SIMILARITIES OF THE HEME ENVIRONMENT IN VERTEBRATE AND NON-VERTEBRATE OXYGEN-BINDING HEMOPROTEINS

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1. Introduction

In the search for structural features essential for the functional properties of oxygen binding, hemoproteins, hemoglobins and myoglobins from a great variety of species have been investigated. The results to date indicate that there is an essentially complete lack of conservation of amino acid sequence which would distinguish the oxygen-binding hemoproteins from hemoproteins with different functional properties [1–3]. On the other hand, the presently known X-ray data reveal closely related protein conformations in vertebrate and non-vertebrate hemoglobins and myoglobins [2, 4–10], indicating that invariant structural properties of oxygen-binding hemoproteins might be more clearly manifested in the three-dimensional arrangement of the protein than in the amino acid sequence. In this paper we report evidence obtained by high resolution proton nuclear magnetic resonance (NMR) spectroscopy that an aliphatic amino acid residue is located very close by to the distal side of the heme plane in chironomus hemoglobin and aplysia myoglobin. The same had previously been observed in single crystals and solutions of vertebrate hemoglobins and myoglobins [4, 5, 11], and in single crystals of chironomus hemoglobin [2]. Thus from the present evidence the close approach of an aliphatic amino acid residue to the distal side of the heme plane might turn out to be one of the features common to the molecular conformations in oxygen-binding hemoproteins.

2. Materials and methods

Chironomus hemoglobin and aplysia myoglobin were prepared as described previously [3, 12]. For the NMR studies ca. 8×10^{-3} M solutions of the ferric proteins in 0.1 M deuterated phosphate buffer, pD = 7.0, were obtained by ultra-filtration. Horse heart ferrimyoglobin was purchased from Pentex Inc. The ferrous carbon monoxide compounds ($Mb^{II}CO$, $Hb^{II}CO$) were obtained by reduction with dithionite in a carbon monoxide atmosphere, and the completion of the reaction was checked by optical spectroscopy.

High resolution proton NMR spectra were recorded on a Varian HR-220 spectrometer equipped with a standard temperature control unit and a Varian 1024 computer of average transients for data accumulation. Chemical shifts are expressed in parts per million (ppm) from the position of internal sodium-2,2-dimethyl-2-silapentane-5-sulfonate (DSS), where shifts to low field at constant radio frequency are assigned negative values.

3. Results

In the proton NMR spectrum of chironomus $Hb^{II}CO$ (fig. 1), where the strongly overlapping resonances between 0 and −9 ppm correspond to ca. 900 protons of the polypeptide chain and the heme group, several rather well resolved lines are in the spectral regions from −9 to −10.5, and 0 to 2.5 ppm.

Prominent NMR spectral features in proteins can arise from the local ring current fields in aromatic rings [13, 14]. Comparison with the amino acid resonance positions in denatured proteins [15] shows that the resonance at 2.3 ppm in chironomus $Hb^{II}CO$, which corresponds in intensity to ca. 2 protons, has to be shifted upfield by at least 3.5 ppm under the influence of ring current fields. From the known dependence of direction and extent of ring current shifts on the size of the aromatic ring and the relative positions of the observed protons and the ring [13, 14] this resonance has to come from an aliphatic amino acid side chain located very close by to the face of the heme group. Using the atomic coordinates [2] the resonance shifts due to the local ring current fields can be estimated for all the protons in chironomus $Hb^{II}CO$ [11]. These calculations indicate that the resonance at + 2.3 ppm corresponds to protons of isoleucine E-11. Furthermore, evidence is obtained that the resonances of ca. 18 protons between 0.7 and 1.0 ppm come from aliphatic amino acid side chains surrounding the heme group (among these: leucine B-10, isoleucine C-4, valine FG-5, isoleucine E-11), and that the quite well resolved resonances of total intensity ca. 4 protons between −9 and −10.5 ppm are due to the meso-protons of protoheme IX.

In fig. 2 the spectral regions from 0 to 4 and from −8 to −12 ppm in the NMR spectra of aplysia $Mb^{II}CO$, Horse $Mb^{II}CO$ and chironomus $Hb^{II}CO$ are compared. In all 3 species a resonance is observed in an outstanding high field position. In aplysia $Mb^{II}CO$ a line of 2–3 protons is at 3.0 ppm, in horse $Mb^{II}CO$ a resonance of 3 protons at 2.6 ppm, and in chironomus $Hb^{II}CO$ there is the resonance at 2.3 ppm. The only plausible explanation is that in all 3 species an aliphatic amino acid residue is located very close to the center of the heme plane [14]. For all the 3 species the spectra in fig. 2 show further that there are several resonances between 0 and 1 ppm, and that the resonances of the mesoprotons of protoheme IX are quite well resolved between −9 and −10.5 ppm.

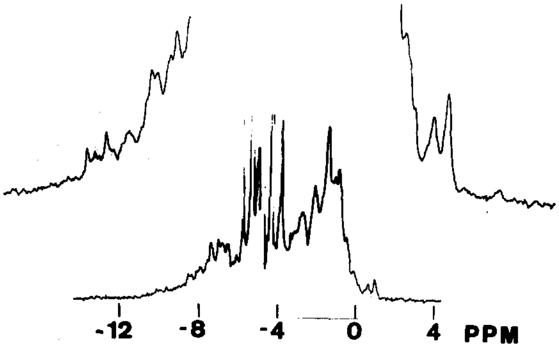

Fig. 1. Proton NMR spectrum at 220 MHz of chironomus $Hb^{II}CO$, T = 25°. The intense lines between −3.8 and −6.0 ppm correspond to the resonance of the residual protons of the solvent and its spinning side bands. The spectral regions from 0 to 4 and −8 to −12 ppm are shown with two different scales.

The high field lines in aplysia $Mb^{II}CO$ and chironomus $Hb^{II}CO$ are somewhat broader than the corresponding resonance in horse $Mb^{II}CO$ (fig. 2). From studies of the ferric cyanide derivatives and from observations in the region of the heme mesoproton resonances it appears that this might arise because more than one molecular conformation contributes to the NMR spectra of these 2 non-vertebrate proteins [16]. The sequence data of chironomus hemoglobin [2, 17] provide indeed evidence for heterogeneity of the crystalline material.

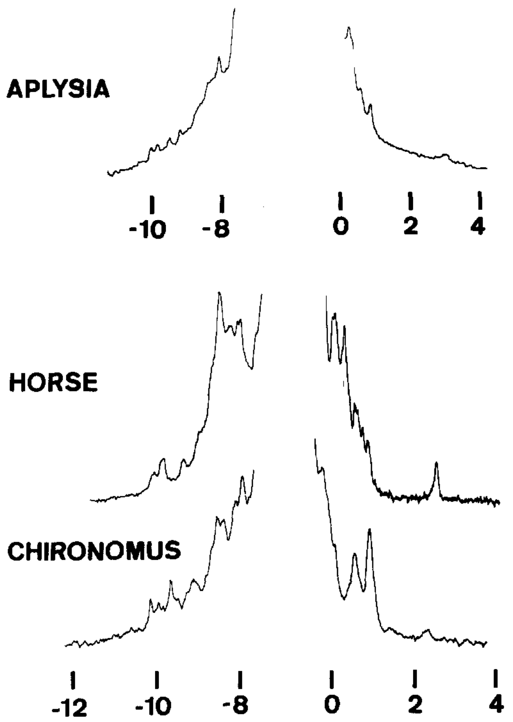

Fig. 2. Spectral regions from 0 to 4, and −8 to −12 ppm in the proton NMR spectra at 220 MHz of aplysia Mb^{II}CO, horse Mb^{II}CO, and chironomus Hb^{II}CO, T = 25°.

4. Discussion

The presently available data [11, 18] indicate that the appearance of a high field proton resonance in the range + 2 to + 3 ppm is a common feature of the diamagnetic low spin ferrous form of "normal" hemoglobins and myoglobins. In the 2 species which have been examined in more detail, sperm whale myoglobin [11] and chironomus hemoglobin [16], this resonance comes, respectively, from valine E-11 and isoleucine E-11, which are both located very near the center of the distal side of the heme plane [2, 4]. If it is assumed that the only histidyl residue in aplysia myoglobin is the proximal ligand of the heme iron, which is very likely in view of the spectral properties of the heme group and the preliminary sequence data [3, 16], it seems safe to assign the high field line in this protein to an aliphatic amino acid side chain located on the distal side of the heme group. For, with histidine in the position of the proximal ligand, no amino acid residue on the proximal side could conceivably be located sufficiently close to the center of the heme plane for any of its resonances to be shifted to a position near + 3 ppm. More specific resonance assignments in aplysia Mb^{II}CO will probably have to await the availability of the atomic coordinates in this protein.

The present study thus provides some evidence that the invariance of the general pattern of apolar heme contacts found in the comparison of chironomus hemoglobin and vertebrate myoglobins [2] holds also in aplysia myoglobin. This would be in line with the well recognised fact that the hydrophobicity of the heme environment might be an important factor for the stability of both the pentacoordinated deoxy form and the ferrous complex with O_2 in hemoglobins and myoglobins [19, 20].

Acknowledgement

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