Ultra-fast Recombination in Nanosecond Laser Photolysis of Carbonylhaemoglobin

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Summary The transient absorption change observed in nanosecond laser photolysis of carbonylhaemoglobin which was previously attributed to a tertiary structural change is shown to arise from ultra-fast ligand recombination.

ALPERT *et al.*[1] observed transient absorption changes immediately following the flash in nanosecond photolysis of liganded haemoglobin solutions. They concluded that these were due to tertiary structural changes and that for the carbon monoxide complexes, known to have high photodissociation quantum yields,[2] the final product on this time scale was completely ligand free. We have undertaken a detailed study of the spectra of this transient species and final product and their variation with temperature. The results show that the published interpretation cannot be correct.

50 μmol l^{-1} solutions of human carbonylhaemoglobin (HbCO) in 0·1 mol l^{-1} potassium phosphate buffer at pH 7 in a thermostatted cell were photolysed with 30 ns pulses from a frequency doubled ruby laser giving up to 0·5 J at 347 nm. Transient absorption changes were monitored with an oscilloscope.

At room temperature the kinetic trace observed is exactly as reported[1] but a change is observed when the temperature is varied. Whereas the initial transient absorption remains unchanged, within experimental error, the final level varies with temperature so that the difference between the initial and final levels increases with decreasing temperature. For illustration, the absorbances of the transient and final level, compared to that of HbCO, are given in the Table for wavelengths 416 and 438 nm. Traces are shown in the Figure. At 416 nm the transient absorption is lower than that of the ground state, *i.e.*, there is net transient bleaching whereas at 438 nm there is net transient absorption. In both cases the final level is intermediate between the ground state and the transient absorptions.

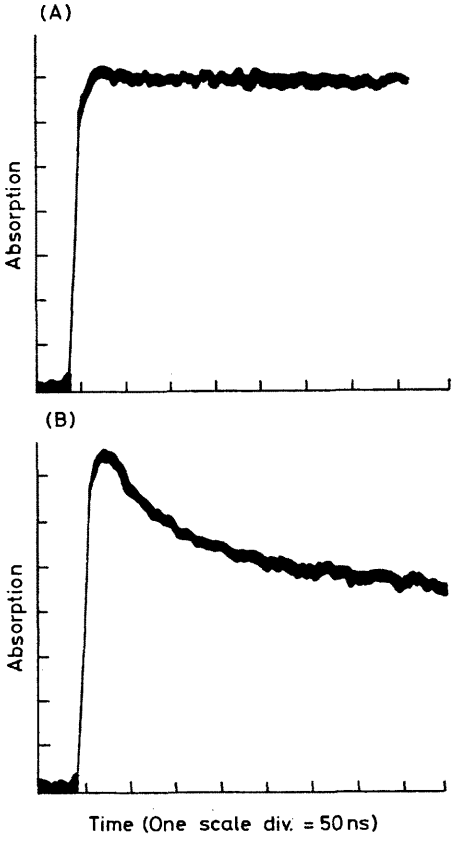

FIGURE. Kinetic traces observed in the 347 nm laser photolysis of carbonylhaemoglobin in aqueous buffer at pH 7·0. Wavelength 438 nm, temperature: (A) 328 K, (B) 277 K.

The fact that the magnitude of the transient absorption change decreases with increasing temperature is not in accord with it being due to conformational change. Evidence that the transient absorption change can be attributed

TABLE. Comparison of absorbances of transient and product with those of HbCO, Hb, and Hb*.

	λ = 416 nm	λ = 438 nm
HbCO	1·18	0·20
Hb[a]	0·64	0·60
Hb*[b]	0·61	0·63
Transient 277 K ..	0·70	0·54
Final level[c] 277 K ..	0·94	0·38
% Recombination ..	50	47
Transient 298 K ..	0·67	0·50
Final level[c] 298 K ..	0·80	0·44
% Recombination ..	25	20
Transient 328 K ..	0·70	0·50
Final level[c] 328 K ..	0·73	0·49
% Recombination ..	6	3

[a] Measured independently from a solution of the same concentration as that of HbCO. [b] Calculated from the value of Hb on the basis of data given in ref. 3. [c] 350 ns after the laser pulse.

to recombination of the carbon monoxide comes from comparison of the values of absorbance of the transient and final level with that of Hb* which is the immediate product of complete photodissociation observed in microsecond photolysis[3] and would be the expected final product of nanosecond photolysis with the high energy pulses used in this work. Hb* is ligand free haemoglobin retaining the quaternary conformation of the liganded form.[3] It is clear that at the lower temperatures the final absorption does not correspond to that of Hb* but that within experimental error the transient absorption does. Therefore, we propose that the immediate product of photodissociation is Hb* but that this undergoes partial recombination with CO. As the temperature is raised more of the CO escapes from the vicinity of the haem and so less recombines. Percentages of recombination calculated on this assumption are given in the Table and show reasonable agreement for the two wavelengths.

In microsecond photolysis experiments using high pulse energies this ultra-fast recombination would not be detected because the recombined CO would be continuously re-photolysed and all of the CO would have escaped from the haem pocket by the end of the pulse. However, the recombination would affect quantum yield determinations and in particular would explain the variation of quantum yield with temperature recently reported.[4]

A full account of these experiments together with a discussion of their implications will be reported later.

(*Received, 5th October* 1978; *Com.* 1068.)

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[3] C. A. Sawicki and Q. H. Gibson, *J. Biol. Chem.*, 1976, **251**, 1533.
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