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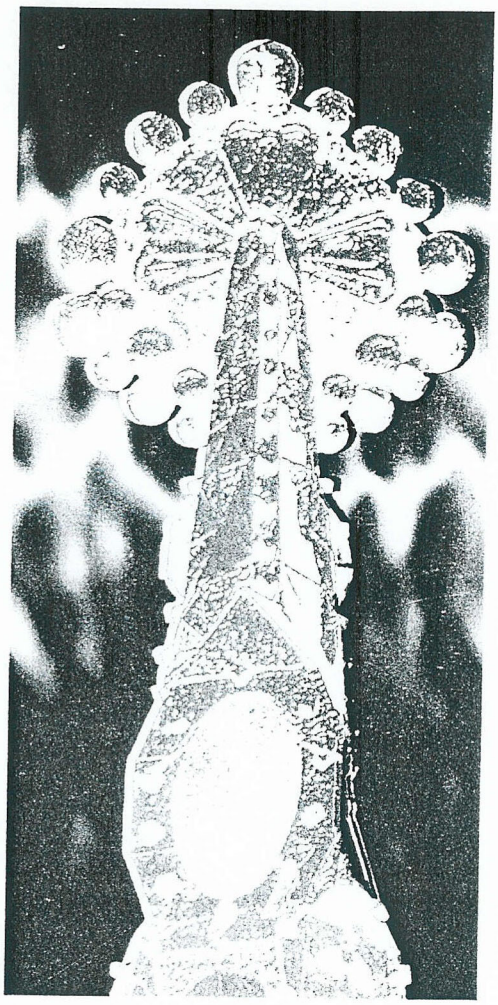

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BOOK OF ABSTRACTS

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THE ABSENCE OF AN INTERMEDIATE AFTER CO PHOTOLYSIS OF CARBONYL HORSERADISH PEROXIDASE DEMONSTRATED BY LASER-INDUCED OPTOACOUSTIC SPECTROSCOPY

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The study of CO binding to heme proteins allows to obtain detailed information about the protein environment surrounding the heme, which is the biologically active site. Both the FeCO geometry in the CO complexes of heme proteins [1] and the dynamics of CO binding/dissociation [2,3] are influenced by the surrounding aminoacids.

We have studied the photodissociation of CO from a prototypical heme protein - horseradish peroxidase isozyme C (HRPC) - by laser-induced optoacoustic spectroscopy (LIOAS). This technique allows to measure both enthalpy and volume changes in photochemical reactions and to resolve them in a time window typically lying between 10^{-8} - 10^{-5} s [4]. In order to detect structural volume changes, which originate in the molecular rearrangement following photoexcitation, it is necessary to experimentally separate them from thermal volume changes which also contribute to the LIOAS signal. This separation is performed by varying the thermoelastic parameters of the solvent, which means varying the temperature for aqueous solutions, and measuring the signals of the sample and of a calorimetric reference under identical experimental conditions.

HRPC-CO samples were irradiated at 532 nm with 10 ns pulses. $KMnO_4$ or ferric HRPC solutions were used as calorimetric references with identical results. The LIOAS signals of both sample and reference were measured in the temperature range 20 - 4.5 °C. The results show that a positive volume change on the order of 1 - 2 ml/mol takes place upon CO photodissociaton. This expansion is faster than the time resolution of our LIOAS setup. A further observation was that the expansion did not take place if the CO complex was prepared at pH 3.

The signal time dependence strongly differs from what was observed by LIOAS for the CO complex with myoglobin (MbCO) [5], where two components were observed in the LIOAS signal decay: a fast one, corresponding to a geminate pair formation, and a slower one (700 ns at 20°C), possibly related to the geminate pair decay and complete dissociation of CO from the protein. We measured MbCO samples with our setup for comparison and we observed the same decay time for the slower component, even if the relative amplitudes of the two components were different than in Ref. [5]. On the other hand, the HRPC-CO LIOAS signals at all temperatures only display a single, prompt component. This points to the lack of an intermediate species following photoexcitation. The absence

of a geminate pair formation at room temperature in HRPC-CO was also observed in a flash photolysis study [6]. Concerning the microscopic origin of the structural volume change, it is possible that the observed expansion is related to the breakage of the H bonds between CO and the aminoacids arginine 38 and histidine 42. The dependence on pH of the structural volume change suggests that the change is related to an interaction which does not occur at low pH. A conformational transition from neutral to acid pH was in fact observed for HRPC-CO, leading to a reduced interaction between CO and the surrounding aminoacids [7]. An alternative explanation is that the quantum yield for photodissociation is strongly reduced at low pH.

The structural difference - and therefore the observed different features - between HRPC-CO and Mb-CO could be related to the absence of a main docking site for the photodissociated CO in HRPC-CO. A docking site in Mb-CO was observed in several time-resolved spectroscopic studies [8,9]. Interestingly, a direct comparison between Mb-CO and the CO complex of microperoxidase [2] shows that the docking site in Mb-CO slows the CO recombination rate by more than 10^4 times. The structural difference might have a biological relevance, being related to the different functions of peroxidases - enzymatic activity involving fast ligand binding and dissociation - and of globins - oxygen transport, involving stabilised ligand binding.

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