PHOTOLYSIS KINETICS BY FREQUENCY DOUBLED LASER EMISSION

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1. Introduction

In recent years the use of crystal lasers for the activation of photosynthetic reactions has been developed [1,2] and has provided unique experimental data on the cytochrome *c*-chlorophyll reaction in photosynthetic bacteria over a wide temperature range. The chlorophyll and carotenoids in photosynthetic bacteria and plants absorb laser light with a high quantum efficiency [3]. Thus, only 1–10 mJ of ruby laser light is needed to fully activate these systems. Photolysis of haemoprotein-CO compounds is a much more difficult problem since their absorption coefficients at 694 nm are much lower than that of chlorophyll. A frequency doubling technique has been used in this work to obtain 347 nm radiation, a wavelength at which haemoproteins have higher extinction coefficients. Although the peaks of the Soret bands are in the 400–450 nm region, the absorption at 347 nm is, for carbonmonoxy-haemoglobin, 6 times as great as at the Soret band peak and 800 times as great as at 694 nm. The absorbancy changes caused by the laser photolysis were read in the 400–500 nm region.

Among the principal advantages of laser photolysis over the conventional xenon flash technique include the shorter laser flashes at comparable numbers of quanta [4], the greater ease in spectrally separating the exciting and measuring light, the highly specific excitation obtainable with the approximately monochromatic laser light and the ease of operating the laser energy generator some distance from the detector because of the high degree of collimation of the laser beam, thereby minimizing electrical and acoustic interference.

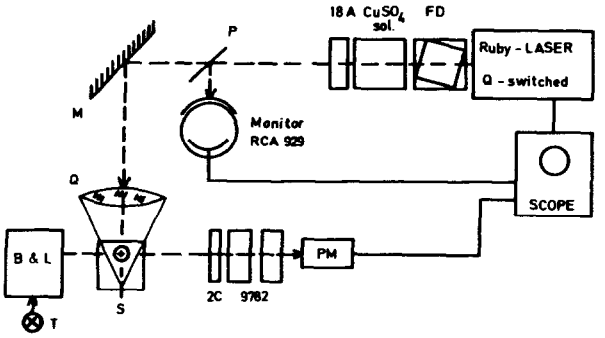

Fig. 1. Block diagram of apparatus. B&L, Bausch & Lomb monochromator; FD, frequency doubler; M, front-surface mirror; P, quartz plate; PM, photomultiplier; Q, quartz lens; S, sample; T, tungsten lamp; LC, 18A and 9782, filters.

2. Experimental

The experimental set-up is shown in fig. 1, it consisted of a Q-switched ruby laser followed by a frequency doubler. The 694 nm fundamental wavelength in the actinic light was eliminated by 2 cm of saturated $CuSO_4$ solution which was followed by a Wratten

18 A glass filter, with a transmission of 80% at 347 nm and less than 0.01% in the 400–650 nm region, to eliminate the pump light which accompanied the laser flash. A quartz plate was inserted into the beam to reflect a part of the laser light onto a monitoring phototube which was covered with a thin fluorescent tissue or a phosphorescent paste. A quartz lens was used to focus the exciting light beam through the surface of the liquid sample which was contained in a Lucite cuvette. The measuring light beam from a tungsten lamp and a Bausch & Lomb $\frac{1}{4}$ meter monochromator was passed through the sample perpendicular to the exciting beam, onto a photomultiplier, through 2C and Corning 9782 filters, which effectively protected the photomultiplier from the intense scattered laser light. The characteristics of all the filters are shown in fig. 2. The signals from the monitoring phototube and the photomultiplier were displayed on a Tektronix Type 564 storage oscilloscope with a Type 3A74 plug-in unit. The oscilloscope sweep was triggered simultaneously with the pumping light.

Two checks were made of the photomultiplier response under extreme conditions. First, to check for space charge effects, the cuvette was filled with water and the monochromator slits opened wide, to produce a large number of photoelectrons from the measuring light, while the photomultiplier voltage was set at the lowest value used in the experiments. A laser pulse in these conditions did not cause a substantial increase in the pulse width, as would be expected when space charge limitation sets in. Second, to check for photocathode fatigue due to stray 347 nm light, the cuvette was replaced with reflecting white paper. In both these tests the performance of the detection system was entirely satisfactory and the response to small electrical pickup effects was very much faster than the shortest time constant measured. Furthermore, no measurable photodissociation of the CO complexes was observed due to the measuring light.

The main features of the laser used (Lear Siegler, Type 140 Q) were a Brewster angle ruby $\frac{3}{8}$ inch in diameter in an elliptical cavity, with a resonant reflector at the front and a Brewster angle prism at the back. A saturable filter (pumped uranyl glass) was inserted in the optical path as a Q-switch. The frequency doubler was a KDP (potassium dihydrogen phosphate) crystal suspended in glycerine and mounted in a gimbal unit provided with a micrometer adjustment. In this the non-linearity in the electric susceptibility of the piezoelectric crystal is used to achieve second harmonic generation [5]. Approximate phase matching between the 2nd harmonic and the fundamental was accomplished by scanning the crystal with the micrometer adjustment. The 2nd harmonic energy was measured with a Type 101 ballistic thermopile (TRG Corp.) connected to a Keithley μV meter (Model No. 149 with amplifier), the output of which was displayed on a galvanometer-type chart recorder. The 2nd harmonic was found to have an energy of about 3 mJ at the cuvette with the unfocussed principal beam and to have approximately the same collimation angle as the principal beam. The anode resistor of the monitor phototube was adjusted until the output of the monitor equalled 3 V for an energy at the cuvette of 3 mJ.

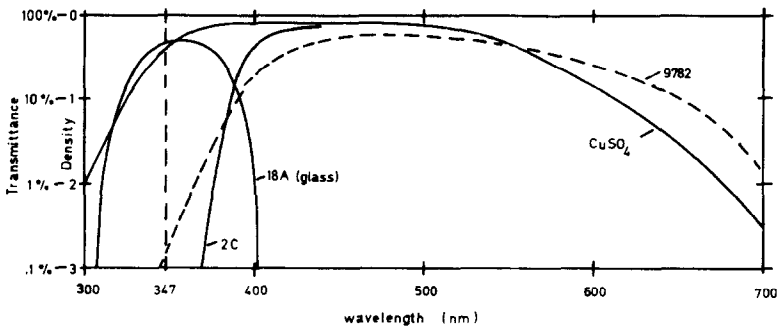

Fig. 2. Characteristics of filters used.

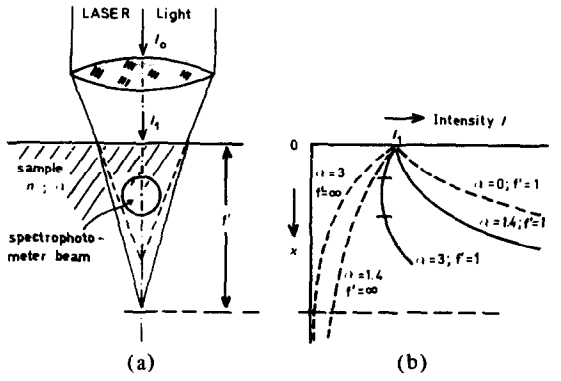

Fig. 3. Intensity distribution of exciting light in the sample. a) Geometry of light beam in sample; b) Calculated intensity distribution for several cases. α, absorption coefficient of sample; I_0 and I_1, light intensities; n, refractive index of sample; x, distance from surface of sample (cm).

Special attention was given to the arrangement of the focusing lens and sample. The f-number and the location of the focus were chosen such that, by a combination of the absorbing properties of the sample and the increasing intensity along the converging excitation beam, a region with an almost uniform spatial distribution of quanta in the area of the spectrophotometer beam was obtained. The power density I as a function of the coordinate x measured from the sample surface can easily be derived from fig. 3a:

$$I(x) = I_1(1 - \frac{x}{f'})^{-2} \exp.(-\alpha x)$$

where the absorption coefficient of the sample is $\alpha(cm^{-1})$, I_1 is the power density at $x = 0$, and f' (cm) is the true focal distance in the sample (refractive index n). Intensity distributions for several cases are shown in fig. 3b.

3. Results and Discussion

The technique was tested and applied to six carbonmonoxy haemoproteins with the results shown in table 1.

In these solutions the CO concentration was about

Table 1
Recombination rates of CO in the presence of $Na_2S_2O_4$. pH near neutrality.

carbonmonoxy compounds	conc. (μM)	monitoring wavelength (nm)	τ(msec)
myoglobin	28	436	2.3
horse-radish peroxidase	26	436	310
turnip peroxidase	10	436	140
cytochrome-c peroxidase (a)	6	436	5.7 640
pigeon heart mitochondria	–	450	43
cytochrome oxidase (b)	–	430	30

(a) We are indebted to Dr. T.Yonetani for samples of this substance.
(b) Excitation with normal ruby line (694 nm). Such excitation produced no effect with myoglobin. The sample was generously provided by Dr. Q.H.Gibson.

the saturation value of 10^{-3} M and the pH was kept near neutrality with dilute buffers. Hence the rate constants for the re-association reactions are obtainable from $k(M^{-1}\ sec^{-1}) = 10^3\ \tau^{-1}$, since the reactions are pseudo-first order. Good agreement was found between values of 5.5×10^5 reported by Gibson [6] from flash photolysis and $4.4 \times 10^5\ M^{-1}\ sec^{-1}$ calculated from table 1 for the combination of CO with myoglobin. Equally good agreement was found for horse-radish peroxidase, with rate constant 3×10^3 from table 1 and $1 \times 10^3\ M^{-1}\ sec^{-1}$ reported by Chance [7] from measurements in a stopped flow reactor using pH 4.5 acetate buffer. The underlying assumption that the effect of the dissociation rate constant on τ is negligible is based on the small equilibrium dissociation constants reported for horse-radish peroxidase-CO, cytochrome-c-peroxidase-CO and myoglobin-CO. In the absence of CO, turnip peroxidase showed an absorption change after laser flash excitation with a time constant of about 7 sec. The other solutions showed no such phenomenon.

Because of the uniform distribution of the excitation light in the monitored portion of the sample and assuming a quantum efficiency of one, a rough com-

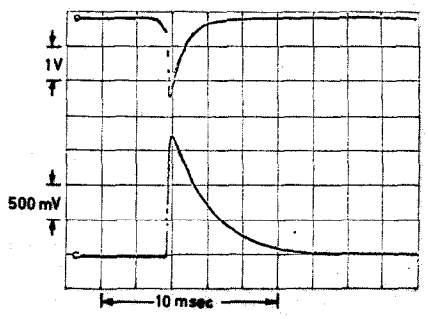

Fig. 4. Myoglobin-CO. Upper trace, laser beam monitor; lower trace, spectrophotometer reading 436 nm.

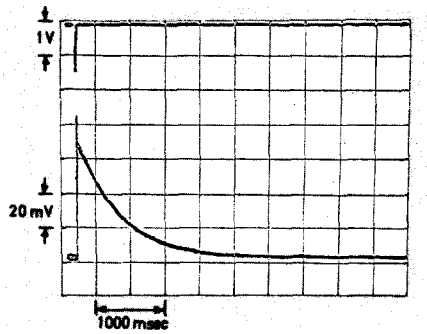

Fig. 5. Cytochrome *c* peroxidase. Upper trace, laser beam monitor; lower trace, spectrophotometer reading 436 nm.

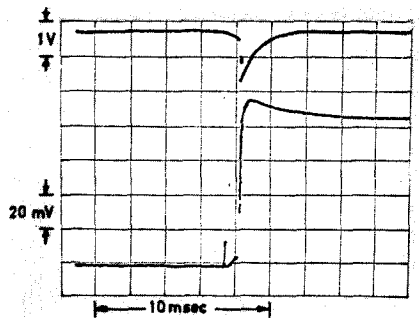

Fig. 6. Cytochrome *c* peroxidase. Same as fig. 5, different time scale.

parison between quanta absorbed and molecules present can be made. With myoglobin and horseradish peroxidase a saturation effect was observed when the ratios of quanta absorbed to molecules present were near unity. When the concentration of myoglobin was doubled this effect could no longer be achieved with the energy available.

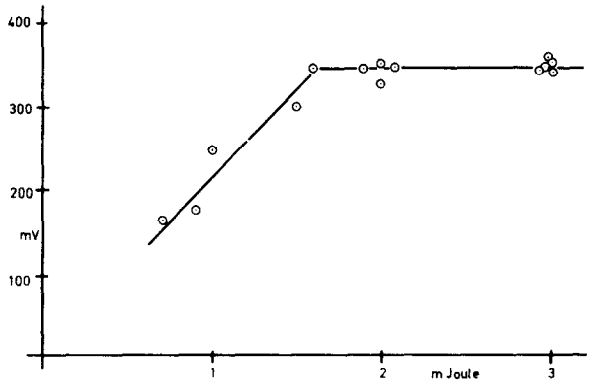

Fig. 7. Pigeon heart mitochondria-CO. Ordinate, transmission change at 450 nm; abscissa, energy input.

In mitochondria the cytochrome oxidase combines readily with CO to form a CO compound [8] the photolysis of which reactivates respiration [9], which can be observed from changes in the absorbancy of cytochrome a_3 [10]. Fig. 7 shows that a saturation in the photolysis of this compound was obtained with 2 mJ energy input. The ordinate shows the maximum initial deflection observed by the photomultiplier. Points at maximum and minimum energies were obtained at both the beginning and the end of the sequence.

Faster processes with τ as low as 0.2 msec would have been observed in all cases described here but none were found.

Acknowledgements

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