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Complex Formation Between Biliverdin and Apomyoglobin

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Summary. The intermolecular interactions of biliverdin with apomyoglobin were investigated using UV-VIS spectroscopy and chiroptical methods. Biliverdin is bound reversibly in the heme pocket of the apoprotein. The structural implications of the spectroscopic findings are discussed.

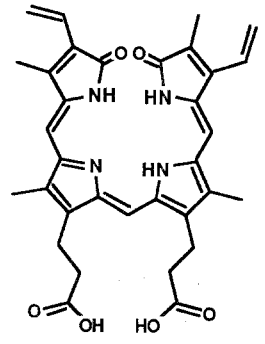
Keywords. Apomyoglobin; Biliverdin; Intermolecular interaction.

Komplexbildung zwischen Biliverdin und Apomyoglobin

Zusammenfassung. Die intermolekularen Wechselwirkungen von Biliverdin mit Apomyoglobin wurden mittels UV-VIS-Spektroskopie und chiroptischen Methoden untersucht. Biliverdin wird reversibel in der Häm-Tasche des Apoproteins gebunden. Strukturelle Implikationen der spektroskopischen Resultate werden diskutiert.

Introduction

In a recent paper we have reported investigations on intermolecular complex formation between bile pigments and two small proteins [1]. These efforts and the present investigation have been motivated by the various instances of close interactions of bile pigments and proteins in the animal and plant kingdoms. Recently Huber et al. have studied the crystal structure of the bile pigment binding protein of *Pieris brassicae* [2], which non-covalently binds pterobilin (biliverdin-IXγ). The phycobiliproteins and phytochrome, in which there is a covalent linkage between the bile pigment and the apoprotein, are functionally important as light harvesting and photomorphogenesis inducing factors [3]. Interestingly enough, the apoproteins in the phycobiliproteins exhibit considerable sequence homology and structural similarity to globins, especially myoglobin, as has been shown recently [4, 5].



BV

Therefore it seemed worthwhile to investigate the rather easily accessible system of biliverdin (BV) and apomyoglobin as a model for the interactions and structural changes encountered in the less readily available naturally occurring verdin-protein complexes. Analogous systems may be found in the literature: complex formation of apomyoglobin with bilirubin has been described [6] and a complex between chlorophyll and apomyoglobin has been studied [7].

Materials and Methods

Biliverdin was prepared by oxidation of bilirubin (Sigma) with 2,3-dichloro-5,6-dicyanobenzoquinone [8]. Apomyoglobin was prepared from sperm whale myoglobin (Sigma) by acid cleavage [9] and purified by gel chromatography. Heme was prepared from bovine hemoglobin following a well established procedure [10].

UV-VIS spectra were recorded on a Perkin-Elmer 330 spectrophotometer, CD-spectra on an Auto-Dichrograph Mark V (Jobin-Yvon).

Stoichiometric compositions were determined by the method of Job [11]. Formation constants were calculated using quantitative CD-spectroscopy and also by quantitative interpretation of UV-VIS difference spectra.

Preparation of the BV-apomyoglobin complex: 0.5 mg BV are dissolved in the minimum amount (approximately 0.15 ml) of 0.1 *N* NaOH, diluted with 1 ml 0.01 *M* KH_2PO_4, and the *pH* is adjusted with 0.1 *M* H_3PO_4 to 5.9, then 14 mg freshly prepared lyophilized apomyoglobin is added. The complex is separated from free biliverdin, desalted by gel chromatography (Sephadex G15), and lyophilized subsequently.

Results and Discussion

The formation of a biliverdin-apomyoglobin complex is immediately evident from the pronounced changes in the UV-VIS and CD-spectra of the chromophore upon addition of apomyoglobin (Fig. 1).

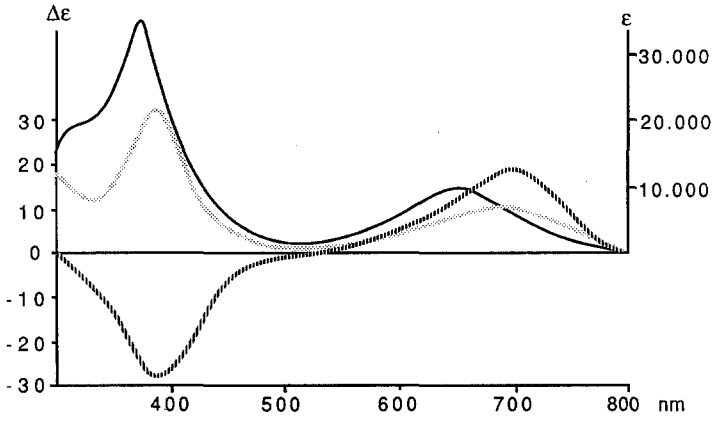


Fig. 1. UV-VIS spectrum of BV (full line), and UV-VIS (grey line) and CD (dashed line) spectra of the BV apomyoglobin complex at *pH* 5.9

The occurrence of a Cotton effect in the absorption range of the verdin chromophore is a clear indication of intermolecular interaction as free BV exhibits no chiroptical signal since the two rapidly interconverting helix enantiomers are present in equal populations. Bathochromic shifts of the long and short wavelength absorption bands by nearly 50 nm and 25 nm, respectively, are observed in the UV-VIS spectrum. Based on semi-empirical calculations [12] these relatively large shifts can be interpreted as indications of a flattening or compression of the helix (i.e. reduction of the torsions at the exocyclic chromophore double bonds) induced by the fitting of the bile pigment conformation to the shape of the heme pocket (*vide infra*). From the conservation of the ratio of long to short wavelength absorption coefficients it can be concluded that the circular helical structure of the bilindione chromophore persists in the complex.

From the signs of the CD bands one may deduce the helicity of the chromophore as (*P*), by applying the sign rule for C_2 symmetric chromophores [13]. The large Cotton effects observed indicate a considerable degree of enantiomeric discrimination. Based on values for the CD of pure bilindione helix-enantiomers [2, 14, 15] an enantiomeric excess of approximately 35% can be estimated. This value has to be considered with caution and might even be larger, because the helix in this complex appears to be flatter than the one in the chromophores from which the "pure" $\Delta\varepsilon$ values have been derived [2, 14, 15].

Monitoring titration experiments by means of UV-VIS-difference- (Fig. 2) and CD-spectra the stoichiometry of the complex is found to be equimolar and its formation constant can be deduced as $5 \cdot 10^5 \mathrm{l\,mol^{-1}}$ at *pH* 5.9. This constant is similar to the ones reported for the artificial complexes of apomyoglobin with bilirubin ($9 \cdot 10^5 \mathrm{l\,mol^{-1}}$) and protoporphyrin ($5.5 \cdot 10^6 \mathrm{l\,mol^{-1}}$) [6, 16]. For comparison, the binding constant for the native prosthetic group hemin is estimated as 10^{12} to $10^{15} \mathrm{l\,mol^{-1}}$ [16].

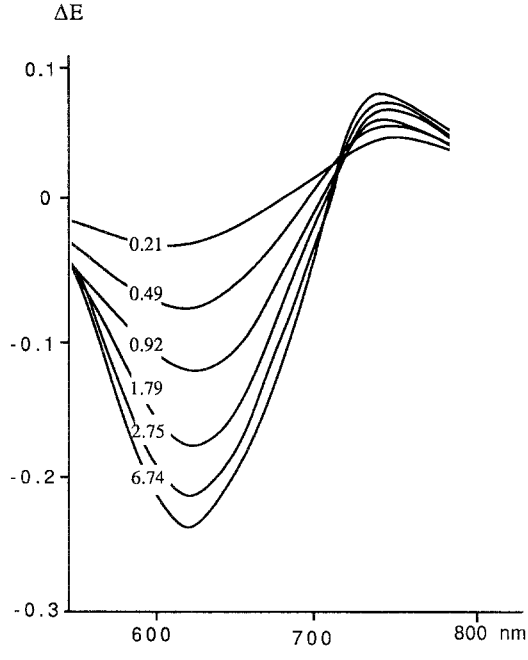


Fig. 2. UV-VIS titration difference spectra; parameters: $C_{apomyoglobin}/C_{BV}$

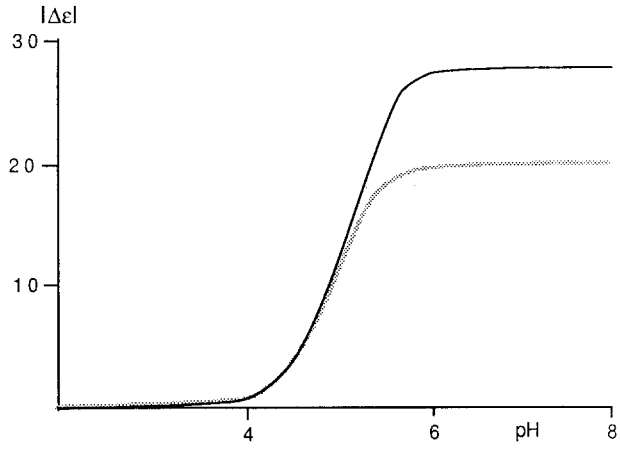


Fig. 3. Plots of *pH* dependence of the long (grey line) and short (full line) wavelength CD bands of the BV-apomyoglobin complex

The *pH* dependence of the binding constant can most effectively be investigated by observing the *pH* dependence of the CD bands (Fig. 3). Below *pH* 4 practically no binding occurs as judged from the absence of a Cotton effect. Above a steep rise of the signals saturation is reached at *pH* 6. No decrease of the chiroptical signals is observed up to *pH* 8.

The BV-apomyoglobin complex is kinetically more inert than the complexes of BV with the pancreatic trypsin inhibitor and lysozyme investigated in our previous communication [1]. It can readily be purified by gel chromatography without decomposition. Addition of hemin to solutions of the complex, however, leads to immediate displacement of the bile pigment. This finding indicates that both prosthetic groups compete for the same site and hence that biliverdin is bound within the heme pocket of the apoprotein. This result is also corroborated by the finding that biliverdin does not bind to native myoglobin even when added in large excess.

Acknowledgement

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