ENERGY TRANSFER BETWEEN CAROTENOIDS AND BACTERIOCHLOROPHYLL IN CHROMATOPHORES OF PURPLE BACTERIA

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SUMMARY

Measurement of the action spectrum of fluorescence of *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum* showed no difference between intact bacteria and chromatophores prepared therefrom in energy transfer from carotenoids to bacteriochlorophyll. Also an increase of osmotic pressure or ionic strength, obtained by the addition of sugar or sodium chloride, did not have any effect on this energy transfer. Hence most probably these treatments do not influence the relative spatial distribution of carotenoids and bacteriochlorophyll.

The efficiency of light absorbed by carotenoids in *R. spheroides* was found to be 90 % and in *R. rubrum* 30 %.

Extraction of a large fraction of the carotenoids from chromatophores of *R. rubrum* does not result in an increase in efficiency of the remaining fraction. It thus seems unlikely that the low value of energy transfer in *R. rubrum* can be explained by the assumption of two different carotenoid systems.

INTRODUCTION

The action spectra for excitation of fluorescence of the photosynthetic purple bacteria *Rhodospirillum rubrum*, *Chromatium* and *Rhodospirillum molischianum* were measured by DUYSENS[1], who found that part of the light energy absorbed by the carotenoid pigments is transferred, probably by inductive resonance, to bacteriochlorophyll. The efficiency of this energy transfer was calculated to be 35 to 40 %, expressed in quanta of incident light. The action spectrum of phototaxis—measured as a reversal of the direction of bacterial motion at a boundary between light fields of different intensities and colors—was found to be similar to that for fluorescence.

With *R. rubrum* measurements of the action spectrum of photosynthesis determined by CO_2 uptake by THOMAS[2], of the action spectrum of phototaxis by MANTEN[3], and of the relation between photosynthesis and phototaxis by THOMAS AND GOEDHEER[4] indicated that in both these processes light absorbed by the carotenoids was partially effective and approximately of the same efficiency as mentioned above.

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These observations indicated that the efficiency with which the light absorbed by the carotenoids is used for bacterial photosynthesis can be found by measuring the fluorescence intensity as a function of the wavelength of the incident light.

The above mentioned experiments all were done with living bacteria. BERGERON[5] mentioned that light absorbed by the carotenoids isolated from purple bacteria is only very slightly effective in promoting photophosphorylation, much less than could be expected from an energy transfer of 50 %. This might be explained by the assumption either that extraction of the chromatophores from the living cell changes the energy transfer, or that light absorbed by carotenoids shows an inhibitory effect on photophosphorylation.

In this connection, it should be remarked that the action spectrum of photophosphorylation of spinach chloroplasts measured by JAGENDORF *et al.*[6] suggests that light absorbed by carotenoids of green plants is used to an appreciable extent in photophosphorylation. In their action spectrum, the absorbed light energy is presented in ergs/cm^2/sec. Conversion of the absorbed light energy into quanta/cm^2/sec and scale adjustment does not seem to affect the results appreciably. The activity of light absorbed by carotenoids for photosynthesis and the Hill reaction of algae and chloroplasts of green plants was established by measurements of EMERSON AND LEWIS[7], HAXO AND BLINKS[8] and CHEN[9]. Activity with respect to chlorophyll fluorescence was measured by DUYSENS[1].

We have studied the influence of the extraction of chromatophores on the energy transfer between carotenoids and bacteriochlorophyll. To do this, action spectra of fluorescence were determined for intact bacteria of *Rhodospirillum rubrum* strain III and *Rhodopseudomonas spheroides* and for chromatophores obtained from the same cultures. From these spectra the efficiency of energy transfer from carotenoids to bacteriochlorophyll was calculated both for intact bacteria and for chromatophores.

ANDERSON, FULLER AND BERGERON[10] found an effect of osmotic strength on the photophosphorylation of chromatophores in light absorbed by the carotenoids. They suggest that the spatial relationship between carotenoids and bacteriochlorophyll may be affected by the osmotic strength of the surrounding medium, which in turn affects the energy transfer between the two pigments. This might result in a changed action spectrum of fluorescence. Experiments with algal cells by MENKE[11] and GOEDHEER[12] showed that an increase in osmotic pressure by addition of sugar to the medium resulted in a dehydration of the chloroplasts. Such a treatment could well influence the spatial distribution of the pigments and hence the energy transfer. We therefore investigated the addition of sugar or of sodium chloride thus influencing either osmotic pressure or ionic strength on the energy transfer both in intact bacteria and in chromatophores.

An energy transfer with an efficiency smaller than unity may also be explained by the assumption that from some of the carotenoid molecules the energy is not transferred at all, while from others it is transferred with an efficiency of one. As mentioned earlier (GOEDHEER[13]), carotenoids can be extracted from *R. rubrum* by treating the lyophilized chromatophores with petroleum ether. However, a small fraction of the carotenoids could not be extracted with this method. The fluorescence action spectrum of petroleum ether-treated chromatophores of *R. rubrum* was measured in order to investigate whether the remaining carotenoids exhibit the same or a higher efficiency of energy transfer.

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METHODS

Monochromatic light at a series of wavelengths obtained from a grating monochromator was used to excite fluorescence. The half-width value of the transmitted band was 5 mμ. A filter containing a 6 % copper sulfate solution (2 cm thick) was placed in the incident light beam in order to absorb scattered light of longer wavelengths than 650 mμ. A low voltage incandescent lamp (3.5 V;72A) was used as a light source. The light detector was a DuMont 6911 photomultiplier tube connected to an a.c. amplifier. Scattered incident light was absorbed by a Polaroid XRN-5X55 filter transmitting wavelengths longer than 800 mμ and placed immediately in front of the multiplier.

Absorption spectra were measured with a Beckman DK-2 recording spectrophotometer. With intact bacteria the effects of scattering on the absorption spectrum were diminished by the use of opal glass.

In some cases as much as 50 % increase in the fluorescence was found in the first 15 sec after illumination of colloidal extracts. After this induction period the fluorescence was constant. Therefore at each wavelength we waited 10–15 sec until the fluorescence did not increase any more. After four consecutive readings at different wavelengths the value of fluorescence excited by 587 mμ was read again. The intensity of the fluorescence was a linear function within 10 % of the intensity of the incident light in the intensity range used. The intensity of the incident light was measured with a thermopile. From the number of incident light quanta calculated from this intensity and the measured fluorescence intensity the fluorescence action spectrum was derived.

A correction for the distortion introduced by the inactive pigments was obtained by the use of an equation presented by FRENCH AND YOUNG[14]. For this it was assumed that at 590 mμ light was absorbed by bacteriochlorophyll only. Both fluorescence action spectrum and absorption spectrum were then plotted on an absorbance scale.

The calculation of the efficiency of energy transfer was carried out by comparing the ratios of absorption at one of the carotenoid peaks to the absorption at the 587 mμ peak of bacteriochlorophyll with the ratios of fluorescence at these wavelengths. The absorption spectra of the colloidal extracts, which showed less scattering than the spectra of intact bacteria measured with the opal glass method, were used for this calculation. A correction for the overlap of bacteriochlorophyll and carotenoid absorption was applied. For this correction the absorption curve of bacteriochlorophyll in chromatophores was supposed to be similar to that of bacteriochlorophyll in organic solvents in this spectral region.

In order to estimate the correction for effects due to concentration—such as scattering and reabsorption of fluorescence—action spectra of fluorescence and calculation of energy transfer were made with extracts and suspensions, which showed a maximal absorption in the carotenoid region of about 85 %, and with the same suspensions diluted so much that the maximum absorption was under 40 %.

The bacteria were grown in a medium of yeast extract and casaminoacids, illuminated with incandescent light of approximately 600 f.c., and harvested one week after inoculation. A sample was taken for measurements on living bacteria; the rest was pressed through a needle-valve homogenizer. The homogenized juice provided, after intact bacteria and cell debris had been removed by centrifugation, the chromatophore suspension.

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RESULTS

Rhodopseudomonas spheroides

In Fig. 1a the fluorescence action spectrum and the absorption spectrum of a colloidal extract of *R. spheroides* are presented. These spectra are given in absorbance units. Fig. 1b shows the spectra of the living bacteria of this species. In these figures the values for the fluorescence curves are adjusted to make the fluorescence and absorption curves coincide at 587 mμ. The maximum absorption in the carotenoid region amounts to approximately 85 %. Action spectra made with a maximum absorption of about 40 % did not deviate much from the ones presented here.

The efficiency of energy transfer from carotenoids to bacteriochlorophyll, calculated for both intact bacteria and colloidal extracts, is given in Table I. The values derived from the carotenoid peaks at 512 and 477 mμ are in fair agreement with each other. The lower values at 445 mμ may be explained by assuming the presence of

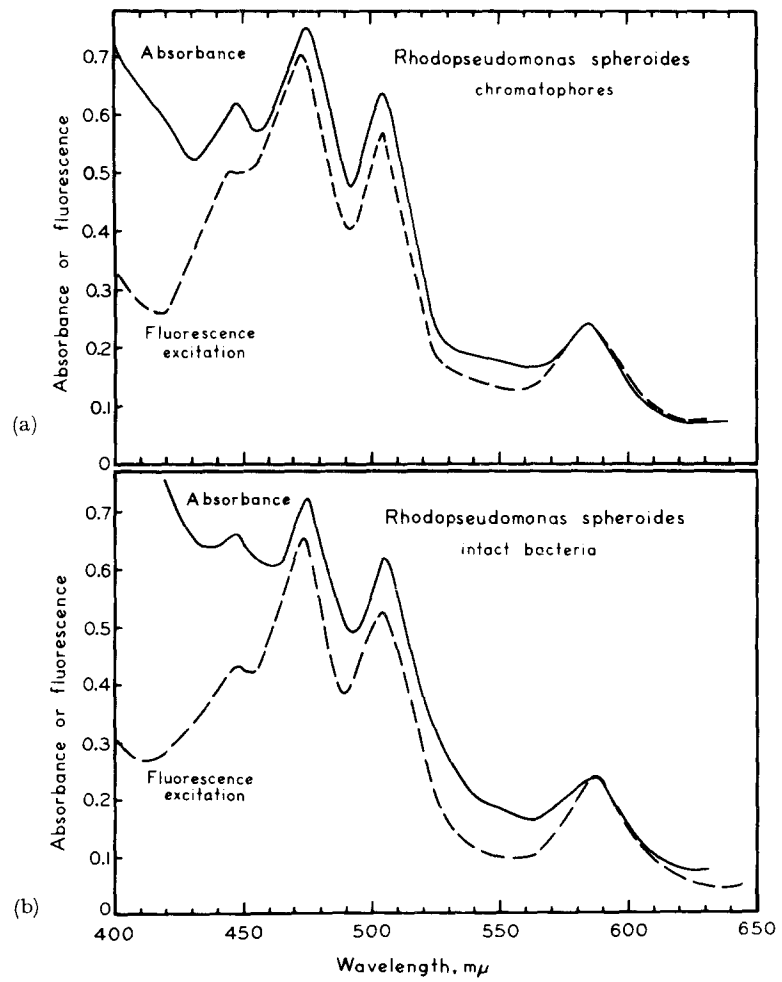

Fig. 1. Absorption spectrum and action spectrum for exciting fluorescence of (a) chromatophores of *Rhodopseudomonas spheroides*, (b) intact bacteria of *Rhodopseudomonas spheroides*.

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TABLE I

EFFICIENCY OF ENERGY TRANSFER IN CHROMATOPHORES AND INTACT BACTERIA OF *Rhodopseudomonas spheroides*

The efficiency is calculated from the values of fluorescence and absorption at the maxima of carotenoid absorption at 512, 477 and 445 mμ and at the 587 mμ maximum due to bacteriochlorophyll at which 100% efficiency is assumed. Maximum absorption of the concentrated suspensions is 85%; maximum absorption of the diluted suspensions is 40%.

Wavelength mμ	*Chromatophores*		*Bacteria*	
	Concentrated	*Diluted*	*Concentrated*	*Diluted*
	Transfer efficiency relative to that at 587 mμ			
512	0.89	0.96	0.86	0.91
477	0.91	0.92	0.90	0.92
455	0.75	0.75	0.73	0.75

TABLE II

INFLUENCE OF SODIUM CHLORIDE AND SUCROSE ON THE ENERGY TRANSFER IN LIVE CELLS AND CHROMATOPHORE SUSPENSIONS OF *Rhodopseudomonas spheroides*

Wavelength mμ	*Chromatophores*			*Bacteria*		
	No addition	*10% NaCl*	*10% Sucrose*	*No addition*	*10% NaCl*	*10% Sucrose*
	Transfer efficiency relative to that at 587 mμ					
512	0.87	0.87	0.85	0.87	0.89	0.87
477	0.95	0.93	0.93	0.93	0.91	0.94

inactive absorbing pigments at this wavelength, different from carotenoids and bacteriochlorophyll. As shown in Fig. 1, such inactive absorption increases towards shorter wavelengths.

Table II presents the effect of the addition of sucrose and sodium chloride on the energy transfer between carotenoids and bacteriochlorophyll. These additions do not seem to have any effect. Action spectra measured after such additions were, within the limits of accuracy of the measurements and in the spectral region measured, similar to the ones without any additions.

The values of energy transfer found with different cultures were between 0.85 and 0.95. Storage of the chromatophore suspension in the refrigerator for several months did not influence the energy transfer.

The accuracy of the measurement of fluorescence intensity was within 3% in the yellow and within 10% in the violet part of the spectrum.

Rhodospirillum rubrum

The action spectrum of fluorescence and the absorption spectrum of a colloidal extract of *Rhodospirillum rubrum* strain 111 are given in Fig. 2a, while Fig. 2b presents the spectra of the living bacteria. The efficiency of the carotenoids is much less than in *Rhodopseudomonas spheroides*. Table III gives the efficiency of energy transfer calculated from the maxima in the carotenoid region located at 545, 512 and 485 mμ. Here also the values are given for both chromatophores and intact bacteria at two concentrations. No difference of any importance exists between the chromatophores and the bacteria.

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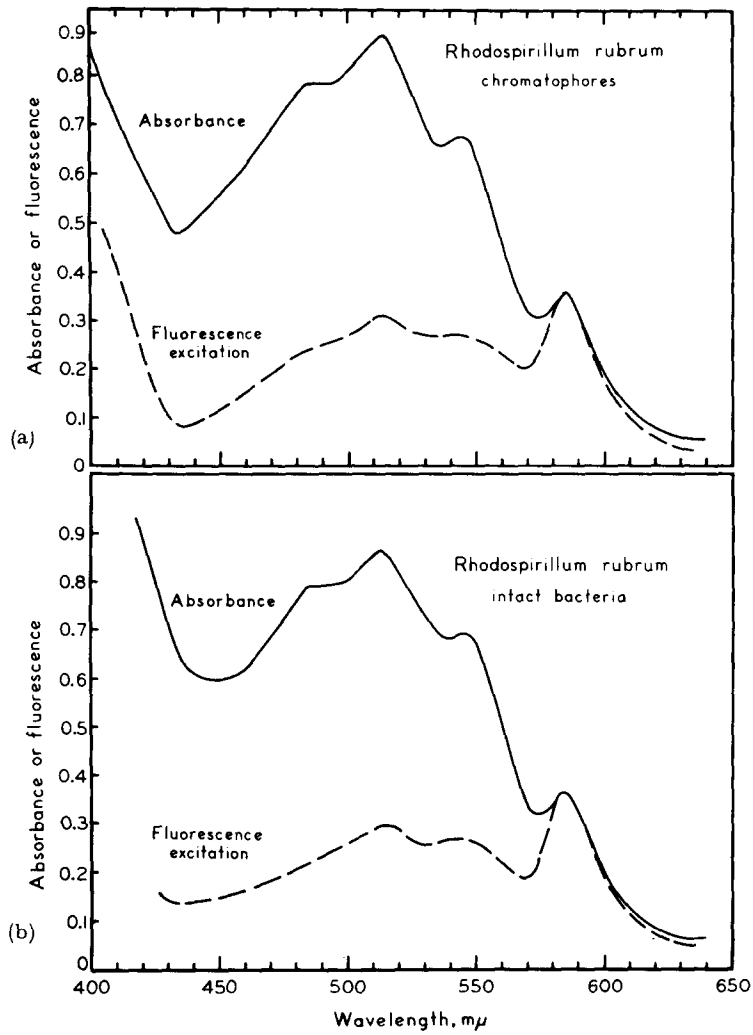

Fig. 2. Absorption spectrum and action spectrum for exciting fluorescence of (a) chromatophores of *Rhodospirillum rubrum* (strain 111), (b) intact bacteria of *Rhodospirillum rubrum* (strain 111).

TABLE III

EFFICIENCY OF ENERGY TRANSFER IN CHROMATOPHORES AND INTACT BACTERIA OF *Rhodospirillum rubrum* (STRAIN 111)

The efficiency is calculated from the values of fluorescence and absorption at the maxima of carotenoid absorption (at 545, 512 and 487 mμ) and at the 587 mμ absorption maximum of bacteriochlorophyll. Maximum absorption of the concentrated suspensions is 85%; maximum absorption of the diluted suspensions is 45%.

Wavelength mμ	*Chromatophores*		*Bacteria*	
	Concentrated	*Diluted*	*Concentrated*	*Diluted*
	Transfer efficiency relative to that at 587 mμ			
545	0.335	0.365	0.32	0.30
512	0.31	0.29	0.31	0.28
485	0.27	0.24	0.26	0.24

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In 5, 10, and 20 % sucrose solution and in 10 % sodium chloride solution the action spectrum of fluorescence of either the chromatophores or the bacteria does not deviate more from the original action spectrum than the accuracy of measurement.

The fluorescence intensity of intact *R. rubrum* was found to be less than half that of *R. spheroides* for an equal number of quanta absorbed at 590 mμ, if cultures of the same age and growth conditions were used.

With *R. rubrum* chromatophores also the action spectrum of fluorescence was determined after the carotenoids had been removed to a great extent. This was done by addition of a mixture of 97 % petroleum ether (b.p. 50–70°) and 3 % acetone to the lyophilized chromatophores[13]. The "solubility" of the colloidal extract is strongly decreased by this treatment, as is the intensity of fluorescence. Fig. 3 shows the fluorescence action spectrum and absorption spectrum of chromatophores treated in this way.

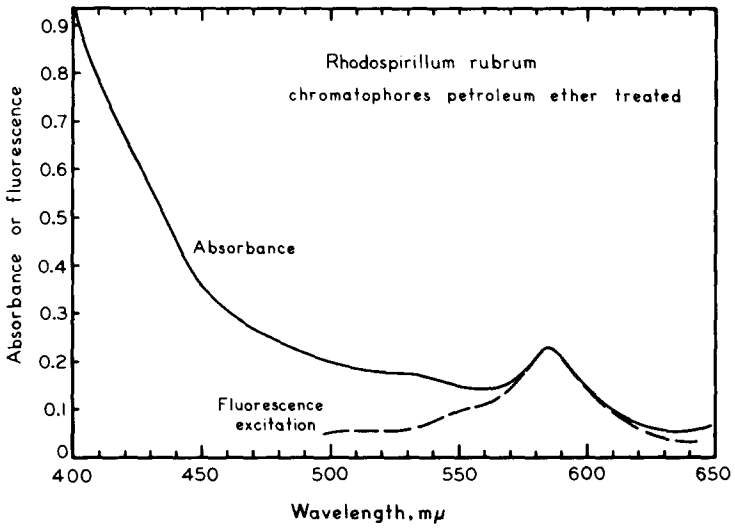

Fig. 3. Absorption spectrum and action spectrum for exciting fluorescence of *Rhodospirillum rubrum* chromatophores from which most of the carotenoids had been removed by petroleum ether.

DISCUSSION

The quantum efficiency of energy transfer from carotenoids to bacteriochlorophyll in our experiments is around 90 % with *Rhodopseudomonas spheroides* and around 30 % with *Rhodospirillum rubrum* strain III. The value found with *R. spheroides* is unexpectedly high. The values of energy transfer measured by DUYSENS[1] with *Rhodospirillum rubrum* strain 4, *Chromatium* strain D, and *Rhodospirillum molischanum* were all around 40 %. Whether the use of a different strain or different culture conditions accounts for the relatively small difference in value with *Rhodospirillum rubrum* cannot be decided. In an action spectrum of phototaxis of strain III determined by CLAYTON[15] the ratio carotenoid bacteriochlorophyll action is lower than the one in a similar spectrum determined by MANTEN[3] with strain 4, which would correspond to an energy transfer of about 30 % in CLAYTON's experiment. Besides the difference in bacterial strain, the measurements of CLAYTON and MANTEN also differed as regards light intensity and bacterial conditions.

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The appreciable difference in efficiency between *R. spheroides* and *R. rubrum* suggests a closer spatial relationship between the carotenoid pigments and bacteriochlorophyll in the first species, and probably a difference in molecular structure between the chromatophores of the two bacterial types.

Extraction of the chromatophores from the cells seems to have little or no influence on the efficiency of energy transfer. In the theory of energy transfer by inductive resonance (*cf.* FORSTER[16]) the probability of energy transfer is inversely proportional to the sixth power of the distance between the two types of molecules involved. Extraction of the chromatophore from the cells seems to leave the spatial structure in the immediate vicinity of the pigments unaltered.

This also seems to be true for the addition of various concentrations of sugar or sodium chloride. From experiments with cells of the green alga *Mougeotia* it was known that addition of sugar affected the chloroplasts, probably by a dehydration of the proteinaceous layers. This was measured as a change of birefringence in polarized light (GOEDHEER[13]). If this effect also occurs in bacteria, it most probably does not affect the spatial relationship between carotenoids and bacteriochlorophyll. The ionic strength also does not have such an effect on the chromatophores.

The low efficiency of energy transfer from carotenoids to bacteriochlorophyll in *Rhodospirillum rubrum* as measured by fluorescence makes this species less suitable for an investigation of a possible effect of light absorbed by carotenoids on bacterial photophosphorylation.

The measurements with petroleum ether-extracted *R. rubrum* chromatophores to remove carotenoids indicate that the remaining carotenoids do not possess a higher efficiency of energy transfer than the easily extracted ones. In fact, the fluorescence action spectrum measured is nearly identical to the absorption spectrum of bacteriochlorophyll in the spectral range considered. It thus seems unlikely that the low value of energy transfer in *R. rubrum* is due to two different systems of carotenoids, one with a high and one with a low efficiency.

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