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Strong vibronic coupling in heme proteins

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Abstract

We report the near infrared absorption spectra of cyanomethemoglobin and cyanometmyoglobin in two different solvents (deuterated solutions containing 65% v/v glycerol$(OD)_3$ or 65% v/v ethylene glycol$(OD)_2$). At 25 K the spectra show a clearly resolved fine structure that can be accounted for by considering a strong coupling of the porphyrin-to-iron charge transfer transitions with a single vibrational mode at 365 cm^{-1}. The coupling constants depend on both the specific electronic transition and the protein surrounding the chromophore, indicating once more the specificity of heme globin interactions.

Keywords: Protein stereodynamics; Low temperature optical spectroscopy; Vibronic coupling; Heme proteins; Hemoglobin; Myoglobin

1. Introduction

The effect of vibrational coupling on the optical absorption spectra of metal proteins and other biomolecules has recently received increasing attention [1–9]. The interest of such studies stems from their contribution to provide information on the electronic structure of the active site and on the dynamic properties of the protein, in the proximity of the chromophore. Recent methodological advances in the analysis (transform [10] and time-correlator [11] techniques) permit to relate the absorption bands to the resonance Raman excitation profiles and therefore to determine normal mode frequencies and vibronic coupling constants and also to investigate various sources of spectral broadening in biomolecules. In particular the absorption spectra of β-carotene [3], of cytochrome-*c* [1] and of deoxy and carbonmonoxy-myoglobin [2] have been analyzed.

For metal proteins investigated to date, however, only weak coupling (linear coupling constants $S < 1$) with several vibrational modes has been observed, so that the optical spectra remain deceivingly smooth and without any clearly resolved vibronic structure even at cryogenic temperatures [1,2,5–9].

We report here the low temperature ($T = 25$ K) near infrared absorption (NIR) spectra of the two low spin ferric heme proteins cyanomethemoglobin (Hb-CN) and cyanometmyoglobin (Mb-CN), in two different solvents. These spectra exhibit a well resolved fine structure; a suitable analysis, within the framework of (harmonic) Franck–Condon approximations taking into account vibronic coupling, shows that they are com-

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patible with four electronic transitions strongly coupled to a single vibrational mode at 365 cm^{-1}. To our knowledge this is the first report of strong vibronic coupling in the spectra of metallo-proteins.

2. Materials and methods

Hemoglobin was prepared from the blood of a single donor, as already described [12]. Concentrated oxyhemoglobin was oxidized by addition of a 4-fold molar excess of potassium ferrycianide; excess ferrycianide and ferrocyanides were removed by dialysis. The oxidized protein was deuterated by prolonged dialysis against 0.1 *M* phosphate buffer in D_2O. Ferric myoglobin (from horse skeletal muscle), purchased from Sigma, was dissolved in 0.1 *M* phosphate buffer in D_2O and centrifuged to remove any precipitate; the protein was deuterated as for hemoglobin. The cyanomet forms were obtained by addition of 0.1 *M* KCN. For optical measurements samples were deuterium oxide solutions containing 1.5 m*M* protein (in heme), 0.1 *M* phosphate buffer ($KD_2PO_4 + K_2DPO_4$, pD 7 in D_2O at room temperature) and 65% v/v glycerol $(OD)_3$ or ethylene glycol $(OD)_2$ (Lots N 323-K, 97.8 atom %D and 6148-M, 98.9 atom %D, respectively) purchased from MSD isotopes (Montreal-Canada). Absorption spectra were measured with a Varian 2300 spectrophotometer interfaced to a IBM PC. Spectral data acquisition was performed at 2.5 nm steps; the bandwidth was less than 2 nm in the whole spectral range. The experimental setup for optical measurements at cryogenic temperatures is described in Ref. [5]; our samples are homogeneous and transparent also at 25 K without cracking. The absorption spectra, both at 290 K and at 25 K, of the cuvette (methacrylate, Kartell) plus solvent were measured in separate experiments and subtracted from sample spectra.

3. Results and discussion

Near infrared absorption spectra (1800–800 nm) of Hb-CN and Mb-CN in D_2O solutions at

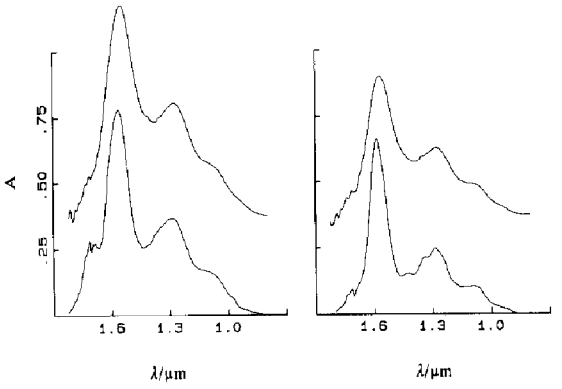

Fig. 1. Near infrared spectra of Hb-CN (left) and Mb-CN (right) in 65% v/v glycerol $(OD)_3/D_2O$ solutions at 25 K and at 290 K. The spectra at high temperature have been shifted along the vertical axis for clarity.

room temperature were already reported by Eaton and coworkers [13–15], who attribute the observed bands to charge transfer transitions from the top four filled porphyrin π orbitals to the d_{yz} iron orbitals [15]. This assignment is also supported from magnetic circular dichroism [13] and single crystal polarized absorption spectroscopy [16]. It must be noted that other electronic transitions are not expected in this frequency range since crystal field calculations [15] show that the lowest frequency for a spin allowed *d–d* transition is about 1.7×10^4 cm^{-1}, i.e. far outside the (10^3–10^4 cm^{-1}) range studied here.

Figures 1 and 2 show the NIR spectra at 25 K of Hb-CN and Mb-CN, in 65% v/v glycerol $(OD)_3/D_2O$ and 65% v/v ethylene glycol $(OD)_2/D_2O$ respectively, with the respective spectra at room temperature for comparison. At

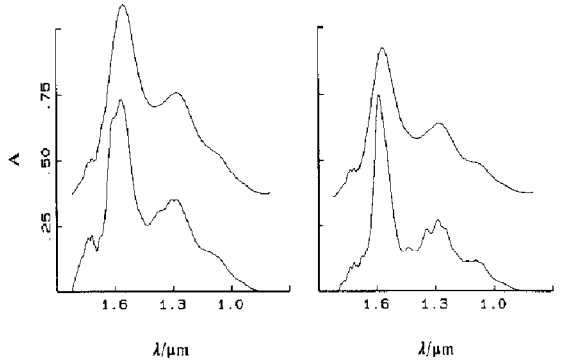

Fig. 2. Same as in Fig. 1, for samples in 65% v/v ethylene glycol $(OD)_2/D_2O$ solutions.

low temperature the spectral bands are narrower and fine structures are clearly resolved. These structures remain unaltered even if the spectra of carbonmonoxy derivatives are used as baseline; this rules out eventual contribution from the globin's vibrational spectrum [14]. The whole set of spectra can be made consistent with the suggested assignment by taking into account a strong vibronic coupling.

For a single electronic transition coupled to N vibrational modes of the nearby nuclei the following expression for the absorption lineshape, in the limit of $T \to 0$, is predicted [17,18].

$$A(\nu) = M\nu \sum_{m_1,m_j,m_N=0}^{\infty} \prod_{j=1}^{N} S_j^{m_j} \exp(-S_j)/m_j! \times F\left(\nu - \nu_0 - \sum_j^N m_j \Omega_j\right) \quad (1)$$

where M is a constant proportional to the electronic transition moment, ν_0 is the frequency of the 0–0 line, Ω_j, m_j and S_j are the frequency, the occupation number and the linear coupling constant of the jth vibrational mode respectively, F is the functional form of the absorption lineshape for which various shapes can be found in the literature: in particular Lorentzian [3], Gaussian [3] or Gaussian convolution of Lorentzians (Voigtian [18,19]) have been widely used. An expression similar to eq. (1) has been used to account for the vibrational structure of the optical absorption spectrum of β-carotene (that arises from a single electronic transition) in n-hexane at room temperature [3]. In that case the frequencies of the more strongly coupled vibrational modes and the relative S_j values were taken from resonance Raman excitation profiles and Gaussian lineshapes were assumed, leaving as optimization parameters only the intensity, peak position and halfwidth of the fundamental electronic transition. In our case the situation is less straightforward than that described by eq. (1) since four distinct electronic transitions are present and frequencies and coupling strengths of the vibrational modes are not known, due to lack of resonance Raman spectra with exciting frequencies in the range of 6000–12000 cm^{-1}. In order to minimize

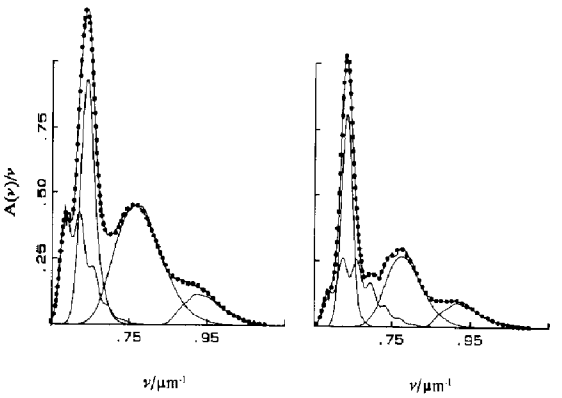

Fig. 3. Fittings of the spectra at 25 K to eq. (2). Left: Hb-CN; right: Mb-CN. Samples in 65% v/v glycerol $(OD)_3/D_2O$ solutions. The continuous lines represent experimental spectra and the component structures relative to each electronic transition. Dots represent the lineshape synthesized according to eq. (2). Rms deviation values are 1.2×10^{-4} and 1×10^{-4} for Hb-CN and Mb-CN, respectively.

the number of parameters in fitting the spectra in Figs. 1 and 2 we made the assumptions that the four electronic transitions are strongly coupled with a single vibrational mode and that the functional form F of the absorption lineshape is Gaussian.

Under these assumptions eq. (1) becomes:

$$A(\nu)/\nu = \sum_{k=1}^{4} I_k \exp(-S_k) \sum_{m_1=0}^{10} S_k^{m_1}/m_1! \times \exp\left[-(\nu - \nu_{0k} - m_1\Omega_1)^2/2\sigma_k^2\right] \quad (2)$$

where I_k, S_k, ν_{0k}, σ_k and Ω_1 are the parameters to be optimized. The contributions to the linewidth arising from homogeneous broadening, inhomogeneous broadening and weak vibrational coupling with a bath of low frequency modes are included in the parameters σ_k [1].

The fittings of the low temperature spectra with eq. (2) are reported in Figs. 3 and 4; their quality validate our assumptions. The values of the fitting parameters are reported in Table 1 and show that Ω_1 is 365 cm^{-1} for both Hb-CN and Mb-CN, in both solvents. It is also clear that S_k depend on the electronic transition and, quite interestingly, on the particular protein; in fact bands I, III and IV are strongly coupled ($S = 1$–3) whereas for band II the coupling is definitely

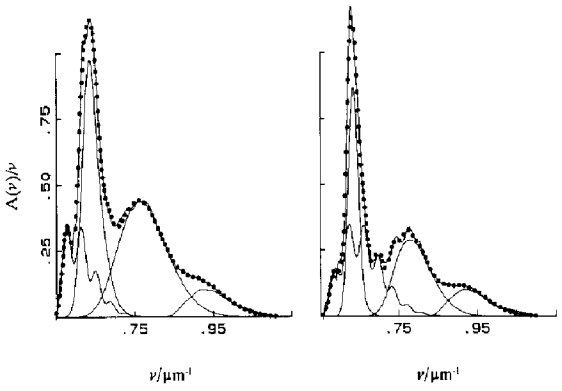

Fig. 4. Same as in Fig. 3, for samples in 65% v/v ethylene glycol $(OD)_2/D_2O$ solutions. Rms deviations values are 3.7×10^{-4} and 5×10^{-4} for Hb-CN and Mb-CN, respectively.

weaker ($S<1$); the coupling constant of band I is larger for myoglobin than for hemoglobin while those of bands II and III are smaller for the former protein. A further interesting feature evidenced from Figs. 3 and 4, and from the σ_2 values reported in Table 1 is that the main band is, in both solvents, broader for Hb than for Mb. This is indicative of a greater spectral heterogeneity of hemoglobin with respect to myoglobin: differences between α and β chains could, in principle, be responsible for this effect.

Since the mode at 365 cm^{-1} is not much populated even at room temperature ($kT=215$ cm^{-1} at 300 K), eq. (2) can be safely used to fit the spectra at 290 K; indeed such fitting has been successfully performed (rms deviation $<10^{-4}$). The solvent and temperature dependence of the NIR spectra of Hb-CN and Mb-CN will be reported in detail elsewhere.

Resonance Raman spectra (Soret excitation) of heme(Fe^{3+})-CN complexes [20] and of cyanomet insect hemoglobin [21] indicate the Fe-CN stretching mode (identified by isotope substitution studies) to be at about 450 cm^{-1}, i.e. at a frequency value substantially larger than that reported in Table 1. On the other hand, in the resonance Raman spectra of heme proteins and of metal porphyrins [22,23] pyrrole tilting and peripheral substituents out of plane deformation modes are detected at about 350–380 cm^{-1}; these modes are likely to be the strongly coupled ones responsible for the behavior of the spectra reported here. It is interesting to note that while the Soret bands of Hb-CO and Mb-CO (that are attributed to $\pi\rightarrow\pi^*$ electronic transitions) result to be essentially coupled to the in plane "ring breathing" mode of the heme at about 1370 cm^{-1}

Table 1

Values of the parameters obtained by fitting eq. (2) to the experimental data reported in Fig. 1 ($T=25$ K). Here ν_{0j}, σ_j and Ω_1 are in cm^{-1}. The uncertainties in the parameters values were obtained from the fitting by inversion of the curvature matrix [24].

Parameter	Hb-CN Glycerol	Mb-CN Glycerol	Hb-CN Ethylene glycol	Mb-CN Ethylene glycol
I_1	1.12± 0.02	0.91± 0.02	0.93± 0.02	1.25± 0.02
ν_{01}	5845± 2	5864± 3	5771± 2	5874± 4
σ_1	134± 2	131± 2	116± 2	122± 2
S_1	1.00± 0.04	1.96± 0.06	1.00± 0.04	1.96± 0.06
I_2	1.07± 0.02	0.83± 0.02	1.20± 0.02	0.89± 0.02
ν_{02}	6395± 2	6331± 2	6344± 2	6344± 2
σ_2	168± 2	141± 2	177± 2	125± 2
S_2	0.15± 0.02	0.030± 0.002	0.24± 0.02	0.025± 0.002
I_3	0.96± 0.02	0.41± 0.02	0.82± 0.02	0.50± 0.02
ν_{03}	7000± 5	7500± 5	7000± 5	7500± 5
σ_3	281±18	316± 7	326±32	262±15
S_3	2.16± 0.02	1.00± 0.02	2.10± 0.03	1.14± 0.03
I_4	0.31± 0.08	0.23± 0.02	0.28± 0.02	0.26± 0.04
ν_{04}	8838±28	8784±27	8779±60	8770±70
σ_4	189±20	184±19	187±39	191±52
S_4	1.72± 0.08	1.54± 0.09	1.88± 0.18	1.66± 0.20
Ω_1	365± 5	365± 5	365± 5	365± 5

(A. Di Pace et al., work in progress), the NIR bands of Hb-CN and Mb-CN (that are attributed to $\pi \rightarrow d_{yz}$ charge transfer transitions) result to be essentially coupled to out-of-plane deformation modes. It is also worth stressing that the comparison of hemoglobin and myoglobin shows that the coupling between the charge transfer transitions and the out-of-plane vibrational modes is not a local property of the heme group but is sensitive to heme–globin interactions.

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