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RELATIONS BETWEEN THE LASER-INDUCED OXIDATIONS OF THE HIGH AND LOW POTENTIAL CYTOCHROMES OF *CHROMATIUM* D*

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SUMMARY

1. A single 30-nsec laser flash will oxidize only a limited amount of cytochrome in *Chromatium* D.

2. The cytochrome oxidized will be C553 in dark, anaerobic whole cells and chromatophores or C555 in illuminated, anaerobic whole cells, in aerobic whole cells, and in illuminated chromatophores.

3. This evidence indicates that the electron or energy transport component whose pool-size provides the limitation of Point 1 is common to the electron paths associated with both cytochromes unless a switching mechanism turns one off when the other is on.

4. The quantum efficiency for C553 oxidation is close to 1. The ratio of maximum amount of cytochrome oxidation in a single flash to quantum efficiency is the same for C555 as for C553. This means that both cytochromes are oxidized by centers served by the same light gathering bacteriochlorophyll and suggests that their quantum efficiencies are equal.

5. Oxidation half-times for the dark and illuminated laser-induced cytochromes are 1 μsec and 2–3 μsec, respectively; reduction half-times were >10 sec and 8 msec (34 msec in chromatophores), respectively.

6. Assuming that the laser oxidizes equal amounts of the two cytochromes, the following *in vivo*, reduced-*minus*-oxidized ε_{mM}'s were obtained for C555: 48 at 423 nm and 8.7 at 556 nm.

7. In our cultures the size of the photosynthetic unit was estimated at 140–160 bacteriochlorophyll molecules per cytochrome oxidized by a 30-nsec flash (whole cells).

INTRODUCTION

One of the earliest observations made with laser flashes on *Chromatium* D was that, regardless of the intensity above a very low saturation point, a short (30 nsec, 'Q-switched'; wavelength, 694 nm) flash will oxidize only about 1/5 of the cytochrome that can be oxidized by strong, steady illumination[1–3]. In other

* Paper No. 24 in a list of source papers from the Johnson Research Foundation on *Studies of Photosynthesis using a Pulsed Laser.*

words, the pool of cytochrome is larger than some bottleneck which limits the amount that can be oxidized in a single flash. By 'bottleneck' we mean some pool that can be saturated with a single 30-nsec flash, but which is of such a size that it must turn over several times for all of the cytochrome to be oxidized.

OLSON AND CHANCE[4,5] have shown that different cytochromes in *Chromatium* D can be oxidized depending upon the strength of illumination and upon the presence or absence of oxygen. Thus C553 (C423.5 in their notation) is oxidized almost exclusively in the steady state produced by the lowest light levels and C555 (C422) only at the highest levels. If one assumes quantum efficiencies of the same order of magnitude this steady state behavior correlates with the further fact that C555 has a fast dark reduction rate ($<$0.1 sec) while the reduction rate for C553 is slow (seconds to minutes). Oxygen, if present, oxidizes C553 but not C 555 leaving only the latter to be oxidized by light.

In work previously reported[2,6] the presence of oxygen or the presence of steady background illumination of an intensity just sufficient to oxidize C553 appeared to have little effect on either the extent or the rate of laser-induced cytochrome oxidation leading to a supposition that in all cases the laser oxidizes the same cytochrome and that it is the high potential C555. The fast reduction rate in the presence of background illumination is consistent with this interpretation. However, the slow reduction rate in the absence of background illumination indicates that C553 rather than C555 is oxidized by the flash.

The work reported in this paper was undertaken to identify by spectral means the cytochromes oxidized by laser flash under the various conditions of concern and to measure more accurately the cytochrome reduction rates. We will discuss the implications of these results in relation to those of PARSON AND CASE[7] in their accompanying paper and in relation to the recent proposals by MORITA[8], by SYBESMA *et al.*[9–11], and by CUSANOVICH *et al.*[12] that there may be separate chlorophyll reaction centers for separate cytochromes in *Chromatium* D and *Rhodospirillum rubrum*. A preliminary report of this work was made at the 3rd Intern. Biophys. Congr.[13].

METHODS

Chromatium D was grown in medium described by MORITA *et al.*[14] with the pH adjusted to 7.9. The cells were incubated at 30° for 2 or 3 days (depending on the amount of inoculum which in all cases was less than 5 %) under an average illuminance of about 1700 lux from two 75-W reflecting flood lamps placed at opposite sides of the growing tank. Cells used at this part of the growth cycle (late logarithmic or early stationary) were termed 'normal', and those allowed to continue incubation for 5 more days were called 'starved'. Harvesting was accomplished by centrifugation at 3000 $\times$ *g* for 5 min, and the cells were resuspended in supernatant fluid.

Cultures obtained originally from Dr. H. Schleyer of our laboratory were used in most of the work, but similar spectra and reduction rates were also measured with bacteria from the American Type Culture Collection, Rockville, Md., Collection No. 17899.

Cell-free particulate suspensions or 'chromatophores' were prepared by a method patterned after KIHARA AND DUTTON[15] using 0.1 M Tris–HCl (pH 7.3) buffer. 'Crude', sonicated particles which they described were then centrifuged at

96000 × *g*, washed with buffer, and recentrifuged. The final 'washed particles' were resuspended in a suitable amount of buffer and stored under nitrogen at 4°. Before use, the chromatophores were de-oxygenated by bubbling with nitrogen and in some cases reducing agents added when low redox potentials were desired.

Bacteriochlorophyll (BChl) analyses were made by extraction with methanol, and absorbance measurements were obtained at 772 nm using an extinction coefficient of 42 $mM^{-1} \cdot cm^{-1}$ from SMITH AND BENITEZ[16].

The basic laser apparatus has been described previously[3,17], but we have modified the system by adding a dual wavelength spectrophotometer[18] using interference filters, as shown in Fig. 1, to monitor long term cytochrome oxidation states of the sample. The signal from the photomultiplier and preamplifier, set for the high illumination level needed for fast laser measurements, required further amplification before it could be handled by the regular dual wavelength phase-detector and difference-amplifier circuitry. For a second or two, at the time of laser flash, the sample was exposed to the more intense measuring light of the fast single beam spectrophotometer. Neither this exposure nor the measuring light source used by the dual wavelength spectrophotometer caused significant cytochrome oxidation. The more intense signal obtained during the fast measurement was recorded photographically from an oscilloscope (Tektronix Model 547). The preamplifier used in the fastest measurements was of a new design employing a Fairchild μA733C integrated circuit and had a rise time of 50 nsec. For slower measurements the signal went through appropriate low pass filters to reduce the noise. Provisions were also made for steady illumination of the sample through a Wratten 88A filter.

Laser output energies (30-nsec pulses at 694 nm) were measured using a TRG Model 101 ballistic thermopile after the beam had passed through the concave

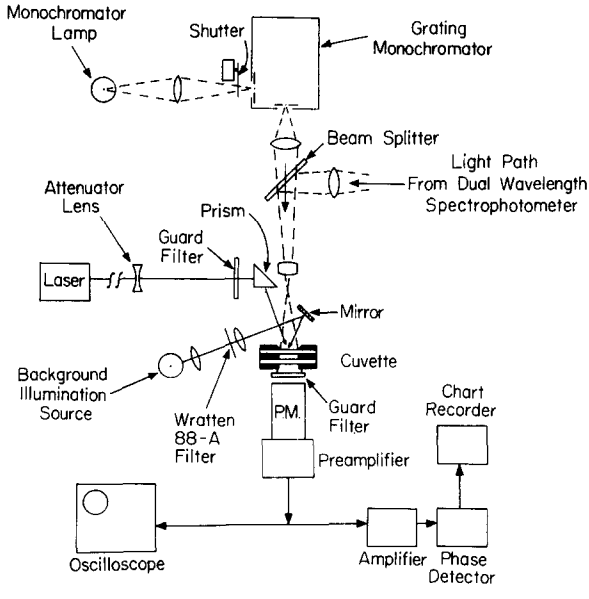

Fig. 1. Schematic representation of the laser single beam and dual wavelength spectrophotometer apparatus.

attenuating lens. Further attenuation of the laser beam for the saturation curve data was accomplished by the use of neutral density filters.

The steady illumination was measured with a Reedeer thermopile which had been calibrated against a standard lamp by Dr. M. Nishimura. A 2-cm water cell was included in the light path along with either a Wratten 87 or 87B filter. These filters were chosen because they closely encompass the spectral region of maximum bacteriochlorophyll absorption. The difference between their respective transmissions approximates a filter passing about 75 % of the light between 770 and 910 nm. The differences between thermopile readings with the two filters, respectively, were converted to units of Einsteins·cm^{-2}·sec^{-1} by assuming a mean wavelength of 850 nm and used directly in Figs. 2–4, and 6 as a measure of the steady illumination intensity. These values, however, are only expected to be proportional to the actual actinicity of the light, and a further calibration is necessary to obtain the proportionality factor. Assuming a quantum efficiency of 1, we can use the oxidation of C553 as an actinometer. Column C in Table I gives the initial rate of cytochrome C553 oxidation in one sample at three different steady illumination levels calculated using $\Delta\varepsilon_{mM}$ (423 nm) = 70 (ref. 19)*. The last column shows that the actual actinicity of the steady illumination is about 3 times that indicated by the thermopile measurements.

TABLE I

ACTUAL ACTINICITY OF THE CALIBRATED LIGHT SOURCE

Light level (*A*)	*Energy between Wratten 87 and Wratten 87B* (*nEinstein·cm⁻²·sec⁻¹*) (*B*)	*Initial rate of cytochrome oxidation in typical samples of Chromatium D** (*nmole·cm⁻²·sec⁻¹*) (*C*)	*Ratio* (*C/B*)
3	0.0055	0.0169	3.07
4 × 1/2	0.0134	0.0379	2.83
4	0.0269	0.0972	3.61

* Bacteriochlorophyll concentration, 0.175 mM; light path, 1.6 mm.

An 'integrating sphere' (icosahedron), built by Dr. W. Parson (at this laboratory) and containing 20 solar cells (International Resistance Co. No. S1020E4-PL) was used to determine the absolute absorption of two samples. Measurements were taken at 694 nm with the experimental cuvette containing either water (A), India ink (B), or the *Chromatium* D whole cell sample (C). The absorption was calculated by substituting the combined output of the solar cells for Cases A, B, and C into the following equation[5]: Absorption = (A–C)/(A–B). Previous investigation had shown that the use of a scatterer (milk) in the A measurement instead of water is likely to affect the resulting absorption by less than 0.04.

RESULTS

Fig. 2 shows, in the upper trace, a dual wavelength recording of cytochrome oxidation in *Chromatium* D whole cells induced by steady illumination as indicated

* The value given for the reduced-*minus*-oxidized *Chromatium* D cytochrome C553 ε_{mM} (423 nm) in their Table II is in error and should read 141 (2 hemes) in agreement with their Fig. 1 on p. 445 (personal communication from Dr. R. BARTSCH).

across the top and by laser flashes at A, B, and C. The lower traces are the oscillographic records of responses to laser flashes on the same sample under conditions identical to those at times A, B, and C, respectively. With no background illumination the cytochrome oxidized by laser flash A takes seconds to reduce. The level of illumination used at the time of flash C is the amount that saturates the oxidation of C553

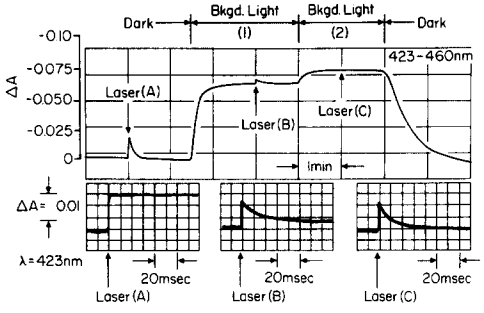

Fig. 2. Top: Dual wavelength spectrophotometer trace (423–460 nm) showing laser-induced cytochrome oxidation in the dark, A, and laser-induced cytochrome oxidation on top of steady state cytochrome oxidation in the presence of two different background illumination intensities, B and C. The background intensity in Range 1 was about $1.7 \cdot 10^{-11}$ Einsteins·cm^{-2}·sec^{-1} and that of Range 2 was about $4.6 \cdot 10^{-11}$ Einsteins·cm^{-2}·sec^{-1}. Bottom: Single beam traces at Points A, B, and C monitored at 423 nm. Cytochrome oxidation is indicated by an upward deflection in the above traces. Sample, anaerobic whole-cell suspension; light path, 1.6 mm; BChl concentration, 0.154 mM.

as observed by OLSON AND CHANCE[5]. The further oxidation produced by flash C recovers so fast that it does not show on the dual wavelength trace. At the intermediate level of illumination used at flash B, it is seen that the steady state level of oxidation of C553 is about 82 % of saturation and that the laser-induced oxidation recovers with a mixture of fast and slow phases. The slow recovery portion produces a blip on the dual wavelength trace whose height is 21–28 % of the difference in steady state levels at B and C. The dark, slow recovery laser component in A has 21 % of the absorbance change observed for the steady state level at C. This suggests that the laser oxidizes only 1/4–1/5 of the available C553 if we assume that the slow recovery component is to be identified as such, and about 1/12 of the total cytochrome.

In Fig. 2A the laser-induced rise half-time occurs in 1 μsec (refs. 3, 6) as determined in other experiments. Rise half-times were a little longer in the case of Fig. 2C (2–3 μsec). The total recovery time of the dark, laser-induced reaction varies from 10 to 360 sec and may show both first and zero order kinetics. The half-time for the first order phase ranges from 10 to 30 sec, and the recovery time of the zero order phase is between 0 and 330 sec. The recovery half-time with background illumination as in C, averages 8.2 ± 0.4* msec (mean of nine experiments).

Fig. 3 shows the results of a 'light-titration' of the effects described in Fig. 2. The background illumination was varied as indicated on the abscissa. The ordinates are explained in the figure legend. The terms cycled and continuous used on Fig. 3 itself refer to the history of the background illumination. By cycled we mean that

* All deviations are standard deviations of the mean.

the steady light was turned on before and off after each laser shot (the dual wavelength trace was allowed to reach the dark steady state value before a new cycle was started). Continuous simply means that the steady illumination was on during the whole experiment and the points taken consecutively as the intensity was increased (low to high) or decreased (high to low). The laser flashes were of saturating intensity (about 2 nEinsteins/cm^2 for 694-nm light).

Laser-induced difference spectra corresponding to Figs. 2A and 2C are seen in Fig. 4. Each point represents the change in absorbance caused by the laser measured 1 msec after the laser was fired (the same results were obtained 50 μsec after the flash)*. With no background illumination the peak was at 408 nm and the troughs at 423.5 and 553 nm. In the presence of background illumination the peak shifted to 410 nm and the troughs to 422 and 555–556 nm, respectively. This evidence indicates that the dark laser-induced reaction corresponds to the oxidation of cytochrome C553 and the background illumination laser reaction to oxidation of C555. The dark spectrum might contain some hidden C555, but the absence of any fast recovery component in Fig. 2A is evidence against any large proportion of C555 being present, at least after a few milliseconds. (We can't rule out a small initial amount of C555 being reduced before it would show up as a fast-reducing component.)

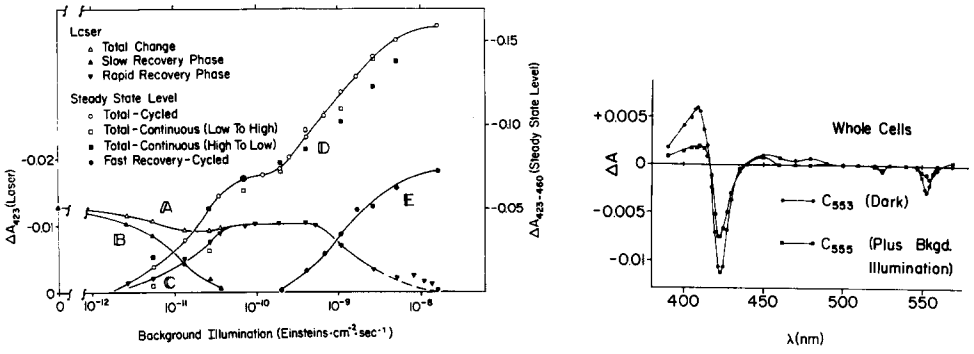

Fig. 3. The amount of laser-induced cytochrome oxidation and the steady state cytochrome oxidation level produced in an anaerobic whole-cell suspension as a function of incident background light intensity. Curve A is the total laser-induced change; Curve B, the slow (>1 sec) recovery laser component (C553); Curve C, the rapid (8 msec) recovery laser component (C555); Curve D, the total steady state level; and Curve E, the fast (<0.1 sec) recovery portion of D. The points which fall above the dashed line at the high intensity end of Curve C are due to the fact that while the entire sample was being flashed by the laser, a small part of it was not being directly illuminated by the continuous light. The laser points were measured by extrapolating the traces back to the time at which the laser was fired. Light path, 1.6 mm; bacteriochlorophyll concentration, 0.175 mM.

Fig. 4. Laser-induced cytochrome oxidation spectra for anaerobic whole-cell suspensions obtained by extrapolating the traces back to the time at which the laser was fired. The cytochrome oxidized in the dark (●—●) was identified as C553 by virtue of its peak at 408 nm and troughs at 423.5 and 553 nm; that oxidized in the presence of about $4.3 \cdot 10^{-11}$ Einsteins·cm^{-2}·sec^{-1} (■—■) was C555 with a peak at 410 nm and troughs at 422 and 555–556 nm. Light path, 1.6 mm; bacteriochlorophyll concentration, 0.15 mM; bandwidth of monochromator, 1.6 nm.

* The '50-μsec effect' reported previously[6] has been found to be an instrumental artifact associated with a particular photomultiplier tube.

Fig. 5 is a plot of the laser-induced absorption changes *versus* incident laser intensity for both of the above cytochromes. Logarithmic plots, not presented, show that the curves are rather accurately exponential agreeing with the formula given by DUYSENS[20] for single-reaction-center-containing, isolated photosynthetic units. Thus our work on whole cells agrees in this respect with that of PARSON[21].

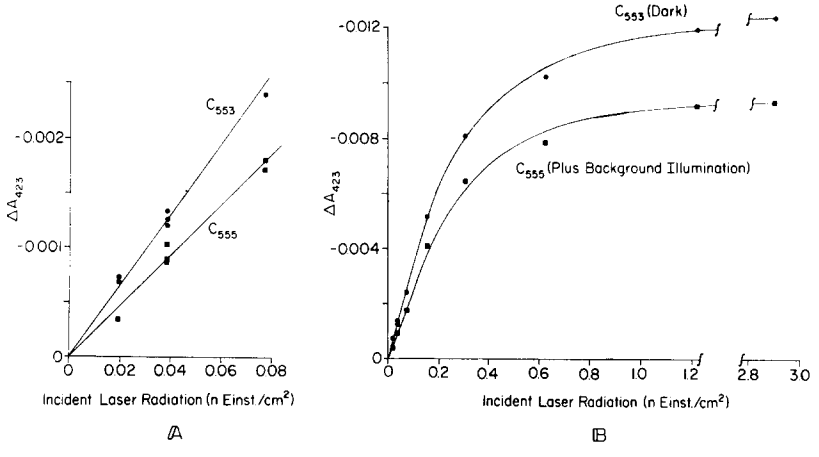

Fig. 5. A. The change of absorbance as a function of incident laser intensity at low intensities for C553 and C555. The quantum efficiency of C553 in this case was 0.90 assuming an ε_{mM} (423 nm) = 70. B. Same as in A but including the entire saturation curve. Light path, 1.6 mm; bacteriochlorophyll concentration, 0.175 mM.

Regardless of the shape of the curve information may be derived from a study of the initial slopes and the saturation asymptotes. The latter necessarily has the value $B_i\varepsilon_i$ where B_i is the size of the 'bottleneck' for cytochrome i (in μmoles/cm^2 cuvette area) and ε_i is the change in millimolar extinction coefficient for oxidation of cytochrome i. The initial slope, dA/dI, where A is the absorbance change and I is the intensity of laser pulse in μEinsteins/cm^2, is equal to $F\phi_i\varepsilon_i$, where F is the fraction of the laser light absorbed by BChl and ϕ_i is the fraction of photons absorbed which can be trapped by reaction centers which can oxidize cytochrome i when all reaction centers of this type contain oxidized acceptor and reduced cytochrome.

Table II exhibits measurements of these parameters when C553 is the cytochrome oxidized (dark) and when C555 is oxidized (background illumination). The ratio of asymptote to initial slope may be called the 'break point', I_{Bi} and is equal to $B_i/(F\phi_i)$. The ratio of Row 3 to Row 4 is the ratio of I_{B5} to I_{B3} and is equal to $B_5\phi_3/(B_3\phi_5)$ where subscript 5 stands for C555 and 3 for C553. This ratio is seen to be close to 1. If $\phi_5 = \phi_3$, then the bottlenecks for both cytochromes are equal in size, if not identical.

Row 6 of Table II presents quantum efficiencies for the laser oxidation of C553. ϕ_3 is seen to be close to 1 except, perhaps, in chromatophores, in agreement with other investigators[21-23]. Thus it is clear that the centers oxidizing both cytochromes share the same light-gathering bacteriochlorophyll. If not, that fraction of photons captured by the centers serving C555 would be lost to C553 system and ϕ_3 would have to be considerably less than 1. Since they share the same light-gathering bacteriochlorophyll and since $\phi_3 = 1$, it seems likely that $\phi_5 = 1$.

TABLE II

DATA USEFUL IN ESTIMATING THE EXTINCTION COEFFICIENT OF C555 AND THE QUANTUM EFFICIENCY OF C553

The information in Rows 1, 2, and 3 was obtained from curves similar to Fig. 5A. That in Row 4 was obtained from points similar to results at the far right hand side of Fig. 5B. Bacteriochlorophyll concentrations: 1, 0.15 mM; 2, 0.154 mM; 3, 0.175 mM; 4, 0.268 mM.

Experiment	*1**	*2**	*3**	*4***
1 Slope of C555 curve at low laser intensity ($-\Delta A \cdot \mu Einsteins^{-1} \cdot cm^2$)	17.8	39.0	23.6	11.8
2 Slope of C553 curve at low laser intensity ($-\Delta A \cdot \mu Einsteins^{-1} \cdot cm^2$)	28.4	49.1	33.1	19.9
3 Ratio of C555 to C 553 curve slopes at low laser intensity	0.626	0.794	0.714	0.59
4 Ratio of C555 to C553 saturation absorbance changes	0.637	0.710	0.755	0.66
5 Absorption of sample at 694 nm (%)	—	56.3***	52.1***	54.3§
6 Quantum efficiency of C553 oxidation§§	—	1.25	0.90	0.52

* Normal whole cells, each column a single experiment.
** Chromatophores, single experiment.
*** Using integrating sphere.
§ Using Zeiss spectrophotometer (sample not turbid).
§§ Assuming $\Delta\varepsilon_{mM}$ (423 nm) = 70.

The reduced-*minus*-oxidized extinction coefficient of C553 at 423 nm is 70 $mM^{-1} \cdot cm^{-1}$ per heme[19]. That for C555 is unknown, but on the assumption that $B_5 = B_3$, it is proportional to that for C553 in the same ratio as the observed absorbance changes. The mean of the six whole-cell absorbance ratios collected in Rows 3 and 4 together with eleven normal cell ratios whose mean is given in Table III (last row) is 0.68 ± 0.03. Multiplying this by the value 70 $mM^{-1} \cdot cm^{-1}$ gives 48 $mM^{-1} \cdot cm^{-1}$ per heme for C555. This may be compared with the value 55 $mM^{-1} \cdot cm^{-1}$ reported by CUSANOVICH AND BARTSCH[24] for a high potential cytochrome isolated from *Chromatium* D and with the value 47 $mM^{-1} \cdot cm^{-1}$ given by BARTSCH[25] for the high potential cytochrome c_2 of *R. rubrum*. It disagrees with the value 95 which can be estimated from the data of VREDENBERG AND DUYSENS[22] and the value 90 estimated by PARSON[21]. We can offer no reason for the discrepancy unless the assumption of equal amounts of C553 and C555 oxidized by laser flash is wrong.

Starved *Chromatium* D cells were found to behave in a manner similar to that of normal cells if one takes care to eliminate oxygen from the cell suspension by bubbling several minutes with nitrogen. The laser-induced spectra were essentially the same as those presented in Fig. 4. The recovery half-time of the C555, however, increased to 63 ± 8 msec (three experiments). If one does not bubble the starved cells with nitrogen, the laser-induced α-peak was at 555–556 nm in the dark as well as with background illumination.

Chromatophores also gave results similar to those of normal cells if small amounts of 1% sodium dithionite in 10 mM NaOH were added to lower the redox potential. Fig. 6 shows laser-induced α-spectra of chromatophores in the dark and with background illumination. The shift of the α-peak from 553 nm in the dark to 556 nm (between 556 and 557 nm in some experiments) when illuminated is

apparent. Dark recovery of C553 showed zero and first order kinetics in the same time range as in whole cells, but the C555 recovery half-time was 34 ± 6 msec (five experiments), about 4 times longer than in normal whole cells.

Table III summarizes other laser-induced spectral data in the form of means. Figures in parentheses are the number of experiments averaged into each result. Rows 1 and 3 give γ/α trough extinction coefficient ratios from oxidized-*minus*-reduced difference spectra without and with background illumination, respectively.

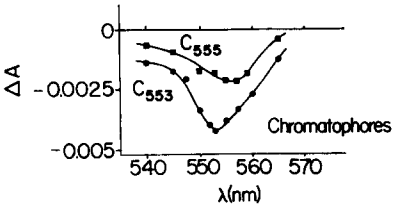

Fig. 6. Laser-induced cytochrome oxidation difference spectra for deaerated chromatophores to which small amounts of sodium dithionite were added to lower the redox potential (no redox buffering capacity added). ●—●, was obtained in the dark and its trough at 553 nm indicates C553 oxidation. ■—■, obtained in the presence of low level background light (about $4.3 \cdot 10^{-11}$ Einsteins·cm^{-2}·sec^{-1}) with a trough at 556 nm identifies the cytochrome as C555. Light path, 1.6 mm; bacteriochlorophyll concentration, 0.286 mM; bandwidth of monochromator, 1.6 nm.

TABLE III

EFFECT OF A SATURATING LASER FLASH ON C553 AND C555

	Normal cells	*Starved cells*	*Chromatophores*
$\Delta A_{423\,nm}/\Delta A_{553\,nm}$ (C553)	3.89 ± 0.22 (7)	4.55 ± 0.1 (3)	4.23 ± 0.22 (6)
Bacteriochlorophyll/C553*	137 ± 7 (14)	200 ± 28 (6)	295 ± 31 (7)
$\Delta A_{423\,nm}/\Delta A_{556\,nm}$ (C555)	5.61 ± 0.74 (5)	5.43 ± 0.33 (3)	4.52 ± 0.18 (2)
Bacteriochlorophyll/C555**	166 ± 17 (12)	240 ± 17 (4)	284 ± 37 (3)
$\Delta A_{423\,nm}$ (C555)/$\Delta A_{423\,nm}$ (C553)	0.67 ± 0.04 (11)	0.63 ± 0.06 (5)	0.63 ± 0.05 (3)

* Mole per mole assuming ε_{mM} (C553, reduced-*minus*-oxidized, 423 nm) = 70.
** Mole per mole assuming ε_{mM} (C555, reduced-*minus*-oxidized, 423 nm) = 48.

The results for C553 may be compared with the value, 3.6, obtained for extracted C553 (ref. 19) and those for C555 with the value, 5.5, for extracted high potential cytochrome[24]. The starved cell ratio in the first row seems a little high, but it may be indicative of inadequate endogenous reducing power so that some C555 was present. The low ratio for chromatophores in the third row could indicate that the intensity of the background illumination was too low so that some C553 was present. Dividing the value in Row 3 for normal cells into the extinction coefficient estimated above for C555 at 423 nm gives an estimate of 8.7 for ε_{mM} (C555, 556 nm). Rows 2 and 4 give estimates of the photosynthetic unit size as judged by the ratio of total bacteriochlorophyll present to cytochrome oxidized in a single flash. Since the extinction coefficient for C555 was estimated from this kind of data the results for the two different cytochromes differ only because of the use of different sets of data. The last line summarizes Soret absorbance changes for the two cytochromes.

DISCUSSION

It is apparent that even a great excess of laser light in a 30-nsec flash will not oxidize more than a limited amount of cytochrome, either C553 or C555, and that the amount of each is considerably less than the amount of the same cytochrome which can be oxidized by steady application of light over a longer time. This limitation in the amount of cytochrome that can be oxidized in one 'stroke' we have called a 'bottleneck'.

What can we say about the nature of the bottleneck? *A priori* it could be a limitation (a) on the size of a pool of cytochrome which alone can react directly with the reaction center bacteriochlorophyll, the rest of the cytochrome being oxidized through it but not before the excess bacteriochlorophyll oxidized by a single laser flash has been reduced by some process (such as return of the electron from the acceptor) which does not oxidize any more cytochrome; (b) in the amount of reaction center bacteriochlorophyll; or (c) in the amount of electron acceptor which alone can take electrons directly from the reaction center bacteriochlorophyll. The data of PARSON[26] makes it possible to distinguish between these possibilities. If the cytochrome were limiting we would expect that excess laser intensity would oxidize excess P890. If the bacteriochlorophyll of the reaction center ('P 890') were limiting we would expect that a second laser pulse flashed as soon as P 890 is reduced by the cytochrome after the first laser flash (2 μsec) would be able to oxidize more cytochrome. PARSON[26] observes neither of the above, and so it appears that the acceptor is the bottleneck. He has determined that in chromatophores it takes 60–80 μsec before a second laser flash is able to oxidize half the P 890 oxidized by the first flash. In whole cells the recovery half-time is much longer being in the order of 1 msec[7,26]. This is interpreted as being the turnover time of the bottleneck-acceptor.

The absence of C555 oxidation by laser flash in the dark (at least after a few milliseconds) requires explanation. Either (1) the bottleneck is common to both cytochromes so that competition for the oxidizing power of the reaction center followed by rapid equilibration between the two cytochromes gives the partitioning of cytochrome oxidation observed, or (2) the two cytochromes have separate bottlenecks but that for C555 is switched off under the conditions which give C553 oxidation as the sole product. The latter mechanism has been suggested to us by Dr. P. L. Dutton. He pointed out that if there are separate bottleneck-acceptors for the two cytochromes the lower redox potential, which prevails when C553 is the cytochrome observed to be oxidized, could reduce and thereby inactivate the acceptor associated with C555. In accordance with this idea, the slight depression in the total laser-induced absorbance decrease (Curve A of Fig. 3) at about $2 \cdot 10^{-11}$ Einsteins$\cdot$cm$^{-2}\cdot$sec^{-1} might be taken as a hint that the C553 pool is becoming oxidized at a lower background light level than that required to oxidize the acceptor for the C555 system. If the background illumination acts by increasing the ambient redox potential, then this would be evidence that the midpoint potential of the acceptor for the C555 system is somewhat higher than the midpoint potential of C553.

Several proposals have recently appeared suggesting that the different cytochromes may be oxidized by separate reaction centers. MORITA[8] concluded such in the case of *Chromatium* D on the basis of differing action spectra for the different cytochrome oxidations. SYBESMA AND FOWLER[9] came to a similar conclusion on

similar evidence for *R. rubrum*, and SYBESMA[10] AND SYBESMA and KOK[11] added to this evidence correlations with the spectral shift at 800 nm. CUSANOVICH *et al.*[12] suggested separate reaction centers in *Chromatium* D based on the observation of a new absorption change at 905 nm appearing only under redox conditions at which C553 is oxidized. These proposals are consistent with the idea of separate, but switchable, acceptors.

If, on the other hand, the acceptor is common, then it is necessary to say that the reaction centers cannot be entirely independent, if indeed there are more than one. If there were a single reaction center, the different action spectra and the different BChl absorption spectral behaviors could possibly be explained by the differing conditions required for their measurement. To observe action spectra and BChl absorption spectra associated with the oxidation of C555 it is necessary to use conditions (background illumination, the presence of oxygen or high ambient redox potential) which allow noticeable oxidation of C555. For the observation of C553 zero background illumination, absence of oxygen, and a redox potential around 0 mV (ref. 12) are the required conditions. SUZUKI *et al.*[27] show that variation of conditions can indeed change action spectra.

The possible models consistent with a common acceptor are summarized in Fig. 7. All three require equilibration between C555 and C553 to account for the complete absence of C555 oxidation (after several milliseconds) when there is no background illumination. The switchable acceptor model would not need this hypothesis. In view of the fact that cytochrome oxidation is faster under conditions eventually oxidizing C553, it would seem that the serial model (C555 oxidized first, then C553) can be retained only by saying that the presence of reduced C553 somehow speeds up the oxidation of C555. Allowing for this possibility, present data does

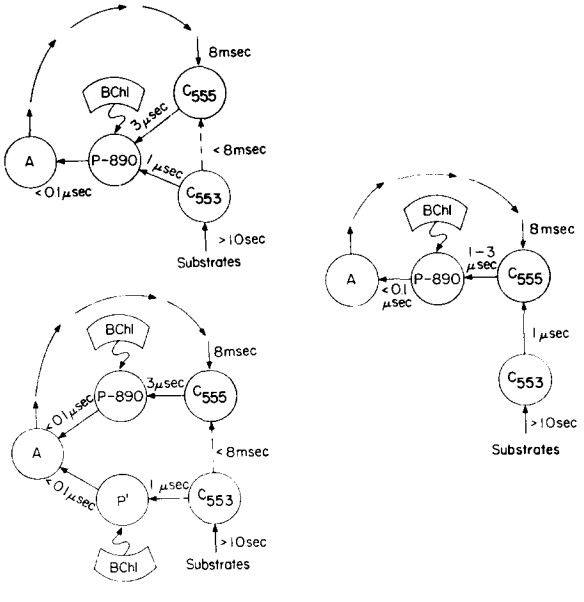

Fig. 7. Possible models incorporating a common acceptor and compatible with the data presented or discussed in this paper. BChl, bacteriochlorophyll.

not seem adequate to make a clear choice between any of the models of Fig. 7 or the switchable acceptor model. However, it seemed worthwhile to present the bottleneck evidence as it stands since eventual clarification will have to take it into account. Work is in progress to further clarify the questions which this investigation raises.

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