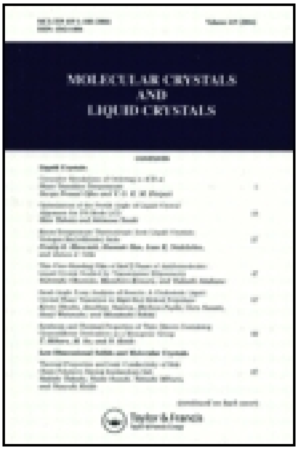

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Spectral Characteristics and Hole Burning in Myoglobin

Takashi Kushida [a] & Atusi Kurita [a]

[a] Department of Physics, Osaka University, Toyonaka, Osaka, 560, Japan

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SPECTRAL CHARACTERISTICS AND HOLE BURNING IN MYOGLOBIN

TAKASHI KUSHIDA and ATUSI KURITA
Department of Physics, Osaka University, Toyonaka, Osaka 560,
Japan

Abstract Temperature dependence of the absorption bandshape and various hole-burning characteristics have been studied in myoglobin. The results indicate that myoglobin has many conformational substates. It has also been found that a deoxy-myoglobin molecule can assume various iron-porphyrin distance at physiological temperatures, while the distribution of this distance becomes fixed around 250 K. The spectral shape of the sideband hole in Zn-substituted myoglobin is concluded to reflect the vibrational modes of the protein, and this shape is shown to be in fair agreement with the result of theoretical calculation based on the normal-mode analysis. The distribution of the barrier height between the conformational sub-states determined from a heat cycle experiment will also be discussed.

INTRODUCTION

Proteins which possess biological functions are considered to be typical molecular systems. It is known that a protein molecule can have a large number of sligtly different overall structures because of its complexity and flexibility. Namely, a protein can assume many conformational substates, i.e., nearly degenerate local minima of the free energy. As a result, a protein shows various glass-like features at low temperatures. The concept of this conformational substate may be important in molecular systems consisting of many atoms and molecules. For example, the photoinduced transformation between the substates is considered to be an origin of the non-photochemical hole burning in dye-doped glassy systems. However, structure of the microscopic environment of a dye molecule in these materials is not known. On the other hand, tertiary structure and its dynamics have been studied fairly well in several proteins. Therefore, it is interesting to investigate the conformational substates and spectral hole burning in proteins. We have studied temperature dependence of the absorption bandshape and several hole-burning characteristics in myoglobin. Myoglobin is one of the most well-studied proteins.

GLASS TRANSITION OF DEOXYMYOGLOBIN

As shown in Fig.1, the intense Soret band of deoxymyoglobin at 23000 cm^{-1} is asymmetric with a longer tail in the high-energy side. This spectral shape has been found to be explained by a configuration-coordinate model in which the envelopes of the minimum energies of the conformational substates in the electronic ground and excited states are expressed as $U_g(Q) = a(Q-Q_0)^2$ and $U_e(Q) = b(Q-Q_1)^2 + E_0$, respectively, where Q is the conformational coordinate.[1] This model accounts also for the energy distribution of the zero-phonon line in dye-doped polymers.[2] The energy difference between the substates is assumed to be sufficiently small and the density of these states to be homogeneous. Then, using the absorption spectrum f(E,E') of a myoglobin molecule in a conformational substate in the ground state for which $U_e(Q)-U_g(Q) = E'$, the absorption spectrum of the sample is expressed as $A(E) = \iint dE'dQ$

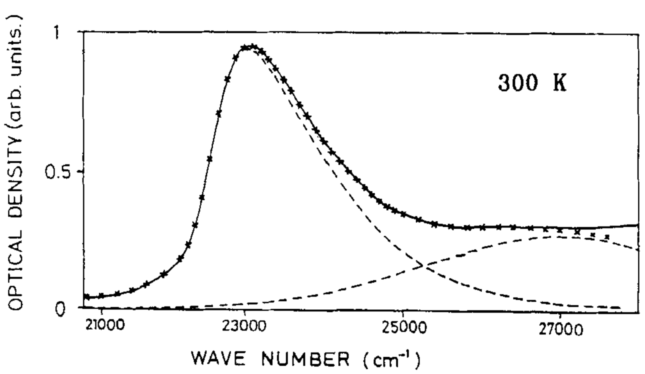

FIGURE 1 Absorption spectrum of deoxymyoglobin in aqueous solution. The dashed lines and crosses show the theoretical spectra.

$\times\ f(E,E')P(Q)\delta(U_e-U_g-E')$, where E is the photon energy and P(Q) is the distribution function of Q in the electronic ground state. The transition strength has been assumed to be insensitive to the conformation. The dashed line for the Soret band in Fig.1 has been calculated by assuming the Boltzmann distribution for P(Q) and a Lorentzian for f(E,E'). The coefficient b has been taken to be larger than a, which explains the spectral asymmetry. As shown by crosses in Fig.1, the absorption spectrum is well reproduced by the sum of the dashed curves, in which the N band at 27000 cm^{-1} has been assumed to be a Gaussian. The absorption spectrum of deoxymyoglobin observed in the 320-250 K temperature range has been explained well by this model. This indicates that this protein fluctuates among substates around physiological temperatures. Below 230 K, however, it has been found necessary to assume the Boltzmann distribution at 250 K. This result is interpreted in terms that deoxymyoglobin exhibits a liquid-glass transition around 250 K. Assuming that Q is the distance between the iron atom and the porphyrin plane in the heme, we can determine the energies of the adiabatic potential curves for the electronic ground and excited states as a function of the iron-porphyrin distance. The result has been found to be consistent with the theoretical calculation by Hopfield.[3] This shows that the distribution of the iron-porphyrin distance becomes fixed around 250 K in deoxymyoglobin.

PERSISTENT SPECTRAL HOLE BURNING IN MYOGLOBIN

A hole burning experiment has been performed in Zn-substituted myoglobin (ZnMb), i.e. myoglobin whose heme iron is replaced with Zn.[4] ZnMb is suitable for the hole burning experiment because narrow holes are burned in the lowest optical absorption band at low temperatures, and intense fluorescence is observed in the red spectral region.

Hole Spectra

Figure 2 shows the hole spectra of ZnMb in a glycerol-water (3:1) mixture and ZnMb freeze-dried powder. They consist of a narrow zero-phonon hole and a broad sideband hole. These spectra are very similar to those of dye-doped polymers.[5] Since the hole spectra of Fig.2 are almost identical with each other, the spectral shape of the sideband hole is considered to reflect the internal motion of myoglobin. We have calculated the spectral shape of this sideband using the density of vibrational modes obtained by the normal-mode analysis.[6] The Coulomb interaction between the electrons of the chromophore and the charges of the surrounding atoms has been assumed to be the cause of the electron-phonon coupling. The result has been found to be in fair agreement with the experiment. When a second hole is burned around the first hole, the area of the first hole has decreased remarkably. This result suggests that the dominant hole-burning mechanism in ZnMb is non-photochemical in character. Namely, it is considered to be the photoinduced conformational change in the polypeptide chain which surrounds the Zn-substituted protoporphyrin IX.

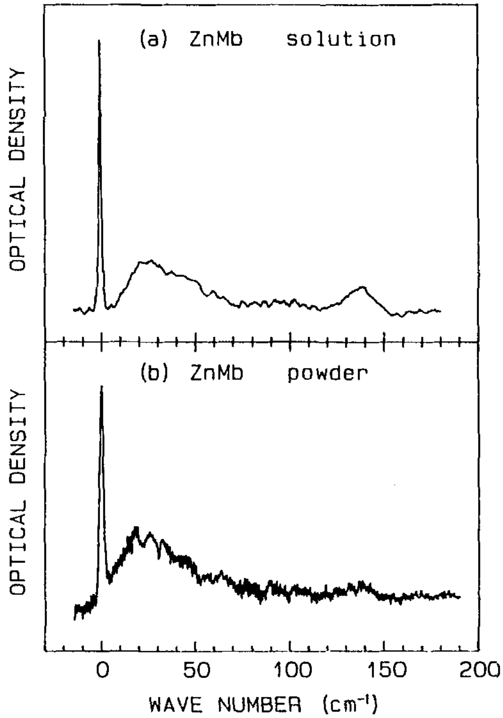

FIGURE 2 Hole spectra of ZnMb burned at 2 K.

Time Evolution of the Hole Depth

As shown by the dots in Fig.3, the depth of the zero-phonon hole in ZnMb grows non-exponentially with burning time. This time dependence of the hole depth has been found to be explained, as in the case of dye-doped polymers,[7-8] by taking into account the dispersion of the burning rate.[4-5] Namely, it can be explained by assuming that the burning rate is given as $k = k_0\exp(-\lambda)$ with a constant k_0 and that the distribution of λ is expressed by a Gaussian with the standard deviation of ~4 (the solid curve in Fig.3). This result is interpreted in terms that the photoinduced transformation rate between the conformational substates in the electronic excited state has a broad distribution in proteins as well as in dye-doped polymers.

Temperature Dependence of the Hole Area

The hole filling process across the potential barriers between the conformational substates in the electronic ground state has been studied by a heat cycle experiment.[4] The

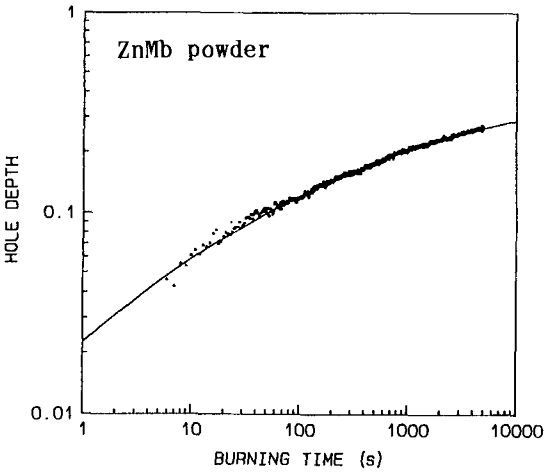

FIGURE 3 Burning-time dependence of the zero-phonon hole depth in ZnMb.

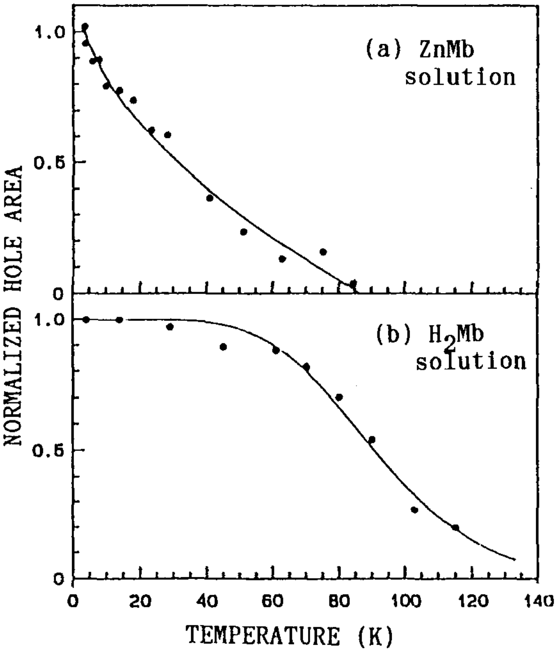

FIGURE 4 Cycling-temperature dependence of the hole area burned at 4 K.

potential barrier height has been found to have a broad distribution. As shown in Fig.4a, the hole area of ZnMb is expressed as A - $B\sqrt{T}$, where A and B are constants. This is the same result as observed in many dye-doped glassy systems, and is explained by assuming that the distribution of the barrier height V is proportional to $V^{-1/2}$ with a cutoff at V_{max}. The average barrier height between the conformational substates in the electronic ground state of ZnMb, which is related to the hole burning, is much smaller than that related to the glass transition in deoxymyoglobin. This suggests multi-dimensional character or hierarchical character of the conformational substate. In the case of iron-free myoglobin (H_2Mb), the hole area is almost independent of temperature up to about 50 K, and decreases rather abruptly above 60 K (Fig.4b). This difference in the temperature dependence of the hole area is considered to be related to the fact that the dominant hole burning mechanism in H_2Mb is photochemical in character.

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