EFFECT OF CATIONIC DETERGENTS ON FERRIMYOGLOBIN

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1. Introduction

Spectral and magnetic effects produced by the interaction of horse ferrimyoglobin with cationic detergents such as LPC* have been described [1, 2]. The type of light-absorption spectrum obtained in the visible region is similar to that observed in the aqueous systems protonated polybase-FP, alkaline pyridine-FP, or electrolytes-FP, and has been termed 'green complex' [3, 4]. In all these systems, low paramagnetic susceptibility values have been obtained** [5, 6, 2]. Some new spectrophotometric and chromatographic experiments involving the ferrimyoglobin–detergent system are described below. It appears that the prosthetic group separates from ferrimyoglobin under certain conditions.

2. Materials and methods

Materials were obtained from the following sources: Myoglobin (ferri), from sperm whale, crystallized, salt-free, lyophilized, from Koch-Light (Colnbrook); hemin, bovine, twice recrystallized, from Sigma (St. Louis, Mo.); it was further recrystallized twice; cytochrome *c*, from Nutritional Biochemicals (Cleveland, Ohio); laurylpyridinium chloride (Dehyquart C), crystallized (94% pure + 6% water), from Henkel (Düsseldorf); cetyltrimethylammonium bromide, (21.2% Br), from B.D.H; Sephadex (G-200), from Pharmacia (Uppsala); Biogel (P-30), from Bio-Rad (Richmond, California); glass wool, from Merck (Darmstadt).

All other chemicals were reagent grade.

Stock solutions of ferrimyoglobin in water and of hemin in 0.01 M NaOH were stored in the dark at 0–5°C. Solutions containing myoglobin or hemin and detergent were prepared with continuous stirring, adding the detergent last. Absorption spectra were measured at 25 ± 1°C on a Cary Model 14 recording spectrophotometer.

3. Results and discussion

Fig. 1 shows the Soret and other visible-range bands of sperm whale ferrimyoglobin, both in the absence and presence of LPC. A similar spectrum is obtained for the system FP–LPC at pH 7.1 (see also ref. [1] for results at pH 10). The aliphatic detergent CTAB also produces a 'green-type' spectrum at pH 9 with ferrimyoglobin (conditions as given for fig. 1) except for the appearance of a peak instead of a shoulder of the band near 600 nm. Essentially the same spectrum is obtained with FP (6.7×10^{-5} M) in the presence of CTAB (1.2×10^{-2} M) at pH 10 (see also ref. [7]).

An investigation of the pH dependence of the system sperm whale ferrimyoglobin (~ 1×10^{-4} M)-LPC at 25°C showed that at an excess of LPC (molar ratio of over 200) the 'green' spectrum is produced above pH 5 up to at least pH 10.5 (Gersonde et al.

Gersonde et al. [2] explained the spectral changes which occur in the myoglobin–LPC system by polar-

*Abbreviations:
LPC: lauryl pyridinium chloride; CTAB: cetyltrimethylammonium bromide; FP: ferriprotoporphyrin IX.

** The term 'low-spin complexes' used previously (e.g. [5, 6]) should be avoided unless antiferromagnetic types of interaction in the aggregates can be ruled out.

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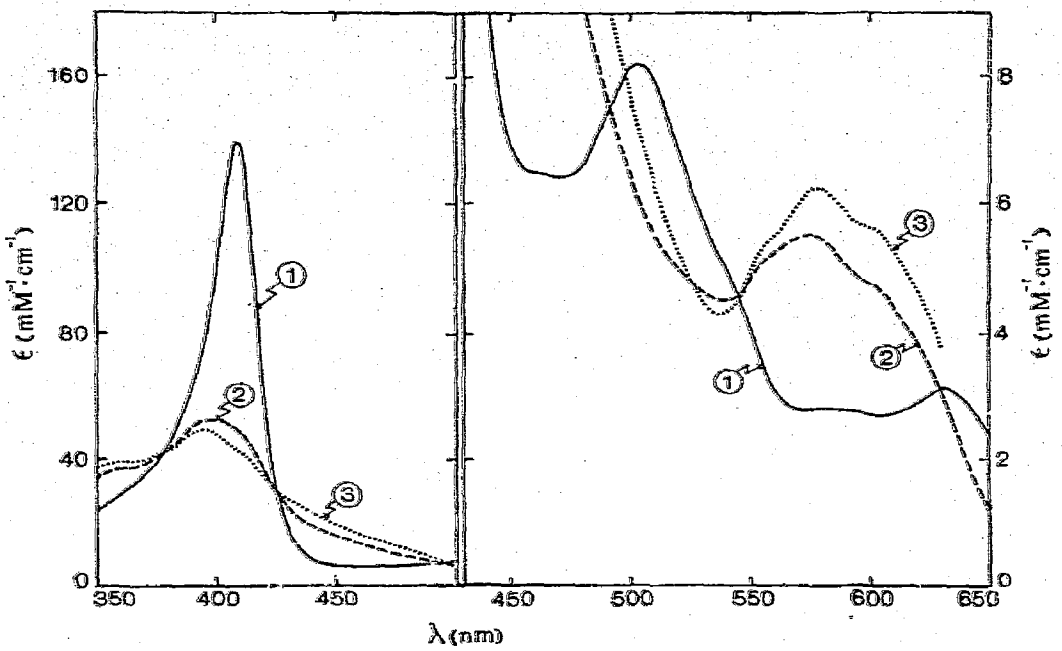

Fig. 1. Light–absorption spectra of ferriprotoporphyrin IX in various systems (including myoglobin). Prosthetic group, 1.0×10^{-4} M; 0.02 M sodium phosphate buffer, pH 7.15; temp., 25°C. Curve ①, ferrimyoglobin. Curve ②, ferrimyoglobin in the presence of 7×10^{-3} M LPC. Curve ③, FP in the presence of 1×10^{-3} M LPC. Water was used as reference solvent.

[2] investigated horse ferrimyoglobin at pH 8.35 only, and Niedick [1] at pH 8.2). At a molar ratio of LPC to ferrimyoglobin of 50, the 'green complex' is completely formed above pH 9.5. However, upon inclusion of 0.5 M NaCl, the 'green complex' is already produced at pH 7.4 under these conditions (see also ref. [8]).

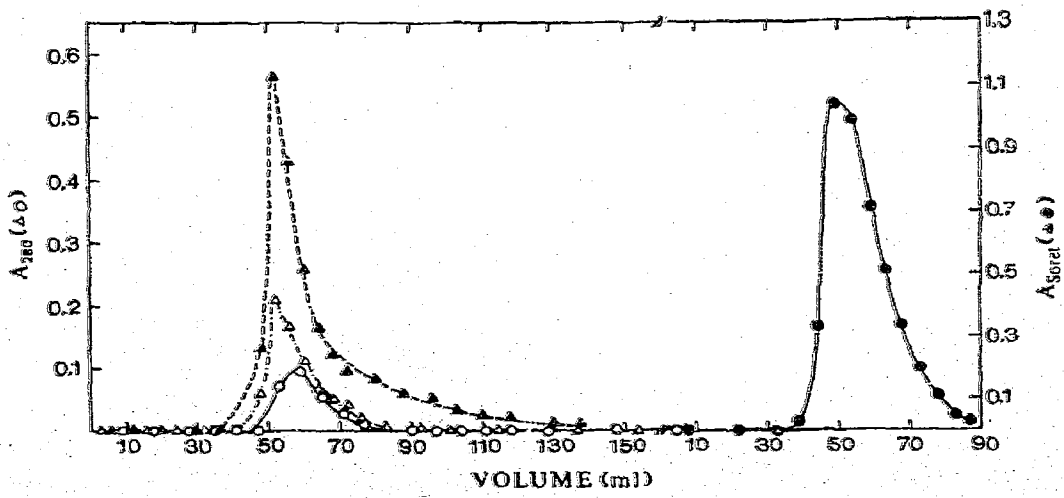

Fig. 2. Elution pattern of sperm whale ferrimyoglobin and of the system ferrimyoglobin–LPC on a column packed with glass wool (1.1 × 45 cm): 0.02 M sodium phosphate buffer, pH 7.16; room temp.; LPC, 7×10^{-3} M; myoglobin, 5×10^{-5} M; A_λ = absorbance per cm at wavelength λ in nm; A_{Soret} refers to the respective maxima at 410 nm for myoglobin and at 395 nm for the fraction eluted with acid. Reference solvents were either water or aqueous LPC solutions; ○, myoglobin–LPC, 4 ml, eluted with buffer–LPC; ●, above system, elution continued with acetic acid–aqueous HCl (50 vol. percent; 0.02 M HCl); ▵▲, myoglobin without LPC, 4 ml, eluted with buffer. The absorbance ratio A_{410}/A_{280} is practically the same as in the original ferrimyoglobin.

ization effects of the quaternary pyridinium ions on the heme π–electron system within the detergent micelle. Also, these authors considered the accompanying magnetic effects to result from direct coordination of protein ligands to the heme ion. These interpretations did not seem very likely to us in view of previous results obtained for the 'green complex', in which direct interactions between aggregated FP molecules were considered to be the cause for both spectral and magnetic changes [4–6, 8, 9]. Direct evidence was, therefore, sought for a possible removal of the FP from the protein through the action of the detergent, which may lead to interactions between FP molecules in the presence of detergent.

The system myoglobin–LPC at pH 7.1–7.2 was passed through a column packed with glass wool. The optical densities of the eluted fractions were measured at both 280 nm (protein peak) and in the Soret region. The results, illustrated in fig. 2, indicate that in the presence of LPC, the first elution band is colorless and contains only the apomyoglobin. After exhaustive dialysis of this fraction against distilled water, the composition of this hydrolyzed fraction was determined on an amino acid analyzer (Beckman 120-C) and found to correspond to apomyoglobin. The FP is strongly adsorbed on the glass wool, as can also be observed visually. It can be eluted by a mixture of acetic acid–aqueous HCl (50 vol percent; 0.02 M HCl; see [10]). FP in 0.02 M sodium phosphate buffer (pH 7.2), in the absence of LPC, is also strongly adsorbed and can be eluted by the same acidic solvent. cytochrome *c*–LPC, passed through the column under the same conditions as the complex myoglobin–LPC, retains its original absorption spectrum upon elution. This would be expected since the heme is covalently bound to the protein in this case [11]. But even ferrimyoglobin, in the absence of LPC, appears to leave the column unchanged (fig. 2). It should be noted that the elution pattern on the column used depends on the mode of packing of the glass wool. In separation experiments on Biogel or Sephadex G-200, the main fraction collected showed a considerably larger absorbance ratio of protein to Soret-band maxima than the original myoglobin–LPC system. It could not be ascertained in this fraction if the Soret absorption was due to unbound (aggregates) or bound FP. However, the data obtained are in qualitative agreement with separation of the FP from the protein in the presence of the detergent.

It may be concluded that the LPC causes at least partial separation of the FP from the apoprotein. In analogy with spectrally similar systems described above, the 'green complex' observed may be constituted of specifically arrayed FP aggregates or FP–LPC interaction products. A more detailed analysis of this system at various concentrations of the interacting species will be published elsewhere.

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