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Photolyses of mammalian oxy-hemoglobin studied by nanosecond photoacoustic calorimetry

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Abstract

Enthalpy and conformational volume changes in photolyses of oxy-hemoglobin (HbO_2) of human, bovine, pig, horse and rabbit are investigated by photoacoustic calorimetry. In the experiment, a pulsed Nd:YAG laser is used as an exciting source, and a PVDF film transducer and a PZT transducer are used to detect the photoacoustic signals. Based on the time scales of the excitation and detection systems as well as the photolysis processes of HbO_2, it can be indicated that the measured enthalpy and conformational volume changes are related to slow geminate recombination and tertiary relaxation in photolyses of HbO_2, which are with the time scale of 30–40 ns and 100–150 ns, respectively. The results show that the enthalpy and conformational volume changes are different for both photolysis processes of HbO_2 and also for various mammals. The different results among the five mammals are analyzed and discussed briefly.

Keywords: Oxy-hemoglobin; Photolysis; Enthalpy change; Conformational volume change; Photoacoustic calorimetry

Hb as the allosteric protein has been researched widely in terms of structures and energetics of the initial and final states for ligand binding. On the other hand, the intermediate states playing a dominant role in cooperative ligand binding are also being studied. Especially, with the development of time-resolved photoacoustic calorimetry (PAC)[1], enthalpy and conformational volume changes in photoinduced ligand dissociations can be directly measured [1–3]. This method has been applied to ligand dissociation in both sperm-whale [1,2] and horse [3] carboxymyoglobin (MbCO) where the dynamics of enthalpy and conformational volume changes associated with photolyses of CO from MbCO are observed in the nanosecond time scale. Meanwhile, this method is successfully employed to investigate enthalpy and conformational volume changes for ligand dissociation to produce triply ligated R-state of human HbCO [4]. But a few studies have been presented on the photolysis reactions of O_2 from HbO_2 by PAC, because the quantum yield of the photolysis reaction of HbO_2 is very low [5].

In the paper, using a PAC system with the time scales of nanoseconds, the enthalpy and conformational volume changes of the slow geminate recombination and tertiary relaxation processes in photolyses of HbO_2 for human, bovine, pig, horse and rabbit are obtained.

Materials and methods

Materials

The samples of Hb for human, pig, horse and rabbit are made by Sigma, USA, and Hb of bovine is from Mingzhu Dongfang Biological Technique, China. They are all in the form of lyophilized powders and have the same water content, iron percentage, rate of purity and solubility. The solutions are prepared according to conventional procedures. The Hb powder is first dialyzed against 50 mM phosphate buffer at pH 7.0, and

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[1] *Abbreviations used:* Hb, Hemoglobin; HbO_2, oxy-hemoglobin; PAC, photoacoustic calorimetry; PVDF, polyvinylidene diflouride; PZT, $PbTi_xZr_{1-x}O_3$; MbCO, carboxymyoglobin; HbCO, carboxyhemoglobin; BCP, bromocresol purple.

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then reduced with sodium dithionate ($Na_2S_2O_4$). HbO_2 solutions are prepared by passing O_2 through solutions at 1 atm pressure. Meanwhile, for calibration, a solution of bromocresol purple (BCP) also in 50 mM phosphate buffer, pH 7.0, is used as a calorimetric reference, which converts the entire photon energy into heat with the response faster than that of the experimental instrument and no reaction volume change. Before each experiment, to check the oxy- and deoxy-form of Hb in solution, the absorption spectra are measured with Shimadzu UV-3100 Spectrophotometer. The optical density of the solutions ranged from 0.15 to 0.25 at 532 nm in a cuvette of $1 \times 1 \times 3$ cm^3, which is low enough for reducing the influence of the multiple scattering light induced by Hbs in the solution, but having high signal to noise ratio (more than 20), and the calorimetric reference solution is matched to the sample.

PAC measurements

The photoacoustic apparatus is shown schematically in Fig. 1. A pulsed Nd:YAG laser beam operated at 532 nm with pulse width 8 ns, repetition frequency 10 Hz and pulse energy about 20 μJ is used as an exciting source. The laser beam is focused in a quartz cuvette by a lens with the focus length of 30 cm, and the illuminated region with the waist diameter about 0.5 mm in the solution. Meanwhile, the energy is measured and monitored by Dig/Rad R-752 radiometer for normalizing the PAC signals. The measured solution is contained in a quartz cuvette with a volume of about $1 \times 1 \times 1$ cm^3 and slightly flowing with the speed about 1 mL/min. The temperature of the solution changes in a range of 7–22 °C and is controlled within ±0.1 °C by using a thermostat installed directly in a corner in the cell.

The heat evolved as a result of the photo-initiated reactions causes a pulsed ultrasonic wave in the solution. The pulsed ultrasonic wave propagates to the cuvette wall and is detected in a time-resolved mode by an ultrasonic transducer bounded on the outside of the wall. In the experiments, two kinds of transducers with different bandwidths or resonant frequencies are used. One is a 9-μm-thick PVDF (polyvinylidene diflouride) film transducer which has a wide bandwidth up to more than 100 MHz and a response time 5–100 ns, and the other is a PZT ($PbTi_xZr_{1-x}O_3$) transducer with a resonant frequency of 1.5 MHz and a response time in several hundred nanoseconds.

The output electrical signal from the transducers is amplified with a preamplifier and then provided to a digital oscilloscope. The displayed photoacoustic signals are usually averaged from 100 signals, and each PAC waveform must be normalized by the illuminating laser energy and the optical density of the solution before the data processing with a computer based on the PAC theory.

Data analysis

The photoacoustic signal is produced by the volume variation of the solution irradiated by the pulsed laser. There are two main contributions to the overall volume change. One is derived from the thermally induced volume change in the solution, ΔV_{th}, which is related to the thermal expansion coefficient β, heat capacity C_p, density ρ of the solution and thermal energy H released to the solution. The other arises from the molecular volume change ΔV_{con}, i.e., the conformational changes between the products and reactants during the photolysis process. Therefore, the acoustic wave amplitude S_{sam} of the resultant photoacoustic effect for the sample HbO_2 is given by [3]

$$S_{sam} = K(\Delta V_{th} + \Delta V_{con}) = K[(\beta/C_p\rho)H + \Delta V_{con}], \quad (1)$$

where K is a function of the instrument response. In order to eliminate K, a reference solution with the same optical density as the sample at the irradiation wavelength is employed, which transfers totally the absorbed photon energy to heat energy. Therefore, the acoustic wave amplitude S_{ref} of the reference is

$$S_{ref} = K(\beta/C_p\rho)E_{h\nu}. \quad (2)$$

The amplitude ratio Φ of acoustic signals of the sample to the reference is expressed as

$$\Phi = S_{sam}/S_{ref} \quad (3)$$

rearranging gives

$$E_{h\nu}\Phi = H + \Delta V_{con}/(\beta/C_p\rho). \quad (4)$$

Since $\beta/C_p\rho$ is dependent upon the temperature, measuring Φ as a function of the temperature allows for the correlation of $E_{h\nu}\Phi$ with $C_p\rho/\beta$ and yields a slope ΔV_{con} and an intercept H. However, the quantum yield Q of the photo-induced chemical reaction must be taken into account for evaluating the related enthalpy change ΔH_T and conformational volume changes ΔV_T of the reaction, which can be obtained according to the following equations:

$$\Delta H_T = (E_{h\nu} - H)/Q. \quad (5)$$

$$\Delta V_T = \Delta V_{con}/Q. \quad (6)$$

Since the detected waveforms are the convolution of the time-dependent photolysis processes and the response of the experimental system, for the photolyses of multi-processes, the detected waveforms should be taken as deconvolution for analyzing the detected processes [6], in which the signal from the reference solution is used as the response of the experimental system.

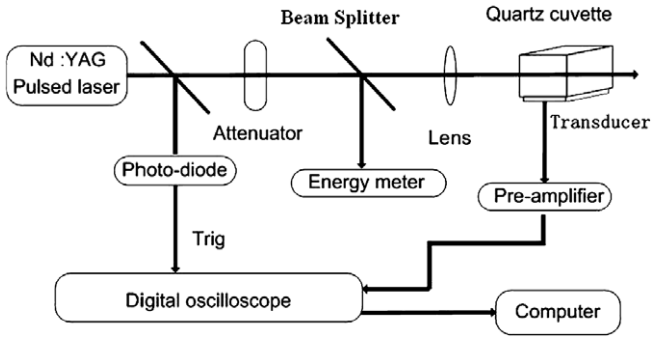

Fig. 1. Experimental setup of photoacoustic calorimetry.

Results

In the PAC experiment, bromocresol purple (BCP) solution is used as a reference, then the photoacoustic signals of the mammalian HbO_2 and BCP are measured. As an example, the photoacoustic signals of horse HbO_2 and BCP solutions (optical density = 0.21) in phosphate buffer at the laser energy of 20 μJ and the temperature 20.6 °C detected by the PVDF film and the PZT transducers are shown in Fig. 2(a) and (b), respectively. In Fig. 2, several signal peaks represent the directly excited (the first) and successively multi-reflected photoacoustic signals in the cuvette. The first peak appeared in a delayed time about 4.4 μs, is induced by the acoustic wave propagation from the excitation location by the laser beam to the transducer. Meanwhile, Fig. 2 shows that the waveforms from the sample and reference are similar and no apparent time shift between them, then the ratio Φ of the acoustic signals of sample to reference can be obtained by the first peak values. Fig. 2 also shows that the observed photolysis processes (peak widths) in the PAC detection systems are strongly dependent on the bandwidth of the detecting transducer used in the experiments as the laser pulse