Time-Resolved Spectroscopy of Hemoglobin and Its Complexes with Subpicosecond Optical Pulses

Abstract. *Subpicosecond optical pulses have been used to study the photolysis of hemoglobin complexes. Photodissociation of carboxyhemoglobin is found to occur in less than 0.5 picosecond. In hemoglobin and oxyhemoglobin a nondissociative excited state recovery in 2.5 picoseconds is observed.*

Recently developed techniques for the generation and utilization of ultrashort optical pulses (*1, 2*) have been applied to the study of the photolysis of hemoglobin. The three systems we have investigated are hemoglobin (Hb) and two of its complexes, oxyhemoglobin (HbO_2) and carboxyhemoglobin (HbCO). Excitation with picosecond light pulses allows us to observe both very rapid relaxation of the excited state and photodissociation.

The fundamental problem of hemoglobin (*3*), the mechanism of its cooperative binding of oxygen, remains unsolved. Hoard (*4*) and Perutz (*5*) have suggested that the binding of oxygen to Hb converts a high-spin iron atom with a coordination number of five, which is displaced 0.75 Å out of the prophyrin plane toward its fifth ligand, the proximal histidine F8, into a low-spin iron atom with a coordination number of six, which is in the plane of the porphyrin ring. Perutz (*5*) proposed that this motion of the iron atom is the trigger that sets off a chain of large-scale atomic displacements in the Hb molecule, ultimately leading to cooperativity. In particular, the proximal histidine moves 0.8 to 0.9 Å away from the porphyrin plane on loss of an O_2 molecule. These structural changes alter the charge distribution around the heme group and hence its absorption spectrum.

The two complexes HbO_2 and HbCO are alike in that they have zero spin in the ground state, whereas Hb has a spin of 2. They also have similar optical absorption spectra because the very intense transitions of the porphyrin ring dominate and make weaker transitions involving metal ions difficult to observe (*6*). In the red portion of the spectrum near 615 nm the absorption of the complexes is considerably less than that of Hb. This particular absorption difference provides us with a means of observing photodissociation.

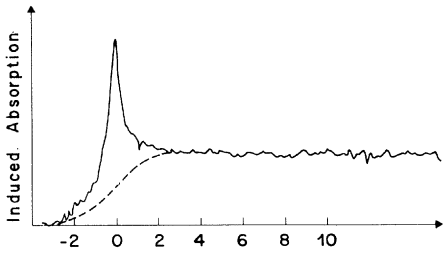

Fig. 1. Induced absorption at 615 nm in HbCO versus time delay between excitation and probe pulse. The dashed line is the response corrected for the optical coherence artifact.

In 1896 Haldane and Smith (*7*) discovered that HbCO can easily be dissociated by light into Hb and CO; whereas HbO_2 hardly dissociates (*8*). Quantitatively, the quantum yield for dissociation is ~ 0.5 for HbCO (*9*) and ~ 0.008 for HbO_2 (*8*). Bucher and Kaspers (*10*) showed that HbCO dissociates with the same quantum yield independent of the photon energy in the range 2.0 to 4.8 ev. The energies of binding per molecule of O_2 and CO are ~ 0.8 and ~ 0.9 ev, respectively (*11*). The quadrupole coupling constant of the ^{57}Fe nucleus is large in HbO_2 but small in HbCO, which suggests grossly different binding at the iron atom (*12*).

In the experiments reported here we used subpicosecond optical pulses at 615 nm (2.0 ev) from a passively mode-locked dye laser (*1*) to optically excite the molecule under study. Each excitation pulse had an energy of 5×10^{-9} joule and was focused to a spot 20 μm in diameter. A much weaker, delayed image of the excitation pulse probed the induced absorption change as a function of time. With a cavity dumping apparatus internal to the mode-locked laser, we were able to provide excitation pulses at rates up to one per microsecond. The high repetition allowed extensive signal averaging during the course of a measurement. Lower rates could be used if necessary to allow for complete recovery of the sample between pulses.

Figure 1 shows the induced absorption as a function of relative delay between the probing and excitation pulses for a water solution of HbCO (*13*) at a concentration of $1.6 \times 10^{-3}M$ saturated with CO at 1 atm. An increase in absorption was observed after passage of the excitation pulse. This increase in absorption at 615 nm is expected because the extinction coefficient of Hb is substantially larger than that of the unphotolyzed HbCO. The pulse repetition rate was one per 100 μsec to allow for diffusion-controlled recombination of Hb with CO between excitations. At higher rates a background signal due to incomplete recovery was observed. The sharp spike in the absorption increase observed near zero delay is the result of an artifact caused by coherence between the pump and probing beams. The detailed interpretation of this artifact has been reported elsewhere (*14, 15*). The dashed line follows the absorption rise corrected for the artifact.

The remarkable result is that the rise of the induced absorption appears in less than 0.5 psec. According to the theory of Zerner *et al.* (*6*), HbCO is easily dissociated because there is a radiationless transition from the initially excited electronic state of the porphyrin π-electrons to a lower state corresponding to a $d_\pi \rightarrow d_{z^2}$ excitation of the iron atom. The d_π wave function forms a bonding orbital with the CO wave functions, but the d_{z^2} is part of an antibonding orbital which results in a repulsive force exerted on the CO molecule. The present experiment shows that the potential curve crossing to a dissociative state takes place on a subpicosecond time scale. This suggests that the high quantum yield for photodissociation of HbCO is due initially to fast dissociation rather than to a slow rate of nondissociative energy dissipation. The constant level in Fig. 1 following dissociation shows no hint of recombination on a time scale of 20 psec. This is consistent with the findings of Frauenfelder and co-workers (*16*) that the final step for recombination of myoglobin and CO is in the time domain of nanoseconds or longer. Thus, the high quantum yield of HbCO is due to a fast rate of dissociation combined with a slow rate of recombination. The extremely rapid appearance of Hb absorption is rather surprising in light of the iron atom and histidine displacement supposedly required. A possible explanation may be that the displacement involved in complex formation is not as large as generally assumed.

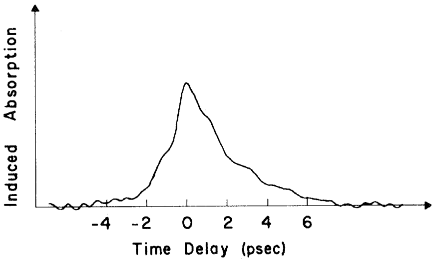

Fig. 2. Induced absorption at 615 nm in HbO_2 versus time delay between excitation and probe pulse.

This conclusion is supported by recent x-ray diffraction studies of HbCO Kansas (*17*).

In Fig. 2 we show the results of a similar experiment with HbO_2. The conditions were the same as before, except that atmospheric air rather than CO was present. Again we see an induced absorption with rapid rise, but in this case the response decays rapidly with a time constant of about 2.5 psec. Additional experiments with deoxygenated Hb gave the same result, indicating that this response is independent of O_2 pressure. Thus, our results indicate that the low quantum yield for photodissociation of HbO_2 is not due to rapid recombination of initially photolyzed molecules but rather to a dissociation rate that is much slower than the observed 2.5-psec nondissociative recovery. Apparently the observed response is due to a short-lived excited state whose absorption is greater than that of the ground state. At present we cannot identify the state, but its measurable lifetime has an important corollary. If this state has the same spin as the ground state, fluorescence should be observable in both Hb and HbO_2, albeit with very low quantum yield, ~ 0.001.

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Valid Climatological Data from Historical Sources by Content Analysis

Abstract. *Content analysis is used to derive dates of freeze-up and break-up from historical descriptions of river estuaries on Hudson Bay between 1714 and 1871. Validity testing of these dates indicates that they are comparable with modern data. It is thus proposed that the method affords potential for the systematic retrieval of a broad array of environmental data from the historical past.*

Current uncertainties about short-term climatic changes are exacerbated not only by the difficulties of projecting present trends into the future, but also by the problem of securing valid information about the past. Among the types of evidence traditionally employed to elucidate past climates, that contained in historical documents has been extensively studied. Manley (*1*) has called certain sources of this nature "non-instrumental diaries" and has classified them into one of five principal categories of evidence for climatic change. More recently, Ladurie (*2*) has appealed for the development of systematic procedures for the acquisition of valid climatic measures from documentary sources. A general procedure of this nature called content analysis has been developed in the social sciences, and has been identified as one of the basic innovations of the social sciences in this century (*3*). We have employed content analysis to derive dates of break-up and freeze-up of river estuaries on Hudson Bay from 18th- and 19th-century descriptions of these estuaries. Testing of these historical dates indicates that they are not inferior in quality to those obtained within the modern Canadian observing network. Our purpose here is to report selected results demonstrating their validity, and to suggest that the method affords the potential to yield a wide array of valid environmental information about the historical past.

The documents subjected to content analysis comprise daily journals kept by personnel of the Hudson's Bay Company in the period 1715 to 1871 at forts lo-

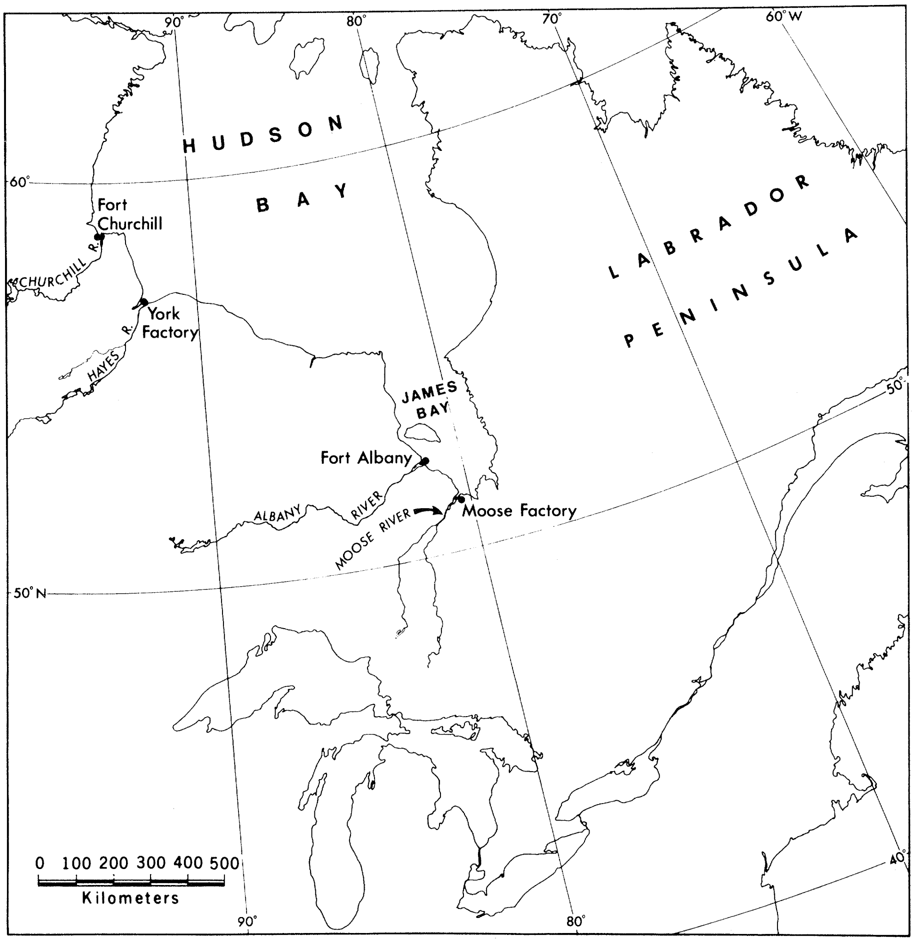

Fig. 1. Location of the estuaries of the Churchill, Hayes, Albany, and Moose rivers.