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On the Chemistry of Pyrrole Pigments, LXXXVII [1]: The Apomyoglobin Heme Pocket as a Reaction Vessel in Bile Pigment Chemistry

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Summary. An apomyoglobin biliverdin complex was reduced to a bilirubin apomyoglobin complex with retention of the helix chirality of the chromophore. Chelation of the mesobiliverdin-XIIIα apomyoglobin complex with zinc ions in aqueous solution yielded an enantiomer of the corresponding derivative. These two systems document the possibility of using the heme pocket of apomyoglobin to execute stereospecific reactions. The chiroptical properties of the two product systems are discussed.

Keywords. Apomyoglobin; Bilindiones; Reduction; Chelation; Absolute Configuration; Chiroptical Property.

Zur Chemie von Pyrrolpigmenten, 87. Mitt. [1]: **Die Häm-Tasche des Apomyoglobins als Reaktionsgefäß für die Chemie von Gallenfarbstoffen**

Zusammenfassung. Der Apomyoglobin-Biliverdin-Komplex wurde zum entsprechenden Apomyoglobin-Bilirubin-Komplex unter Retention der Helixkonfiguration des Chromophors reduziert. Chelierung des Apomyoglobin-Mesobiliverdin-XIIIα-Komplexes mit Zinkionen in wäßriger Lösung lieferte ein Enantiomer des entsprechenden Derivates. Diese beiden Systeme dokumentieren die Verwendbarkeit der Häm-Tasche des Apomyoglobins um stereospezifische Reaktionen durchzuführen. Die chiroptischen Eigenschaften der beiden Produktsysteme werden diskutiert.

Introduction

The area of supramolecular chemistry, which was founded nearly two decades ago [2], has been extremely successful in designing systems capable of executing reactions which otherwise are hampered by kinetic or thermodynamic barriers [3]. The stereochemistry of such reactions also may be influenced in these complexes to a significant extent, thus mimicking the action of enzymes [4]. These systems effect their unique function in most cases by means of embedding the substrate as a guest into a cleft, pocket, or cavity of the host molecule, which may be either of natural origin, as in the case of the cyclodextrins, or of purely synthetic provenance. Thus the host provides orientation of the substrate with respect to an attacking reagent and at the same time shields the substrate in a specific manner.

Recently we prepared a series of supramolecular systems by reconstituting apomyoglobin with several types of linear tetrapyrroles [5, 6]. The structural aspects

of these systems were studied by means of spectroscopic and thermodynamic methods and it was concluded that the bile pigments are embedded into the heme pocket of apomyoglobin [7]. They adopt a defined helical arrangement within the pocket as is common for most verdinoid bile pigments in solution [8]. Their (*P*) configurations are governed mainly by a molecular lever system which results from the anchoring of the pigment's propionic acid side chain(s) to certain amino acid residues chirally located at the heme pocket entrance [6]. These results suggested the interesting possibility of using the heme pocket of apomyoglobin in these supramolecular systems to provide a reaction vessel in order to synthesize reaction products otherwise inaccessible.

The first problem which we thought could be solved by this approach is the reduction of a verdinoid bile pigment of a certain helix configuration to a rubinoid product with a correspondingly defined helical configuration as illustrated schematically in Fig. 1. Such an asymmetric synthesis could also provide experimental evidence of absolute conformation in the rubinoid series. The absolute helical sense of the arrangement of the two dipyrrinone halves of a rubinoid pigment has been hitherto inferred either from the sign rule of the circular dichroism of C_2-symmetric chromophores [9] or the application of exciton coupling calculations [10].

The second problem which could possibly be attacked in this way is the synthesis of a defined chiral metal chelate of a verdinoid bile pigment as indicated in Fig. 2. Metal chelates of this type are chiral [8], however, to our knowledge resoltuion of such a racemate has not yet been achieved.

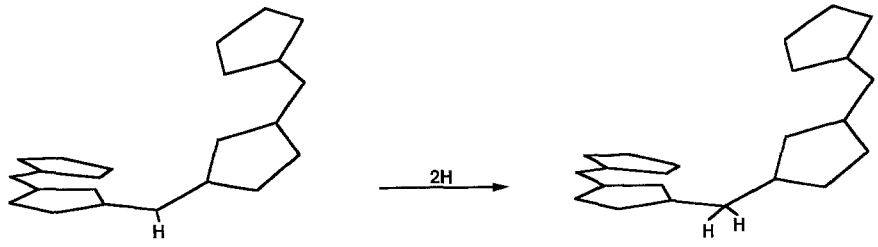


Fig. 1. Reduction (schematic drawing) of a verdinoid bile pigment of helix configuration (*P*) to a rubinoid bichromophoric pigment of configuration (*P*)

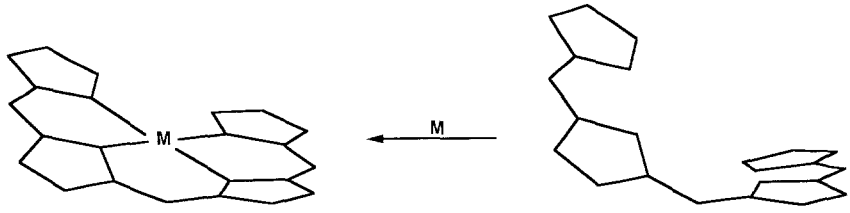


Fig. 2. Chelation (schematic drawing) of a verdinoid bile pigment of helix configuration (*P*) to a metal complex of configuration (*P*)

Experimental

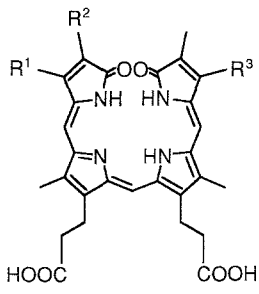
UV-VIS-specra were recorded on a Hitachi U-3210 spectrometer, CD data were obtained by means of an Auto Dichrograph Mark V (Jobin-Yvon), fluorescence was measured using a Zeiss MQ 3 instrument.

Apomyoglobin was prepared from sperm whale myoglobin (Sigma) by acid cleavage [11] and purified by gel chromatography on Sephadex-G 15.

Biliverdin (**1**) was prepared by oxidation of bilirubin (Sigma) as described by McDonagh [12]. The pterobilin type pigment **2** [13] was kindly provided by Prof. Dr. A. Gossauer (Fribourg); mesobiliverdin-XIIIα (**3**) was prepared according to literature [6]. Preparation of the apomyoglobin-pigment complexes of pigments **1**–**3** was achieved according to a recently reported procedure [6]. NADH and NADPH were of commercial origin (Sigma).

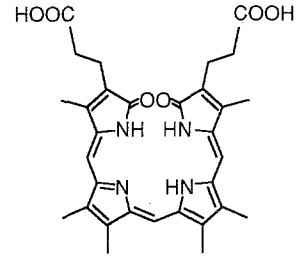
Reductions: To aqueous solutions of the apomyoglobin complexes of **1** and **2** at pH 9 the appropriate aqueous solutions of the reducing agents were added in equimolar amounts. Identity of the produced pigment with bilirubin was proven by isolation after destruction of the complex by adjusting to pH 5.

Zinc chelate of **3**: The apomyoglobin complex of **3** [6] was dissolved in distilled water, the solution adjusted to pH 7.5, and crystalline zinc acetate was added in threefold molar excess. After stirring for one hour (protection with argon) the solution was lyophilized and the lyophilizate purified by gel chromatography on a sephadex G-15 column. Before lyophilization of the product the solution was treated with an ion exchange resin (Chelite C; Serva).



1 $R^1=CH_3$, $R^2=R^3=$ vinyl

3 $R^2=CH_3$, $R^1=R^3=$ ethyl



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Results and Discussions

Reduction

Reduction of the apomyoglobin biliverdin complex to yield the corresponding apomyoglobin bilirubin complex by means of sodium borohydride or sodium dithionite is easily achieved in aqueous solutions of the complex. However, at pH 7 the derived bilirubin apomyoglobin complex is unstable and immediately decomposes. Therefore it is necessary to reduce the biliverdin system at pH 9 as it is known that at this value bilirubin and apomyoglobin form a stable complex [14]. The resulting absorption and CD data are shown in Fig. 3 together with the correspinding spectra of the educt. These bilirubin complex data are identical with those observed on direct reconstitution of bilirubin and apomyoglobin [14]. Since the educt system helix is of configuration (*P*) [5, 6] the configuration of the resulting bilirubin bichromophoric system is also (*P*) (compare Fig. 1). This result follows immediately from the tight dissymmetric anchoring of the propionic acid side chains

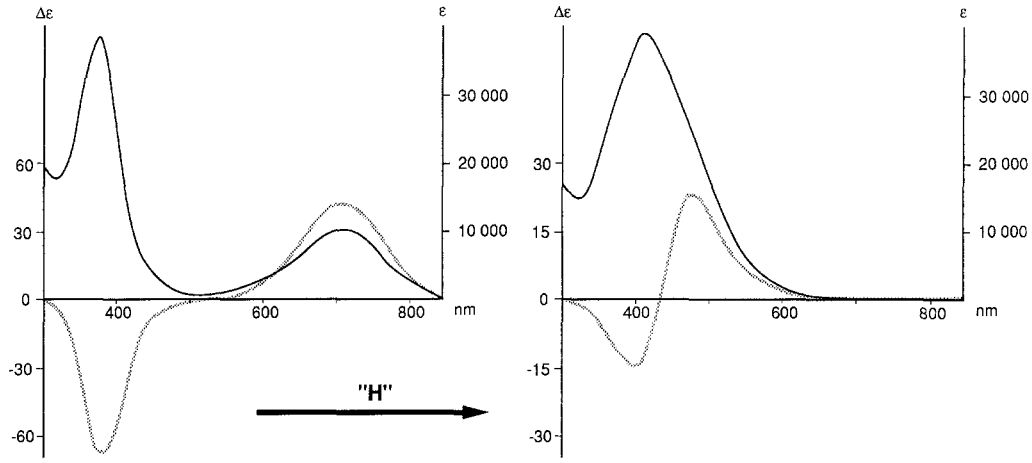

Fig. 3. Absorption (full line) and CD (gray line) spectra of the apomyoglobin biliverdin complex and its reduction product, the bilirubin apomyoglobin complex in water at pH 9

to the entrance of the protein pocket [6] which, of course, does not change in the process of the reduction step. With respect to conformational details the extent of dihedral deformation of the verdinoid and the rubinoid pigments within the pocket (Fig. 1) have to be very similar due to the spatial constraints resulting from the shape of the protein pocket and the anchoring of the propionic acid side chains at its entrance region [6, 7]. Accordingly, in this complex bilirubin exists in a rather flat ridge tile arrangement.

The absolute configuration derived in this way is in accord with the results gained from an application of the sign rule of C_2-symmetric chromophores [9] as well as from model calculations using the exciton theory [10]. Accordingly, an absolute asymmetric synthesis with retention of the helical configuration is achieved using apomyoglobin as a chiral reaction vessel. It is interesting to note that this rather tight and rigid attachment of the educt verdinoid chromophore to the protein pocket is also reflected in a more than doubling of the fluorescence quantum yield (aquesous solution) comparing free and complexed **1**.

Reduction of the biliverdin apomyoglobin complex could not be achieved using NADH or NADPH as the reducing agents. Obviously, this apoprotein cannot be regarded as a model system of biliverdin reductase.

Attempts to add nucleophiles like thiophenol, mercaptoethanol, and glutathione to position 10 of the biliverdin system were unsuccessful due to immediate denaturation of the protein.

In the case of the apomyoglobin complex of the pterobilin derivative (**2**) experiments using sodium borohydride or sodium dithionite as the reducing agents were unsuccessful. Thus, the dipyrrin region of this pigment is effectively shielded within the protein pocket [6] and the reducing reagent species are far too large to enter this pocket.

Chelation

Preparation of the zinc chelate of the apomyoglobin-**3**-complex is easily achieved by adding zinc acetate to an aqueous solution of the pigment protein system. Its

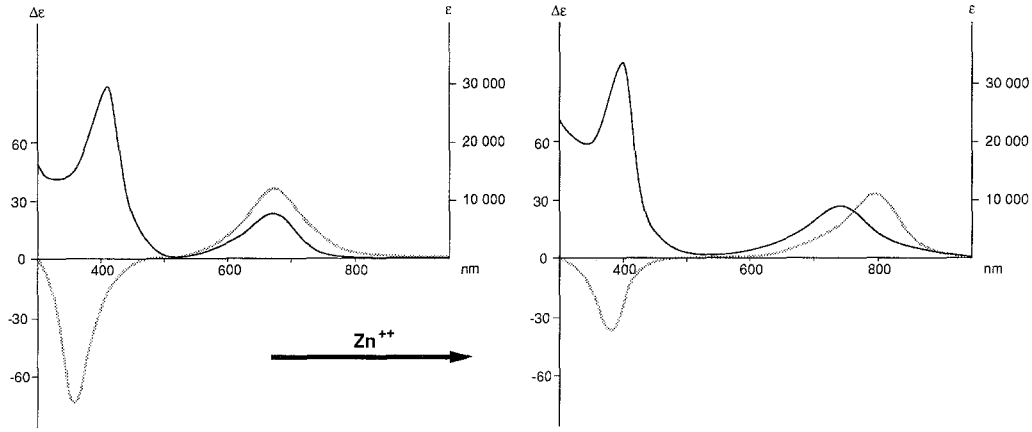

Fig. 4. Absorption (full line) and CD (gray line) spectra of the apomyoglobin complex of **3** and its chelation product with zinc ions in water at pH 6.8

purification and deionization yields the complex chelate system. Its absorption and circular dichroism data are shown in Fig. 4 together with those of the educt system. A strong bathochromic shift of the long wavelength band is observed on chelation. Since the heliciy of the pigment system remains unchanged on complexation, being governed by the anchoring of the propionic acid side chains [6], configuration (*P*) is also associated with a positive sign of the long wavelength band and a negative sign of the short wavelength band of the system. Whereas the intensity of the long wavelength circular dichroism band remains nearly unchanged, the intensity of the short wavelength band circular dichroism is reduced. Thus, it has been possible for the first time to prepare an enantiomer of a verdinoid chelate by asymmetric synthesis and, moreover, to deduce its absolute configuration.

The stability of this system is very high as neither addition of **3** nor of heme leads to a displacement of the chelate from the protein pocket. Additional coordination of the zinc ion with histidine residue 93 [15] within the protein pocket is clearly responsible for this dramatically enhance stability. This stability of the chelate apomyoglobin system should be compared with the stability of verdin metal chelates. These are known to be completely unstable in aqueous solution [8]. Thereby this system could also serve as a model of chelatases.

Acknowledgements

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