STUDIES OF DYNAMICAL PROCESSES IN PHOTODISSOCIATED CARBOXYHEMEPROTEINS USING TIME RESOLVED RESONANCE RAMAN SCATTERING

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INTRODUCTION

Rapid photodissociation of carboxyhemeproteins (Fe^{+2}) results in the production of nonequilibrium species whose nuclear and electronic structures evolve as a function of time. Using time resolved resonance Raman scattering, we have been able to monitor such changes in the iron porphyrin chromophore of photolyzed carboxyhemoglobin (HbCO) and carboxymyoglobin (MbCO). Our findings bear directly on questions regarding the intraprotein dynamics of the photolyzed CO and the structural basis for tertiary and quaternary structural changes in hemoglobin.

METHODS AND MATERIALS

Resonance Raman spectra are generated by a 10 ns dye laser pulse (models UV24 and DL14; Molectron Corp., Sunnyvale, Calif.) fired with an adjustable delay (10 ns–1 ms) after the photodissociating pump pulse from the frequency doubled (5320 Å, 20 mJ) output of a Nd:YAG laser (model 500QG; Holobeam, Inc., Paramus, N. J.). These spectra are obtained using laser excitations between 4,100 and 4,300 Å which results in Soret band resonances. The spectra are accumulated on an optical multichannel analyzer based upon an SEC vidicon tube. The sample configuration is a flowing cell with the temperature and atmosphere under external control. A more detailed account of the apparatus can be found elsewhere (1). Unless otherwise indicated, the samples were under 1 atm of CO and at 10–20°C.

RESULTS AND DISCUSSIONS

Spectra were recorded covering the region 1,225–1,575 cm^{-1}. The most prominent feature in this spectral region for Soret band excitations is the polarized peak that occurs at 1,357 cm^{-1} for deoxyHb, 1,373 cm^{-1} for HbCO, and at 1,377 cm^{-1} for HbO_2 with similar values for Mb. By monitoring this peak in photodissociated HbCO and MbCO we have obtained (1) results pertaining to the origin of the difference in the quantum yields of photolysis in these two similar systems. Firing the dye laser before the photolytic pulse, we find that the spectrum reflects predominantly unphotolyzed material (photodissociation by the probe pulse has been minimized by focusing the beam to a line). Within the first few nanoseconds after photolysis, the HbCO and MbCO peaks have completely disappeared and a peak in the region of the deoxy species has appeared. This observation is consistent with previous results using a single pulse (2–4) and using Coherent Anti-Stokes Raman Scattering (5). At later times a striking difference is observed in the two systems. In the Hb system the HbCO peak regains nearly 50% of its intensity within 100 ns of photolysis. On the same time scale no corresponding change is observed in the Mb spectrum. At much later times ($\geq 100\mu s$) the expected bimolecular recombination takes place in both Hb and Mb. Whereas this slower recombination is observed to be pressure sensitive, the nanosecond recombination dynamics were unaltered upon lowering the CO pressure by two orders of magnitude.

The results at early times and the associated analysis yield striking evidence that the quantum yield for photolysis of HbCO deviates from 100% due to a geminate recombination of photolyzed hemes, with a characteristic time of 65 ± 25 ns. Correcting our results for the recombination taking place in the first 15 ns, we arrive at an estimated quantum yield of 54 ± 4% (i.e., 46% recombination total). Comparing this with the literature value of the quantum yield of 47% (53% unphotolyzed) measured at longer times, we see that the absolute quantum yield for the primary photolysis process (that is, the Fe-CO bond cleavage) is ~90%, which value is similar to the total yield observed in MbCO, where this geminate recombination does not occur.

We have been able to utilze the geminate recombination to generate a long-lived population of half ligated Hb which was used to probe both ligated and deligated porphyrins sites as the Hb undergoes the R-T switch. The temporal evolution of the 1,357 cm^{-1}-Raman line was monitored, because the frequency of this line has been shown (6) to be highly sensitive to the quaternary structure. Furthermore, whereas this peak is shifted ~ -2 cm^{-1} in photolyzed HbCO (R) relative to deoxy Hb (T) no such shift is observed for Mb.[1] By varying the amount of photolysis generated by the probe pulse it was possible to generate spectra of deoxy hemes containing contributions from sites that had temporarily evolved as either deligated or ligated species. It is observed (7) that for the heme sites that remain deligated after the initial photolysis, the corresponding Raman peak undergoes a significant shift towards the equilibrium deoxy Hb value on a submicrosecond time scale. In contrast, the Raman peak associated with the "ligated" sites begins to shift appreciably only after tens of microseconds, which is the time scale for the R-T switch. This difference is temporal behavior for the two sites and is indicative of a trigger-like behavior associated with the propagation from deligated to ligated sites of an alteration in that part of the structure of Hb responsible for the R-T sensitive shift of the 1,357-cm^{-1} peak. Because this Raman peak remains unshifted in the Mb system, it is unlikely that the shifting of this peak in Hb reflects motion of the iron in and out of the porphyrin plane. A more likely explanation based on the electronic interaction model (6) is that the shifting reflects a change in porphyrin-protein contacts initiated by the π electron rearrangement associated with deligation.

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REFERENCES

1. Friedman, J. M., and K. B. Lyons. 1980. Transient Raman study of CO-hemoprotein photolysis: origin of the quantum yield. *Nature (Lond.).* **284**570–572.
2. Lyons, K. B., J. M. Friedman, and P. A. Fleury. 1978. Nanosecond transient Raman spectra of photolyzed carboxyhemoglobin. *Nature (Lond.)* **275**:565–566.
3. Srivastawa, R. B., M. W. Schuyler, L. R. Dosser, F. J. Purcell, and G. H. Atkinson. 1978. Resonance Raman spectra of cytochrome C and oxyhemoglobin using pulsed laser excitation and optical multichannel detection. *Chem. Phys. Lett.* **56**:595–601.
4. Woodruff, W. H., and S. Farquharson. 1978. Time resolved resonance Raman spectrosocpy of hemoglobin derivatives: heme structure changes in nanoseconds. *Science (Wash. D. C.).* **201**:831–833.
5. Dallinger, R. F., J. R. Nestor, and T. G. Spiro. 1978. Resonant coherent anti-Stokes Raman scattering evidence for out of plane heme iron displacement within 6 ns of CO dissociation in CO hemoglobin. *J. Am. Chem. Soc.* **100**:6251–6252.

[1]Friedman, J. M., R. Cone, and R. Stepnoski. Work submitted for publication.

6. Shelnutt, J. A., D. L. Rousseau, J. M. Friedman, and S. R. Simon. 1979. Protein-heme interaction in hemoglobin: evidence from Raman difference spectroscopy. *Proc. Natl. Acad. Sci. U.S.A.* 76:4401–4413.
7. Lyons, K. B., and J. M. Friedman. 1980. Dynamics of carboxyhemoglobin photolysis: time delay Raman scattering. *Proc. Symp. Interact. Iron Proteins in Oxygen Electron Transp. (Airlie, Va.).*

SEGMENTAL FLEXIBILITY OF IMMUNOGLOBULINS

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One of the major functions of antibody is to initiate biological effector functions, such as complement activation, upon binding to antigen. To fulfill these functions, antibody molecules have evolved into a set of related structures with unusual conformations and appropriate dynamic properties. Perhaps the most intriguing feature is the folding of the light and heavy chains into separate and compact domains with homologous conformations (1).

We have used nanosecond fluorescence depolarization measurements of fluorescent hapten-antibody complexes and proteolytic fragments to explore the nature of subunit motions of IgM class antibodies. These molecules are pentamers of IgG-like subunits which, however, contain an additional domain, called Cμ2, between Fab and $(Fc)_5$ in each heavy chain (2).

In native, intact IgM, the Fab moieties were found to display restricted segmental flexibility (3). Heating the IgM to 60° for 30 min significantly reduced the ability to fix or activate complement in the presence of antigen, although binding sites for C1q were still present and the antibodies still bound antigen. Depolarization measurements of the heated IgM and of $(Fab')_2\mu$ fragments showed that segmental flexibility had increased (Table I).

At the same time, a comparison of the circular dichroism of native and heated IgM, Fab, and $(Fab')_2\mu$ fragments showed that a localized alteration of the Cμ2 domains had occurred. However, specific rabbit antibodies elicited against the Cμ2 region of native $(Fab')2\mu$ fragments could still bind to the heated IgM or $(Fab')_2\mu$. Taken together, these results suggest that the interaction between paired Cμ2 domains in each IgM_5 subunit had been altered by exposure to heat.

Similar, although more extensive, changes occurred on exposure of the IgM to 1 M acetic

TABLE I

NANOSECOND FLUORESENCE ANISOTROPY OF IgM AND $(Fab')_2\mu$. SUMMARY OF THE PARAMETERS OBTAINED BY FITTING THE ANISOTROPY TO A SUM OF TWO EXPONENTIALS $[(A(t) = A_0(f_s e^{-t/\phi_s} + f_L e^{-t/\phi_L})]$ BY A WEIGHTED NONLINEAR LEAST-SQUARES ANALYSIS

Dansyl-lysine-anti-dansyl complex	A_0	t^*_{max}	f_s	ϕ_s	f_L	ϕ_L
		(*ns*)		(*ns*)		(*ns*)
Native IgM	0.248 ± 0.002	150	0.49 ± 0.01	34 ± 2	0.51 ± 0.01	241 ± 7
Heat-treated IgM	0.246 ± 0.002	150	0.62 ± 0.08	39 ± 7	0.38 ± 0.08	243 ± 76
Native $(Fab')_2\mu$	0.254 ± 0.005	115	0.30 ± 0.07	22 ± 4	0.70 ± 0.07	85 ± 9
Heat-treated $(Fab')_2\mu$	0.250 ± 0.005	115	0.60 ± 0.07	30 ± 4	0.40 ± 0.07	93 ± 11

*Longest time from which *A(t)* data points were fit.