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STUDIES ON BACTERIAL CHROMATOPHORES

I. REVERSIBLE DISTURBANCE OF TRANSFER OF ELECTRONIC EXCITATION ENERGY BETWEEN BACTERIOCHLOROPHYLL-TYPES IN *CHROMATIUM*

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SUMMARY

Desoxycholate affects the transfer of excitation energy between bacteriochlorophyll-types in *Chromatium* chromatophores It is suggested, that the detergent fragments the chromatophore into subunits and subsequently disturbs the spatial arrangement of these bacteriochlorophyll-types.

INTRODUCTION

Soon after the isolation of pigmented submicroscopic particles from purple bacteria[1], it became probable that in these structures the complete photosynthetic apparatus of these organisms is localized. Photochemical activities and light-induced phosphorylations could be demonstrated and recently anaerobic photophosphorylation

coupled to light-induced oxidation-reduction reactions was reported[2]. Functionally, therefore, bacterial chromatophores seem to be comparable with chloroplasts of higher plants.

About the ultrastructure of the chromatophores very little is known. As they are mainly composed of phospholipoprotein complexes, which have resisted attempts of separation and isolation by various methods[3], a highly organized structure is indicated. The presence of lamellae was indicated by electron microscopy[4,5] The *in vivo* state of bacteriochlorophyll, and of photosynthetic pigments in general, still is an unsolved problem. Several lines of indirect evidence suggest an association of these pigments with some other substance, probably a lipoprotein. For a recent review bearing on this problem, see ref. [6].

For a study of the state of chlorophyll in its functional environment, the purple bacteria are particularly interesting. Their bacteriochlorophyll-carrier complexes show a large absorption shift towards the infrared (from 780 to 890 mμ), when compared with bacteriochlorophyll in organic solvents. Moreover, their chlorophyll, which has been shown to be identical for all species, exhibits *in vivo* absorption properties which are strikingly different for various strains, even within a single strain. As these spectral properties have been described many times before (*cf.* [7]), it may suffice here to mention the intriguing phenomenon of some species showing two or more additional absorption peaks in the 800–850 mμ region This multiplicity of peaks is supposed to be caused by different modes of associations of bacteriochlorophyll with one or more specific carrier molecules. To avoid any suggestion as to the nature of these complexes, they have been named bacteriochlorophyll-types[8]

To enable a more direct study of these types by disruption of their structural organization and, if possible, to isolate them in a physiologically active state, it is necessary to choose mild solubilizing procedures, as the pigment is easily detached from its natural complex. For this purpose detergents, known to have a solubilizing effect on systems rich in lipides, have been used. Preliminary experiments on the action of a non-ionic detergent on chromatophores of the Athiorhodacea *Rhodopseudomonas spheroides* showed the usefulness of such a procedure[9]; the results suggested that one of the bacteriochlorophyll-types could be split off. Unfortunately, however, chromatophores of this species proved to be rather unstable in the presence of the detergent used. Gradual spectral changes are taking place upon standing, even in the dark.

It was found that chromatophores of the Thiorhodacea *Chromatium*, another species with three absorption maxima in the near infrared, behave quite differently. When suspended in desoxycholate, which is without any perceptible effect upon chromatophores of *Rhodopseudomonas*, their pigment systems are stable when kept in the dark and with certain precautions no absorption changes occur upon illumination[10].

Chromatium shows three absorption maxima at approximately 800, 850 and 890 mμ. The bacteriochlorophyll-types responsible for these absorptions will be termed B_{800}, B_{850} and B_{890}, respectively. The fluorescence spectrum of the organism, however, only shows a major peak at about 910 mμ and B_{890} seems to be the only fluorescent pigment type. The fluorescence spectrum is independent of the wavelength of the exciting light. Light quanta absorbed by B_{800} give rise to fluorescence of molecules of B_{890} with about the same efficiency as do quanta absorbed by B_{890} itself[8] When the absorption maximum at 890 mμ, recognizable as a shoulder, is made to disappear by heating, a new fluorescence peak at 880 mμ occurs, indicating the capacity of B_{850}

to emit fluorescence. This fluorescence is absent in the intact organism These findings lend strong support to the concept of transfer of excitation energy between photosynthetic pigments and in *Chromatium* transfer of excitation energy of high efficiency may take place from B_{800} and probably from B_{850} to B_{890}, which seems to be the photosynthetically active pigment system in bacterial photosynthesis[8]. Since the efficiency of energy transfer approaches 100 %, the mechanism of this transfer is presumed to be that of inductive resonance. As the efficiency of this energy transfer is inversely proportional to the sixth power of the distance between the types of molecules[11], changes in the spatial arrangement of the various types in the chromatophore might result in considerable changes in the efficiency of this transfer. Consequently, upon illumination with light which is absorbed by all three types simultaneously, changes in the fluorescence spectrum of structurally disorganized chromatophores may be expected. In the first place the intensity of the fluorescence emitted by B_{890} would be expected to decrease, as it is no longer supplied with energy absorbed by the other types. The B_{850} material on the other hand, no longer able to transfer its excitation energy to B_{890}, will emit part of this energy as fluorescence, resulting in an increase in fluorescence intensity at 880 mμ. If B_{800} is also separated from the other types, it would likewise become able to emit measurable fluorescence and increases of fluorescence intensities around 830 mμ may be envisaged. Under suitable conditions such changes may indeed be induced in the fluorescence pattern of chromatophores by desoxycholate treatment.

MATERIALS AND METHODS

Chemicals and enzyme preparations

Chymotrypsin type II and tris(hydroxymethyl)aminomethane were obtained from the Sigma Chemical Company, crystalline trypsin from Worthington Biochemical Corporation, snake venom (*Crotalus atrox*) from Ross Allen's Reptile Institute, Silver Springs, sodium desoxycholate from the British Drug Houses and L-malate from Nutritional Biochemicals Corporation.

Throughout the experiments 0 05 *M* Tris buffer pH 8.5 was used.

Isolation of chromatophores

Chromatium strain D was grown in a malate-thiosulphate medium. The spectrum of the organism may vary considerably upon subculturing. The relative height of the 850 mμ peak is especially liable to show marked changes Reduction of absorption is accompanied by a shift of the absorption maximum towards shorter wavelengths These changes are not predictable After periods of relative stability, organisms with either a reduced or increased absorption at about 850 mμ may be harvested. In Fig. 1 an example of a spectrum with a high 850 mμ absorption is given. This phenomenon has been described before[12] and its cause is still obscure.

After harvesting and washing with buffer the bacterial cells were disrupted by grinding with carborundum. This method was prefered to disruption by sonic vibration to avoid possible damage to the chromatophore[13]. Whole cells and cell debris were removed by 3 to 4 successive centrifugations of 20 min duration at 10,000 $\times$ *g* and the supernatant once more centrifuged at 25,000 $\times$ *g* for 15 min. Finally, the chromatophores were spun down at 144,000 $\times$ *g* during 1 h and the sediment was

homogenized in a Potter-Elvehjem homogenizer. Concentrated suspensions of chromatophores thus obtained can be stored in a refrigerator in the dark for several weeks without spectral changes occurring.

All preparations used in the experiments were adjusted to an optical density of 2.5–3 (1 cm path length) at 800 mμ, corresponding with a bacteriochlorophyll concentration of 50–60 μg/ml.

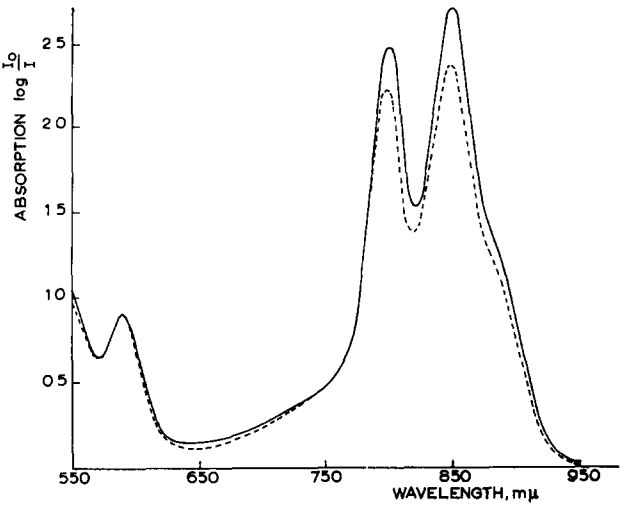

Fig. 1. Spectra of chromatophores in buffer (solid line) and in 0.25% desoxycholate–0.25 *M* KCl (dotted line)

Apparatus

The apparatus for the measurement of fluorescence was the same as described earlier[14], with only slight modifications to improve sensitivity and stability. Fluorescence was excited by irradiation with light of 592 mμ obtained by passing light from a 100 W incandescent lamp through a GAB interference filter. This wavelength was chosen, as a minor absorption band of the three bacteriochlorophyll-types coincides at 590 mμ. Thus, this band is well separated from the infrared peaks. The absorption of a type at this wavelength is proportional to its absorption at the infrared peak[8]. After elimination of scattered light by means of a Kodak Wratten filter 87, the fluorescent light was focussed on an infrared sensitive Maurer-photomultiplier cooled with liquid air The signals were fed into a Honeywell-Brown recorder The cuvette containing the extracts had a width of 0.8 mm. By replacing different filters in front of the photomultiplier, the fluorescence at different wavelengths was determined. For measuring B_{890} fluorescence beyond 900 mμ a bacterial-filter (BF) was used[13]

As these have been found to be negligible in our experiments with partially purified chromatophores, scattering corrections have not been applied. To ascertain these corrections heated and subsequently illuminated extracts of chromatophores have been used. Upon heating, absorption in the 800–890 mμ region disappears and a new peak at about 780 mμ arises. The material responsible for this absorption emits a fluorescence, which is quenched upon illumination. Extracts thus treated did not give rise to any significant signals when irradiated with 592 mμ light.

RESULTS

Absorption and sedimentation characteristics of desoxycholate-treated particles

When desoxycholate is added to a buffered suspension of chromatophores, an instant clarification occurs This increase of light transmission is demonstrated in the absorption spectrum of desoxycholate-treated chromatophores (Fig. 1) Slight shifts of a few mμ of the location of the maxima towards shorter wavelengths are also observed These facts indicate a fragmentation of the particles.

An increasing amount of pigmented material remains in solution, when chromatophore suspensions are submitted to ultracentrifugation at 144,000 $\times$ g for 60 min in the presence of increasing amounts of desoxycholate. This solubilizing effect of desoxycholate is greatly influenced by ionic strength (Fig 2). When chromatophores are suspended in a 0 25 M KCl medium containing 0 25 % desoxycholate solubilization is complete and only a non-pigmented, transparant sediment is obtained upon ultracentrifugation. It will be seen, that the disorganizing action of desoxycholate is similarly dependent upon its concentration and the ionic strength. Therefore, the chromatophores are not merely prevented from sedimentation by enclosing detergent micelles, and thus "solubilized", but a more direct action on the particles seems to be involved. The observation that up to 90 % of the treated particles are not sedimented after rigorous dialysis against buffer to remove most of the detergent, supports this view.

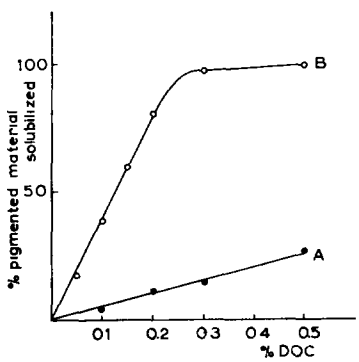

Fig 2 Influence of ionic strength on sedimentation of chromatophore material at 144,000 $\times$ g at increasing desoxycholate concentration A without KCl, B in the presence of 0 25 M KCl

The effect of desoxycholate on transfer of excitation energy between bacteriochlorophyll-types

The changes in the fluorescence pattern of chromatophore suspensions in the 800–910 mμ region due to desoxycholate action are shown in Table I, A and B, which represents the results of an arbitrary experiment A relatively small decrease of B_{890} fluorescence is induced by the detergent, while a significant increase of B_{850} fluorescence is shown. This increase was observed in all preparations tested. The slight decrease of B_{890} fluorescence, however, did occur in most cases, but not in all. The changes of this fluorescence were small in any case, which may be explained as being due to an overlap of the B_{850} and B_{890} fluorescence spectra and of the transmission of the used filters. Consequently, a fraction of the fluorescence emitted by B_{850} is also measured. This explanation is consistent with the observation, that there is a correlation between sign and magnitude of the changes of B_{890} fluorescence intensity and the relative height of the 850 mμ absorption band of the corresponding extracts. In extracts with a high

850 mμ absorption sometimes even a slight increase of this fluorescence intensity was measured. Some reabsorption of fluorescence emitted by B_{850} also occurs, but this effect is of minor importance.

Though no quantitative significance should be attached to the values given in Table I, the value for the fluorescence intensity of detergent-treated particles at 807 mμ is notably low This could be due to either the emission by B_{800} of a fluorescence of low quantum yield or the fact that B_{800} is not spatially separated from the other types and, therefore, is still able to transfer its excitation energy, probably to B_{850}. Of these two possibilities the latter seems to be the most likely[10].

Extracts in the presence of desoxycholate-KCl are remarkably stable as they may be stored for several days in a refrigerator in the dark without any changes in absorption and fluorescence.

Influence of pH, desoxycholate concentration and ionic strength

The action of desoxycholate on the chromatophore is independent of pH between 7 5 and 10. For a maximal effect of the detergent the ratio of its concentration and KCl concentration is critical. In Fig. 3 the influence of desoxycholate concentration at a fixed KCl concentration (0.25 *M*) is shown for chromatophore suspensions diluted as described before. At a concentration of approximately 0.075 %, the detergent starts to exert a perceptable action on the chromatophores and the maximal effect is reached in the presence of about 0.25 % desoxycholate. A further increase in concentration results in quenching of fluorescence. Fig. 4 similarly shows the influence of ionic strength at a fixed detergent concentration (0.25 %). No quenching of fluorescence occurs at high KCl concentration.

In these experiments cysteine has been added to the extracts as a precautionary measure to prevent photooxidation of bacteriochlorophyll as well as light-induced quenching of fluorescence[10].

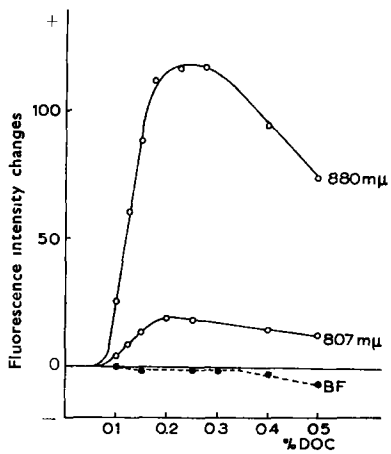

Fig. 3 Influence of desoxycholate (DOC) concentration at a fixed KCl concentration (0.25 *M*) on the fluorescence intensities of chromatophore suspensions at different wavelengths (mμ).

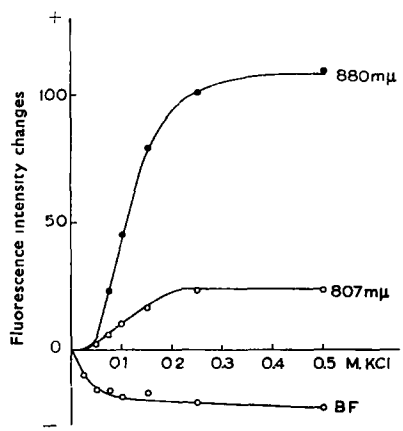

Fig. 4. Influence of ionic strength at a fixed desoxycholate concentration (0 25 %) on the fluorescence intensities of chromatophore suspensions at different wavelengths (mμ)

Reversibility of the disorganizing action of desoxycholate on chromatophores

The effect of desoxycholate with respect to the disturbance of transfer of excitation energy between bacteriochlorophyll-types is fully reversible Dialysis of extracts of detergent-treated particles results in restoration of the original fluorescence pattern of the chromatophores Subsequent readdition of desoxycholate and KCl reproduces the action of the detergent (Table I, C and D). Optimal results were obtained, when the extracts were dialyzed in Visking cellulose tubing for 12 h against 1 l change of

TABLE I

RELATIVE FLUORESCENCE INTENSITIES OF CHROMATOPHORE SUSPENSIONS UNDER VARIOUS CONDITIONS AT DIFFERENT WAVELENGTHS (mμ)

	BF	*880*	*807*
A. In buffer	63	29	2
B. In 0.25% desoxycholate–0.25 *M* KCl, before dialysis	48	117	19
C. Same preparation as B , after dialysis	59	30	2
D. Same preparation as C , desoxycholate and KCl readded	42	109	18

Fluorescence intensities are given in terms of recorder deflections in mm

buffer. Dialysis against buffer of high ionic strength (0.25 *M* KCl) causes the chromatophores to flocculate upon standing, indicating that also some irreversible changes of the chromatophores have been induced by detergent action. This phenomenon is in accordance with ultracentrifugation studies of dialyzed extracts described earlier.

Failure to affect the spatial arrangement of bacteriochlorophyll-types by other chemical and enzymic methods

So far we have failed to obtain similar results as described above by other methods. Trypsin and chymotrypsin, alone or in combination, do not seem to attack either the intact chromatophore or desoxycholate-treated and subsequently dialyzed particles. Spectral changes caused by tryptic digestion as reported by NEWTON AND LEVINE[15] were not observed with the materials used in the present experiments Snake venom, known to exhibit phospholipase activity, likewise was without effect. A dispersing agent like urea also gave negative results.

DISCUSSION

From the described results, it is obvious that desoxycholate has a profound action on *Chromatium* chromatophores Firstly, as evidenced by reduced light absorption of extracts in the presence of the detergent and changed physico-chemical properties of treated and subsequently dialyzed particles, an irreversible fragmentation into subunits seems to take place. Fragmentation of *Chromatium* chromatophores into smaller fragments, obtained by sonic treatment, has been reported by NEWTON AND NEWTON[3] Chromatophores of this same species are rapidly converted into uniform particles, which sediment much less rapidly upon cyanide treatment[16]. It may be mentioned here, that 1 *M* KCN does not affect the spatial arrangement of the bacteriochlorophyll-types. Beautiful electron micrographs of intact chromatophores

and of subunits of *Rhodospirillum rubrum* were published by FRENKEL[2], but the method by which these subunits were obtained was not specified. The complete photosynthetically active system seems to be present in these subunits. This assumption is suggested by the following observations: (1) their spectrum shows the presence of the bacteriochlorophyll-types, (2) when they are obtained by sonic desintegration, transfer of excitation energy is not affected[13] and (3) they are as active photochemically as the larger chromatophore[2].

These data are consistent with the view that chromatophores are made up of assemblies of identical subunits, which may be orderly arranged, *e.g.* in lamellae. Desoxycholate treatment, as does sonic rupture, seems to achieve fragmentation along fragile lines of attachment.

Secondly, desoxycholate has an additional reversible action on the released subunits, demonstrated in changes in transfer of excitation energy, which, among others, requires certain spatial arrangements. Probably a penetration of detergent molecules into the fragments occurs, affecting the spatial relationships of the bacteriochlorophyll-types and thus separating them over a distance too great for energy transfer. This does not seem to involve gross changes in the chemical and physico-chemical state of these complexes, as these would notably affect absorption.

The experimental results may be tentatively explained by assuming that B_{890} is reversibly distantiated from B_{800} and B_{850} while these latter two types still may have retained their natural complexity. As additional manifestations of the action of desoxycholate on chromatophores will be described in a subsequent paper, a further discussion will be postponed until then.

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