pH-induced Oxygen Uptake and Evolution by Aqueous Synthetic Haem–Lipid Solution

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An aqueous solution of a synthetic haem embedded in polymerized liposome reversibly takes up and evolves 3.6 ml of oxygen gas per 100 ml of solution, in a process which is sensitive to ±1 unit of pH change at pH 7.

The oxygen-binding affinity of red blood cells (RBC) decreases [the oxygen pressure at half oxygen binding ($p_{\frac{1}{2}}$) increases] with decrease in the pH of the medium. This phenomenon is called the Bohr effect of RBC; RBC release oxygen more efficiently when the pH of the medium decreases in the presence of carbon dioxide.[1,2] Few studies have been devoted to artificial gaseous molecule transporters or reservoirs which reversibly take up and release gaseous molecules induced by pH changes as RBC do. Aqueous solutions containing such a pH-sensitive gaseous molecule reservoir would be expected to provide, for example, an oxygen concentration-controller to be applied to fermentation.

Recently we achieved reversible oxygen binding in aqueous solution at 37 °C with porphinatoiron (haem) derivatives embedded in a phospholipid liposome.[3–7] We have now prepared a concentrated aqueous solution of the 5,10,15,20-tetra{α,α,α,α-*o*-[2′,2′-dimethyl-20′-(2″-trimethylammonioethylphosphonatoxy)eicosanamido]phenyl}porphinatoiron-(II)–1-laurylimidazole complex[6] embedded in a polymerized lipid liposome, poly[1-{9′-(*p*-vinylbenzoyl)nonanoyl}-2-*O*-octadecyl-*rac*-glycerol-3-phosphocholine], and studied its oxygen uptake and evolution induced by changes in pH.

An aqueous solution (pH 7) containing the haem was prepared as reported by us previously.[3,7] The solution was highly concentrated by ultrafiltration to [haem] = 5 mM and [lipid] = 20 wt%. The solution was physically stable and could be stored for months without precipitation at ambient temperature. The red solution changed to the oxygen adduct solution on exposure to oxygen [u.v.–visible, $\lambda_{max.}$ 426, 535, and 562 (sh) nm for the deoxy solution; 422 and 546 nm for the oxy solution]. By reducing the oxygen partial pressure, the oxy solution immediately reverted to the deoxy solution; reversible oxygen binding and dissociation could be repeated more than a thousand times under the experimental condition (pH 5—10). The oxygen bound to the haem in the solution was determined volumetrically by the van Slyke method to be 11.6 ml of O_2 per 100 ml of solution. One of the advantages of this solution is that the concentration of the haem, *i.e.* the dissolved-oxygen amount, is extremely high and is comparable to that of RBC while the haem is homogeneously solubilized in the solution and the oxygen-binding reaction is rapid and reversible.

The oxygen-binding affinity ($p_{\frac{1}{2}}$) of the solution was determined by the oxygen-binding and -dissociation equilibrium curve measurements. Figure 1 shows the relationship between $p_{\frac{1}{2}}$ and pH of the solution. $p_{\frac{1}{2}}$ Drastically increases with pH, *i.e.* the haem in the solution binds oxygen much more strongly at lower pH. This behaviour is in contrast to the RBC's Bohr effect but the pH dependence of the oxygen-binding amount is more unusual in comparison with that of RBC. While the same pH dependence of the oxygen-binding affinity was observed also for the haem solubilized with a

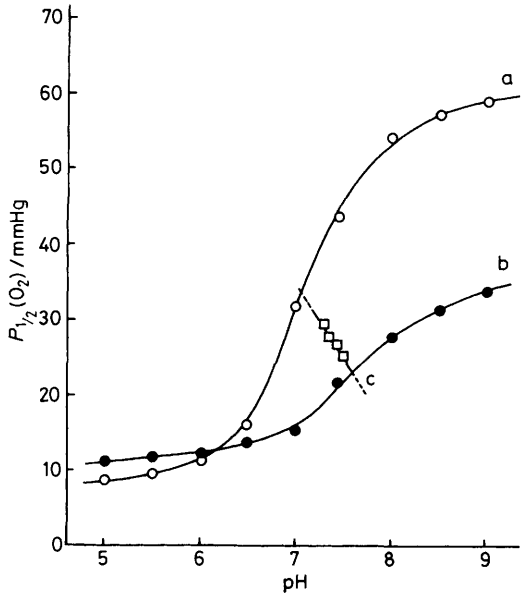
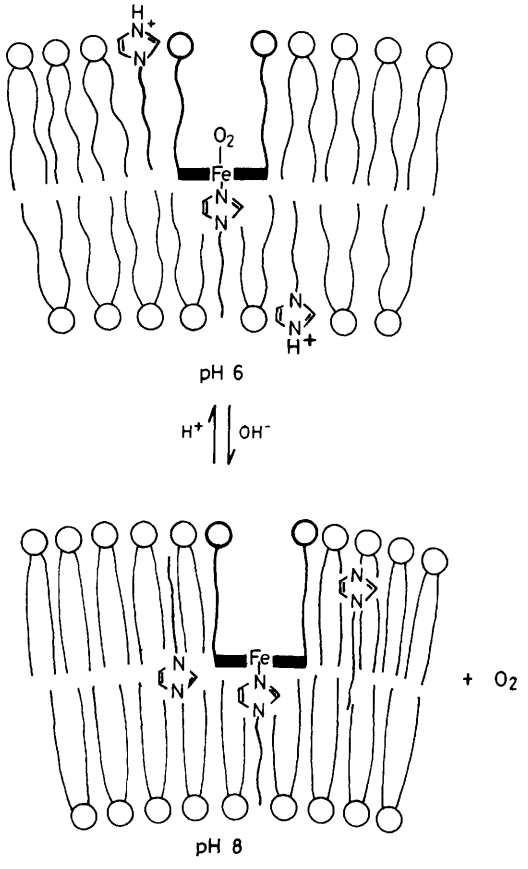


Figure 1. pH dependence of the oxygen-binding affinity of the haem–polymerized liposome at 37 °C. [haem] = 0.1 mM, [haem]/[ligand]/[lipid] = 1/3/50—100, a, haem–polymerized liposome; b, haem–egg yolk lecithin liposome; c, RBC suspension.



Scheme 1. Structural change of the haem–polymerized liposome with change in pH.

natural phospholipid such as egg yolk lecithin or with a synthetic phospholipid such as dimyristoylphosphatidylcholine, it was strongest for that with poly(vinylbenzoylnonanoyl-octadecyl-glycerol-phosphocholine). Furthermore, only for this synthetic phospholipid could the haem solution be highly concentrated without precipitation. The pH dependence curve of the oxygen-binding affinity obtained by measuring $p_{\frac{1}{2}}$ from higher pH by adding acid solution was consistent with that obtained from lower pH by adding alkaline solution. The oxygen-binding equilibrium was attained immediately after the pH equilibrium was reached.

To 100 ml of solution (pH 6) sodium hydroxide solution was added dropwise to increase the pH to 8. The colour of the solution changed simultaneously from dark red ($\lambda_{max.}$ 543 nm; oxy 78%) to brownish red ($\lambda_{max.}$ 539 nm; oxy 43%) and 3.6 ml of oxygen was evolved (calc. 4.28 ml of O_2 per 100 ml of solution, 84.6%, at [haem] = 5 mM and 25 °C). In contrast, on decreasing the pH from 8 to 6, 3.6 ml of oxygen per 100 ml of the medium was taken up. This pH-induced oxygen-evolution and -uptake could be repeated several times at room temperature.

This pH-induced reversible binding could be explained as follows (Scheme 1). The haem–polymerized liposome contains a small excess of 1-laurylimidazole as a ligand of the haem to complete the haem complex formation, and non-coordinated larylimidazole is situated in the bilayer directing the hydrophilic imidazole group outwards. The outward directing imidazole is protonated at lower pH (pK_a of the imidazole 7.20). This protonated imidazole is considered to increase the oxygen-binding affinity of the haem, for the following two reasons. (i) Since oxygen binding involves some charge separation and the oxygen-binding affinity increases with increasing solvent polarity,[8,9] the oxygen bound to the haem–polymerized liposome is stabilized in the presence of the protonated imidazole. (ii) The protonated imidazole destroys the packing structure of the bilayer or increases the mobility of the phospholipid in the bilayer. This change provides an environment around the haem similar to that provided by organic solvents such as toluene and allows the haem complex to adopt a relaxed structure in which it is stable without a structural distortion caused by the surrounding lipid bilayer structure and shows higher oxygen-binding affinity corresponding to that in organic solvents.

A similar pH-induced gas-uptake and -evolution was observed with carbon monoxide. The pH-induced reversible gaseous molecule-binding of the synthetic haem produced by an environmental or a structural change of the surrounding synthetic lipid liposome is a model of the RBC's Bohr effect where pH-induced conformational change of the globin protein induces the reactivity change of natural haem.

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