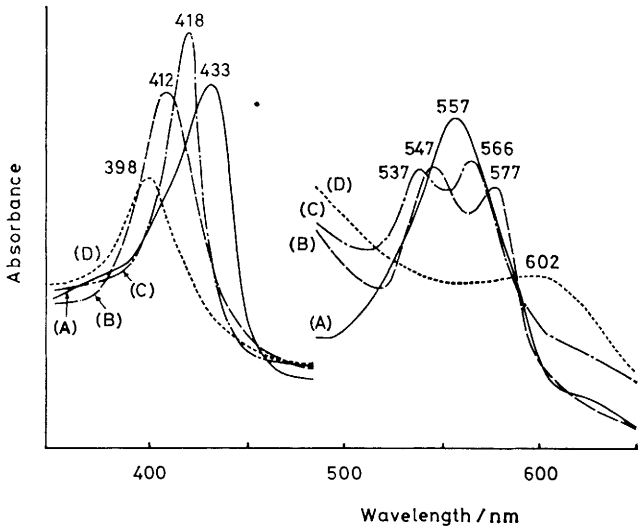



**Figure 1.** U.v. and visible spectra of (**2**). (A) deoxy, (B) oxygen adduct, (C) CO adduct, (D) oxidized; [(**2**)] = 0.1 mM, in pH 7.48 phosphate buffer/ethylene glycol (1/1), at −30 °C.

to the oxygen adduct (Figure 1). Complex (**2**) oxygenated immediately on exposure to oxygen while it took a few minutes for (**1**), corresponding to the structures of the deoxy-complexes. The oxy-spectrum changed to that of the CO adduct (418, 537, 566 nm) on bubbling carbon monoxide through and returned to that of deoxy-heme on bubbling nitrogen through. This oxy–deoxy cycle was repeated three times at −30 °C.

That the reducing agent did not contribute to this reversible oxygenation was shown by the following results. (i) The heme was prepared by reducing hemin with a small excess of sodium dithionite; no trace of it remained before the oxygen exposure. (ii) Where dithionite was added again after the oxygenation, the oxy-heme was reduced to the deoxy-heme in the same manner as the oxy-hemoglobin. (iii) Hemes prepared using organic reductants, such as ascorbic acid or glucose, and the reductase system[9] formed an oxygen adduct with the same absorption spectrum and life-time.

The oxygen adduct was slowly degraded to the iron(III) derivative through isosbestic points at 532 and 561 nm and this degradation obeyed first-order kinetics. The life-times (half-life period) of (**1**) and (**2**) were 80 and 60 min at −30 °C, 10 and 8 min at −15 °C, which were longer than that of PMI-heme (in pH 10 aqueous solution: 24 min at −30 °C).[7] The oxygen adduct was not observed for polymer-non-bound photoheme analogues of (**1**) and (**2**), iron(II)-protoporphyrin IX–mono-*N*-[3-(imidazol-1-yl)propyl]amide and –mono-*N*-[5-(2-methylimidazol-1-yl)pentyl]amide, alone or with 2% poly(1-vinyl-2-pyrrolidone). Reversible oxygen-binding in aqueous medium is efficient when heme is covalently bound to a hydrophobic polymer.

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