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Modification of Separated α and β Subunits of Human Hemoglobin by Iron Tetrasulfonated Phthalocyanine

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In previous papers [1–3] we have shown that it is possible to obtain artificial hemoglobins with metal tetrasulfonated phthalocyanine in the place of heme.

The results presented demonstrate that combination of apoproteins of isolated α and β subunits (apoα and apoβ) with iron tetrasulfonated phthalocyanine (Fe(III)L) leads to the formation of close analogs of the native α and β-chains, Fe(III)L(apoα) and Fe(III)-L(apoβ). Both model complexes exhibit characteristic absorption bands at 650 and 678 nm, for the oxidized and the reduced form, respectively. Incorporation of Fe(III)L into α-globin results in a decrease of its molecular weight to the value corresponding to the monomer (21500). Free α-globin in the same conditions occurs as a dimer. Combination of Fe(III)L with β-globin brings about an increase in molecular size from dimeric to tetrameric (64500). The same is observed in the reaction of heme with α and β-globins. Interaction of Fe(III)L(apoα) with Fe(III)L(apoβ) leads to the formation of tetrameric compound [Fe(III)L(apoα)–Fe(III)L(apoβ)]$_2$.

Combination of α and β-globins with Fe(III)L gives a significant increase of helicity in both proteins, as shown by CD spectra (Fig. 1). In contrast to heme-containing α and β-chains both phthalocyanine derivatives differ in helicity content, that of Fe(III)L(apoβ) being higher. Reconstituted phthalocyanine hemoglobin [Fe(III)L(apoα)–Fe(III)-L(apoβ)]$_2$ exhibits a far UV CD spectrum different from the sum of the individual CD spectra of its subunits. This fact suggests that combination of the modified α and β-chains results in a change in their conformations due to chain–chain interaction, to produce a more ordered or differently ordered structure.

Like native α and β-chains, their phthalocyanine analogs are not equivalent in their properties. It is shown that reduction of the modified β-chain by ascorbic acid occurs at a markedly greater rate than that of the α-chain. Likewise, the rate of oxygen binding is higher in the case of the β-chain. Reconstituted phthalocyanine hemoglobin displays a much higher stability than its subunits.

Recombination of the phthalocyanine modified α and β-subunits with the alternate heme-containing subunits gives hybrid hemoglobins [Fe(III)L(apoα)–heme(apoβ)]$_2$ and [Fe(III)L(apoβ)–heme(apoα)]$_2$. These compounds are stable in their ferric forms.

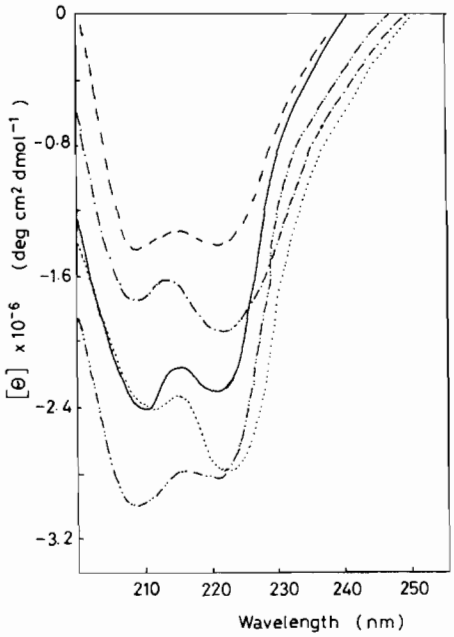

Fig. 1. CD spectra of Fe(III)L(apoα) (– – –), Fe(III)L(apoβ) (– · · –) and [Fe(III)L(apoα)–Fe(III)L(apoβ)]$_2$, compared to α-globin (- - - -) and β-globin (- · - · -).

Reduced with dithionite they combine with molecular oxygen. Deoxygenation by argon bubbling leads to the formation of inactive species which finally undergo irreversible denaturation.

Further studies are in progress.

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Q6

Infrared Spectroscopy of Carbonmonoxide Ligation to the Coelomic Hemoglobins of *Glycera Dibranchiata*

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Glycera dibranchiata is a marine annelid which possesses both a high molecular weight hemoglobin fraction and a monomer hemoglobin fraction of molecular weight 15,590D [1–8]. NMR studies along with companion isoelectric focusing and column chromatography work have established the extensive microheterogeneity of the monomer hemoglobin

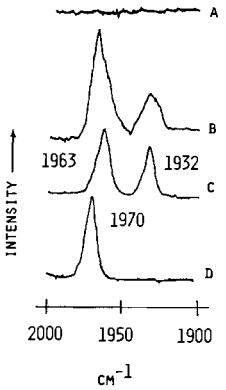

Fig. 1. Infrared spectra in the CO stretching region: A, oxidized, unseparated *Glycera* hemoglobin + CO; B, reduced, unseparated *Glycera* hemoglobin + CO; C, high molecular weight fraction + CO; D, monomer hemoglobin fraction + CO. Note the three frequencies for heme ligated CO in the two separated fractions C, D. Protein concentrations were 2.0 m*M*, 0.1 phosphate buffer, pH 6.8.

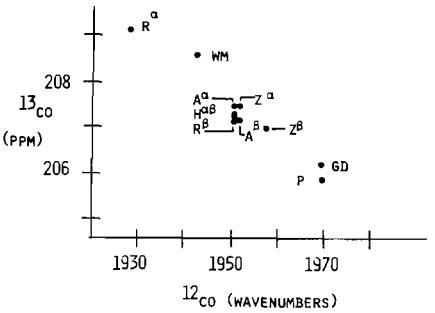

Fig. 2. Correlation between carbon chemical shifts and CO infrared stretching frequencies for ^{13}CO and ^{12}CO ligated heme proteins and imidazole–ferrous porphyrin–CO. Abbreviations used: R = New Zealand white rabbit hemoglobin; Z = hemoglobin Zurich; A = human hemoglobin A; WM = sperm whale myoglobin; GD = *Glycera* monomer hemoglobin which lacks the distal histidine; P = ferrous porphyrin complex. Data in this table are literature values from references 13, 14, 18, and from references therein.

fraction [9]. However, to date, it has been impossible to successfully obtain proton nmr spectra of the high molecular weight fraction. In part, this is due to its ready tendency to aggregate into a gel state. Subsequently, infrared spectroscopy has been used to probe the heme ligand binding site in both of the *Glycera* hemoglobin fractions.

Experimental

Infrared spectra of *Glycera* hemoglobin fractions were obtained using CaF cells. Isolation of *Glycera* hemoglobin fractions has been previously detailed [9], and the proteins were handled in 0.1 *M* phosphate buffer (Mallinkrodt), pH 6.8. ^2H_2O (99.8%, Merck) was selected as the buffer solvent to insure an infrared window between 2000 and 1920 cm^{-1}. Infrared spectra were obtained either on a Nicolet 6000 FTIR or a Perkin Elmer 621 grating spectrometer.

Results and Discussion

Figure 1 reveals the existence of at least three C–O stretching frequencies, indicating multiple heme bound carbon monoxide coordination sites in the unseparated hemoglobin mixture and in the separated higher molecular weight fraction. The separated monomer fraction exhibits only a single C–O infrared frequency at 1970 cm^{-1}, identical to this frequency in ferrous porphyrin–CO [10–12]. This is interesting in view of the fact that X-ray crystallography [3] indicates that at least one of the monomer component hemoglobins lacks the 'ubiquitous' distal histidine (E-7). Because the essential 'myoglobin fold' structure is preserved in the *Glycera* monomer along with a substantial degree of amino acid conservation in the primary sequence of the heme pocket residues [6], this data suggests the influence which a distal residue exerts on the spectroscopic properties of heme bound CO. Likewise, the carbon-13 NMR frequency of heme bound ^{13}CO in the *Glycera dibranchiata* monomer fraction has been reported to be identical to imidazole–ferrous porphyrin–^{13}CO molecules [13, 14].

This data suggests an interesting correlation between infrared stretching frequencies for heme bound ^{12}CO, or ^{13}CO and chemical shifts for the same molecules with heme bound ^{13}CO. The empirical result is shown in Fig. 2. That figure further suggests that the type of distal ligand in heme proteins may be ascertained either by NMR ^{13}CO chemical shift, or by infrared CO frequency.

It is also to be noted that among the proteins which exhibit ν_{CO} near 1950 cm^{-1} there are slight differences. This is obvious for rabbit α chain ν_{CO} and β chain ν_{CO} and between human hemoglobin ν_{CO} and whale myoglobin ν_{CO}. Similar differences occur in the C-13 NMR spectra. It is likely that these differences can be attributed to the degree of distal histidine interaction with heme bound CO. The correlation is one where enhanced interaction reflects downfield ^{13}CO shifts and infrared ν_{CO} bands at lower wavenumbers. Therefore, the correlation of Fig. 2 reflects the distal ligand interaction with heme bound CO as well as the nature of the distal ligand.

Additional pieces of evidence for this view originate with elephant myoglobin [15]. This is a unique mammalian myoglobin in that the distal histidine is replaced by glutamine in the primary sequence [15]. Although no exact values of ^{13}CO chemical shift or ν_{CO} have yet been published, current observations indicate that ν_{CO} lies at lower wavenumbers than ν_{CO} for myoglobin [16]. Similarly, the

unreported ^{13}CO chemical shift appears further downfield than in mammalian hemoglobins [17]. Thus, until specific values are published, what can be said is that elephant myoglobin conforms to the correlation of Fig. 2, in general terms. What can be concluded is that the position of elephant myoglobin–CO in the correlation indicates that the distal residue––CO interaction in this protein is enhanced over normal mammalian hemoglobins and myoglobins. That is, there seem to be 'better' distal ligands than the ubiquitous histidine E-7. A further conclusion is that ^{13}CO chemical shifts or infrared frequencies of CO-protein may be a quick, useful analytical technique for characterizing the distal amino acid in unsequenced b-type heme proteins.

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Q7

Chloroquine Interaction with Ferric Uroporphyrin in Solution

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It is estimated that, in 1979, 80% of the world's population was at risk to *Plasmodium* infection (malaria), primarily in the developing nations [1]. Chemotherapeutic agents useful in treatment include the quinine based drugs and the quinoline derivatives such as chloroquine [2, 3], although their molecular basis of action is not presently well understood. In patients undergoing malaria chemotherapy the drug is found in association with hemozoin pigments in the erythrocyte. Hemozoin is an aggregate of precipitated heme and denatured hemoglobin and this finding has stimulated our interest in the possibility that the antimalarial efficacy originated from association with heme complexes. Fitch *et al.* [4] have, in fact, suggested that erythrocyte localized protohemin IX is the putative receptor of chloroquine.

In this report we present some of our initial results concerning the interaction of chloroquine with iron porphyrins in solution. Urohemin was chosen due to its high solubility in aqueous solutions and because recent raman and nuclear magnetic resonance (NMR) work has allowed us to thoroughly characterize its solution dynamics [5].

Experimental

Urohemin was purchased from Porphyrin Products, Logan, Utah and was further purified by column chromatography. Chloroquine (Sigma) was used without further purification. Titrations of urohemin with the drug were carried out at pH 6 (unbuffered) employing a Cary 219 ultraviolet–visible spectrometer. pH was monitored throughout the experiment.

Results and Discussion

The data of Figs. 1 and 2 reveal that chloroquine does indeed associate with urohemin in solution. Changes in the optical spectrum (Fig. 1) occur, which may be used in an attempt to quantitate equilibrium behavior (Fig. 2).

The result of adding chloroquine to a solution of urohemin monomer (Fig. 1) is a spectrum in which the Soret intensity is lost, characteristic of the dimer (and higher order aggregates). However, unlike the