Picosecond Polarization Studies of Protein Relaxation

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Abstract. Protein relaxation in myoglobin (Mb) following the photoelimination of CO from carbonmonoxymyoglobin (MbCO) is examined using picosecond time resolved circular dichroism (CD) and magnetic circular dichroism (MCD) spectroscopies. The transient CD signal reveals a relaxation process which requires several hundred picoseconds. Comparison with optical absorption studies on the Q-band and near infrared absorption of Mb and linear dichroism studies of the Soret optical absorptions suggest that the CD dynamics reflect a conformation relaxation of the surrounding protein structure.

1. Introduction

Currently there is a substantial effort devoted towards understanding the structure and dynamics of proteins. Advances in NMR spectroscopy, ultrafast time resolved techniques and X-ray crystallography are having a dramatic effect on our understanding of biological systems. Steady state circular dichroism and magnetic circular dichroism spectroscopy have also been extensively used to characterize structural properties of proteins. Thus, in principle, information concerning real time structural relaxations in proteins could be detected through a time resolved measurement of the CD and MCD signals.

In the last decade there have been several reports of instruments which can measure time resolved CD kinetics and spectra [1–3]. Applications of these devices to biological systems have also been reported [4,5]. Recently, we have developed a new technique for collecting time resolved CD data with picosecond resolution [6,7]. As demonstrated in the following sections, this form of spectroscopy can provide information on protein structural relaxations which are not easily detected by more conventional time resolved spectroscopies. In the present discussion, we focus on the relaxation dynamics of Mb following the photodissociation of CO from MbCO. We have also used this technique to examine the evolution of the CD spectrum of bacterial photosynthetic reaction centers and results on that system will be published elsewhere [8].

2. Experimental Details

A detailed description of the experimental apparatus has recently been published. Briefly, a high repetition rate picosecond laser system is used to generate tun-

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able pump and probe pulses. The picosecond probe pulse train is polarization modulated by a piezo-electric modulator switching sequential pulses between left and right circular polarization. The difference in transmission of these probe pulses is detected using phase sensitive detection. The time delay between each pump–probe pulse pair is controlled by a computer driven optical delay line. By scanning the delay line, the transient CD signal can be recorded as a function of delay time following photolysis. On the other hand, for a fixed delay, the dye laser can be scanned in order to record a transient CD spectrum.

Transient MCD kinetics (or spectra) are recorded by placing the sample in an external magnetic field. The details of this technique will appear shortly [9].

A pH 7.0 Tris-bis buffer solution of MbCO was prepared under anaerobic conditions. The sample concentration was 0.9 mM, with an O.D. of 1.7 at 532 nm and > 4 at 355 nm. Under these conditions, the photolysis pulse (50 μJ) excites $> 90\,\%$ of the sample in the focal volume. Probe pulses on the order of 0.1 μJ were used.

3. Relaxation of Myoglobin Following Photoelimination of CO

The photodissociation of MbCO to form Mb and free CO has been the subject of many picosecond time resolved studies [10]. Transient infrared, Raman and absorption studies have revealed that photoinduced bond cleavage occurs within 350 fs. However, in considering the crystal structures of MbCO and Mb, relaxation of the tertiary structure of the protein is also expected. In particular, in the local vicinity of the reaction site, following dissociation, the heme domes, and the iron moves out of the porphyrin ring, causing the distal histidine to tilt and the F-helix to move.

To address the time scale of protein relaxation in Mb following photodissociation of CO, transient CD measurements were carried out on the N-band absorption at 355 nm. The data are shown in Fig. 1. The dashed line is a nor-

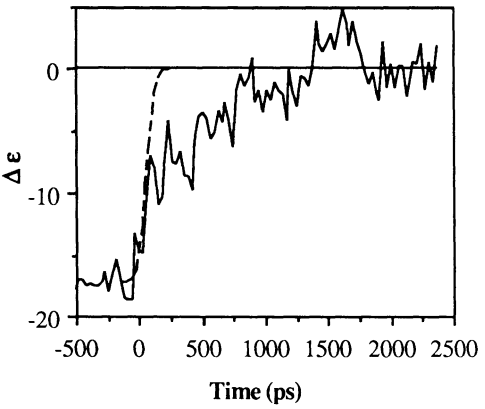

Fig. 1. Transient CD kinetics at 355 nm following photoelimination of CO from MbCO. The dashed line is the corresponding transient absorption measurement and provides a measure of the instrument response

malized transient absorption study on the same sample at the same wavelength, a measure of the instrument response.

The observed level change of the CD signal is consistent with the steady state CD values of MbCO and Mb at 355 nm. The important observation in Fig. 1 is that the evolution of the CD signal is distinctly different from the transient absorption kinetics. The CD kinetics consist of a fast initial rise within the instrument response, followed by a slower rise which reaches zero (the steady state CD value for Mb at 355 nm) at about 500 ps. It is also important to note that both MbCO and Mb have a significant absorption at 355 nm (the N-band for both is centered at 350 nm with the oscillator strength being slightly greater for Mb). The transient CD dynamics reveal a relaxation process which is two orders of magnitude slower than that associated with photoinduced bond cleavage.

In the remainder of this section, several possible interpretations of the CD dynamics are briefly discussed. A detailed study will be reported shortly [11]. Three possible mechanisms are consistent with the observed data: (1) vibrational relaxation in the vicinity of the heme, (2) a time dependent splitting of the degeneracy of the heme transitions, and (3) relaxation of the surrounding protein matrix.

Vibrational relaxation in the vicinity of the heme has been addressed both experimentally and using molecular dynamics simulations [10]. These studies indicate that the heat is dissipated into the protein within a few tens of picoseconds, significantly faster than the relaxation process revealed by the CD data. In order to address the possibility of a time dependent splitting in the degeneracy of the heme transitions, three separate experiments were performed: (1) linear dichroism studies on the Soret absorption bands, (2) transient MCD on the Q-band, and (3) detailed time resolved absorption studies of the Q-band absorption. In these latter two studies, the Q-band dynamics were probed as the Q_0 band is the absorption feature most sensitive to the lifting of the degeneracy of the e_{2g} orbitals [12].

Linear dichroism experiments on the Soret band only reveal a single component corresponding to the overall tumbling of the protein. Furthermore, the initial anisotropy observed is 0.1, that expected for a circular absorber [13]. These observations support the conclusion that there are no fast wobbling motions of the heme nor is there a rotation of the heme transition dipole moments on the time scale of the CD kinetics. In the case of the Q-band absorption, the experimental results show that the shape of the transient spectrum is unchanged from $t = 0$ to a delay of 10 ns, suggesting that there is no time dependent change in the degeneracy of the heme transitions. Finally, transient MCD dynamics recorded at 580 nm reveal an instantaneous change in MCD level following photolysis, with no ensuing dynamics observed within the experimental signal-to-noise ratio. These data support the conclusion that the iron changes its spin state extremely fast (< 1 ps). All three experiments support the conclusion that there is no time dependent evolution in the electronic structure of the heme.

The above discussion signifies that the transient CD kinetics is providing information on the relaxation dynamics of the protein structure following photodissociation. Calculations reported by *Hsu* and *Woody* [14] suggest that the CD of the N-band of Mb is primarily due to coupled oscillator interactions between the Q_x and Q_y transitions on the heme and the $\pi \rightarrow \pi^*$ transitions on the surrounding aromatic amino acids. In addition, aromatic residues as far as 12 Å away from the heme can significantly contribute to the CD spectrum.

One possible motion that could account for the evolution of the CD signal would involve the tilting of the proximal histidine. This can be independently addressed by examining the near infrared absorption band of Mb at 760 nm; this transition arises from a prophyrin-to-iron charge transfer and has been shown to be sensitive to the conformation in the immediate vicinity of the heme ring [15]. This absorption feature is characteristic of five-coordinate ferrous hemes and does not appear in the six-coordinate MbCO spectrum. Detailed dynamical studies of this absorption band reveal no change in band shape or band position from 30 ps (the instrument response) to 10 ns following photodissociation. These results suggest that the tilting of the proximal histidine as well as other structural contributions to this absorption band are not responsible for the observed CD kinetics. Studies on mutant proteins are planned in order to determine the origin of this relaxation process.

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