Observation of sub-100 ps conformational changes in photolyzed carbonmonoxy-myoglobin probed by time-resolved circular dichroism.

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Conformational changes in proteins, which are known to play a paramount role in biophysical processes, are attracting much attention. For example, the change in carboxy-myoglobin (MbCO) after dissociation of the CO has recently been observed on a 100 ps timescale in a time-resolved X-Ray experiment[1]. Shorter time resolution is however out of reach of such experiments. In order to investigate these processes on an ultrashort timescale, we have set up a time-resolved circular dichroism (CD) experiment in MbCO. The principle of the experiment is the following: after excitation with a pump beam, the CO-heme link breaks and a deoxy-heme structure appears very rapidly (< 1 ps)[2]. As the heme CD in the Soret region is very sensitive to the geometrical arrangement of the surrounding aromatic residues[3] (figure 1), measuring the change in the CD spectrum with time allows one to gain insight into the first steps of these conformational changes.

The experiment is carried out on a 10^{-4} M MbCO sample excited with a 400 nm pulse. The CD is measured across the Soret band as a function of time with a sub-picosecond resolution (figure 2). After the initial drop in the CD due to the instantaneous electronic change of the heme, we observe a variation of the signal on a sub-100 picosecond timescale. In order to analyze these results, we have developed a calculation after Applequist's normal mode CD theory[4]. Calculation of the contribution of the main residues to the rotational strength allows us to assert the origin of the signal: in the first 10-20 ps, the CD change comes from the rearrangement of the heme whereas the 100 ps evolution results from the movement of more distant helices.

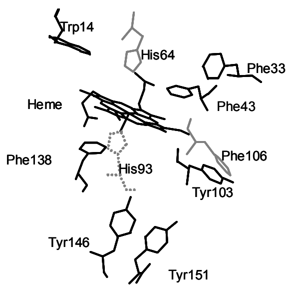

Figure 1

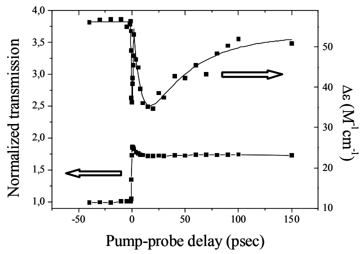

Figure 2

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[2] M. H. Vos and J. L. Martin, Biochimica et Biophysica Acta **1411**, 1-20 (1999).
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[4] F. Hache and T. Dartigalongue, Chem. Phys. **303**, 197-293 (2004).