pH-INDUCED CHANGES OF THE INFRARED ABSORPTION SPECTRA OF PURPLE BACTERIA

by

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INTRODUCTION

It is generally accepted that bacteriochlorophyll of purple bacteria is the same for all studied species. Among others, this view is based on the fact that in organic solvents the infrared absorption spectra coincide. One major peak occurs at about 780 mμ in alcoholic extracts, *cf.* FRENCH[1], and VERMEULEN, WASSINK AND REMAN[2]. In the living cell the situation is different. The infrared absorption spectrum of *e.g.* the Athiorhodacea *Rhodospirillum rubrum* shows a pronounced maximum at about 880 mμ and a weak one about 800 mμ. However, in absorption spectra of *e.g.* the Athiorhodacea *Rhodopseudomonas* and the Thiorhodacea *Chromatium* three maxima, at about 890, 850, and 800 mμ respectively, are found. For details we may refer to VERMEULEN, WASSINK AND REMAN,[2] KATZ AND WASSINK[3], WASSINK, KATZ AND DORRESTEIN[4], FRENCH[1,5].

A number of these spectra were established using aqueous bacterial extracts. In these extracts, of the type first prepared by LEVY, TEISSIER AND WURMSER[6], the *in vivo* spectrum is retained.

WASSINK, KATZ AND DORRESTEIN[4] analysing infrared absorption spectra of various strains of purple bacteria suggested that the three maxima mentioned may represent one and the same bacteriochlorophyll, bound to different proteins, "thus building up different photoactive complexes characterized by somewhat different infrared absorption spectra". There is some evidence for the individuality of these "complexes". The mutual height of the maxima is variable, *cf.* also FRENCH[5]. They are differently influenced by *e.g.* light conditions and pH. In *Chromatium*, the 890 mμ band is preferentially destroyed by heat.

DUYSENS[7,8] used the term "type" instead of "complex", thus avoiding any suggestion about its nature. The types are denominated after the wavelength of maximal absorption. So the infrared absorption spectra with three maxima referred to above are considered to be composed of those of the bacteriochlorophyll types B 800, B 850 and B 890, while the absorption spectra showing only one pronounced infrared maximum suggest the occurrence of B 890 mainly with, perhaps, traces of B 850 and B 800. Actually, the exact location of the maxima varies slightly in different strains. For the sake of simplicity, the same designation was retained in all cases. This is also done in the present study.

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As already mentioned, WASSINK *et al.*[4] suggested that the "three maxima infrared spectrum" may indicate coupling of bacteriochlorophyll to three different proteins. However, it may equally be that, in such a case, bacteriochlorophyll is bound to three chemically different groups of one and the same protein molecule. Moreover, as RABINOWITCH[9] remarked, there are other possibilities including several isomeric or tautomeric forms, small differences in chemical composition or in the reduction level of bacteriochlorophyll.

Thus the nature of the natural bacteriochlorophyll types is still unknown. A more detailed study of pH influence on the absorption spectra of aqueous extracts may yield information about this nature. The present paper deals with such experiments.

MATERIAL

Since the required pH should prevail in the direct neighbourhood of the pigment complexes aqueous extracts were used instead of live bacteria. These extracts were prepared from *Chromatium* strain D, *Rhodospirillum rubrum* strain 4, and *Rhodopseudomonas spheroides*. The first one was cultivated in a malate-thiosulfate medium while the other strains were grown in a peptone-NaCl medium. About 500 ml of a 6 days old culture were centrifuged at 1200 *g* for 30 minutes. The centrifugate was washed with a 0.1 *M* Na_2HPO_4 solution and centrifuged again. The sediment was thoroughly mixed with an equal amount of carborundum, grain size 0.05 mm, and ground in an agate mortar. About 15 ml of the above solution was added, and, after stirring, the silicon and the debris were centrifuged down at 3,000 *g* for about two hours. The supernatant was centrifuged again at 20,000 *g* until all colored matter had sedimented. Usually three hours were needed. Next the sediment was resuspended in glass-distilled water and centrifugation was repeated. Finally the sediment was resuspended in water. In this way a clear, intensely colored, extract was obtained. These extracts were stored in a refrigerator.

METHODS

Each of three 1 cm, 5 ml cuvettes was filled with 4.5 ml of a 0.1 *M* Sörensen citrate buffer solution of the required pH. Depending on its concentration, 0.1 to 0.2 ml of a bacterial extract was added to each cuvette by means of a micropipette. If this was done carefully no mixing took place. Next, the glass covers were placed on the cuvettes. Then, the cuvettes were shaken as quickly as possible till complete mixing had occurred. In the "short term" experiments the absorption at 880, 860 and 802 mμ was measured immediately after mixing.

Apart from these measurements, complete infrared absorption spectra were established. In order to avoid distortion of the measured spectrum by rapid initial changes, an interval of one to two hours was kept between mixing and measuring. These measurements are called "long term" experiments.

RESULTS

Infrared absorption spectra of aqueous extracts from the three species studied are represented in Fig. 1. In that of *Chromatium*, 1*a*, three absorption bands can be distinguished. The spectrum, however, is very variable; during subculturing the mutual heights of the bands may change considerably. This can be seen by comparison with the spectra 1 of Figs. 3*a*, 3*b*, and 4. They coincide with those of the non-buffered

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extracts. The cause of this variability is still obscure. Both technique and conditions of cultivation were kept as constant as possible.

Fig. 1*b* shows the same spectrum for *Rhodopseudomonas*. It is also composed of three absorption bands which are located at about the same wavelengths as the *Chromatium* maxima.

In *Rhodospirillum rubrum*, 1*c*, the B 890 type strongly prevails while the B 800 type is only present at low concentration.

"Short term" influence of pH on the height of the absorption bands is represented in Fig. 2. In the *Chromatium* extract, 2*a*, the peak heights decrease with the same percentage down to pH 3.0. Below this pH the 800 mμ absorption starts to rise. Those at 890 and 850 mμ decrease still further; the former at an increased rate.

For *Rhodopseudomonas* things are different, *cf.* Fig. 2*b*. The height of the three absorption bands are shown to be nearly independent of pH at least down to pH 1.

Rhodospirillum rubrum represents a third type. In contrast with what happens in *Chromatium*, the 890 mμ band is insensitive to pH at least down to pH 1. The small 800 mμ peak decreases, below pH 3.5. The 850 mμ absorption, in any case, is due for the major part to the 890 mμ band and is not affected by pH in the range studied.

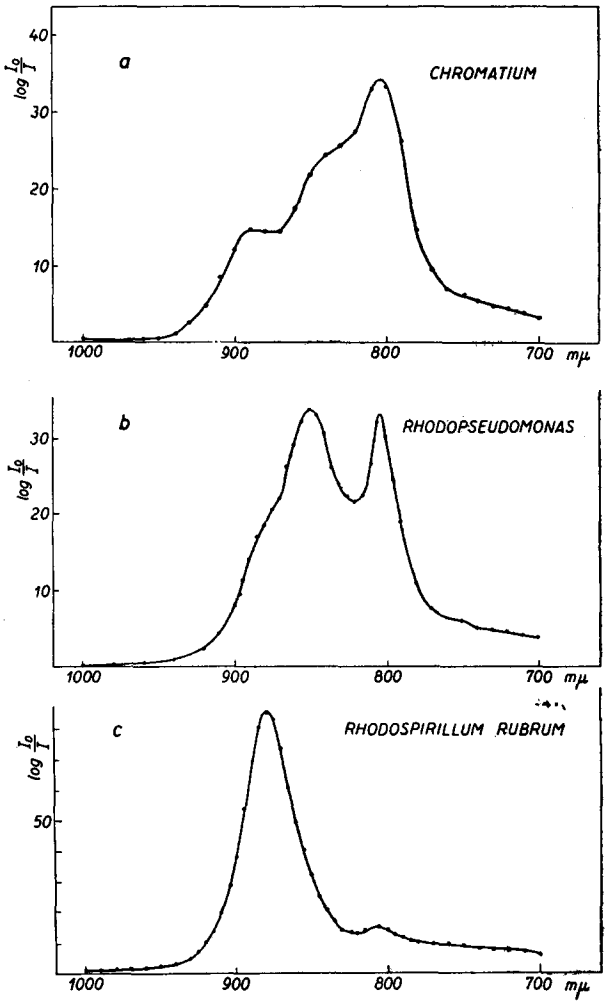

Fig. 1. Infrared absorption spectra of aqueous extracts from *a*, *Chromatium* strain D, *b*, *Rhodopseudomonas spheroides*, and *c*, *Rhodospirillum rubrum* strain 4.

Attention may be drawn to the fact that, in the Athiorhodacea *Rhodopseudomonas* and *Rhodospirillum rubrum* the B 890 type is much more pH-stable than it is in the Thiorhodacea *Chromatium*.

"Long term" influence of pH on the shape of the absorption spectrum of the extracts is shown in Fig. 3. As mentioned above, the *Chromatium* spectrum may vary considerably. In *a*, curve 1, the 890 mμ band is only weakly developed while the height of the 850 mμ maximum nearly equals that at 800 mμ. In the *b* curve the 890

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mμ band is rather distinct, while the presence of an 850 mμ band is only demonstrated by the asymmetry of the 800 mμ maximum.

At pH values 2 or 1, the maxima at 890 and 850 mμ are nearly absent while a new maximum is formed. The location of the latter differs in both graphs. In Fig. 3*a* it occurs at 835 mμ while in Fig. 3*b* it is situated at 818 mμ. This phenomenon may be due to a variation of the distortion of the band as caused by the presence of varying amounts of B 850 material.

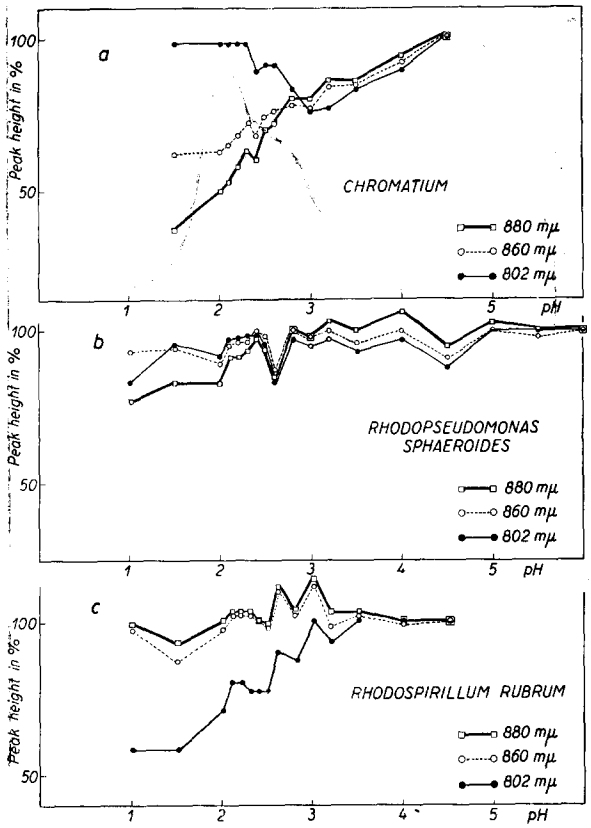

Fig. 2. Short term influence of pH on the height of the infrared absorption bands of aqueous extracts from: *a*, *Chromatium*, *b*, *Rhodopseudomonas spheroides*, and *c*, *Rhodospirillum rubrum*.

According to Fig. 3*c*, pH influences the *Rhodopseudomonas* spectrum in quite a different way. At pH 1 the 890 mμ band is strongly reduced. The same is true of the 800 mμ band, and the 850 mμ maximum is only slightly affected. The height of the latter maximum is somewhat decreased. This can be due to the reduction of the 890 and 800 mμ bands. However, the location of the maximum is slightly shifted to the short wavelength side. Its exact position is influenced by the bases of the neighbouring peaks. The disappearance of the closest one, at 890 mμ, may account for this shift. However, the concentration of the type responsible for the more remote 800 mμ absorption is much higher than that of the 890 mμ type. Thus it is impossible to designate the cause for the shift in question with certainty.

Fig. 3*d* shows the pH-induced spectral changes in a *Rhodospirillum rubrum* extract. At pH 1 the height of the 890 mμ band is reduced with only 23%. The asymmetry of this peak is increased. This is due to a slight increase of absorption at about 840 mμ. The 800 mμ maximum seems to be destroyed. Such a spectrum is also formed after prolonged intense illumination.

Some additional experiments may be mentioned. The formation of an extra absorption maximum with *Chromatium* extracts at low pH may be due either to denaturation of the pigment or to some reversible reaction. To decide between both possibilities the following experiments were done. A sample of an aqueous *Chromatium*

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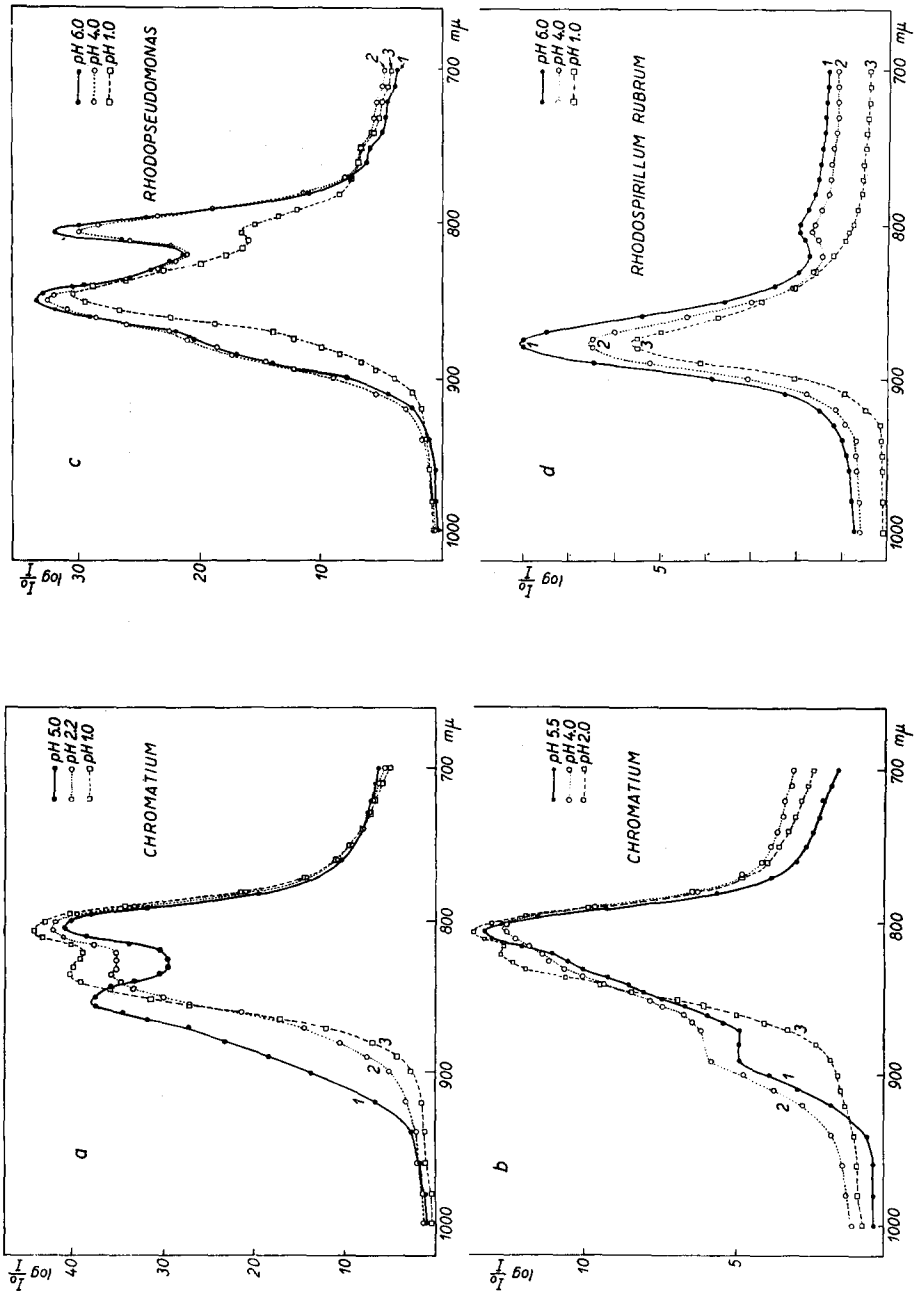

Fig. 3. Long term influence of pH on the infrared absorption spectrum of aqueous extracts from: *a* and *b*, *Chromatium*; *c*, *Rhodopseudomonas spheroides*, and *d*, *Rhodospirillum rubrum*.

extract was buffered at pH 5.0. A second sample was adjusted to pH 2.2. After one hour, the absorption spectra were determined. They are represented in Fig. 4, curves 1 and 2. The additional maximum is evident. Next, the most acid sample was adjusted to pH 5.0. Immediately afterwards both samples were centrifuged until the coloured matter had sedimented. The supernatants were discarded and the sediments

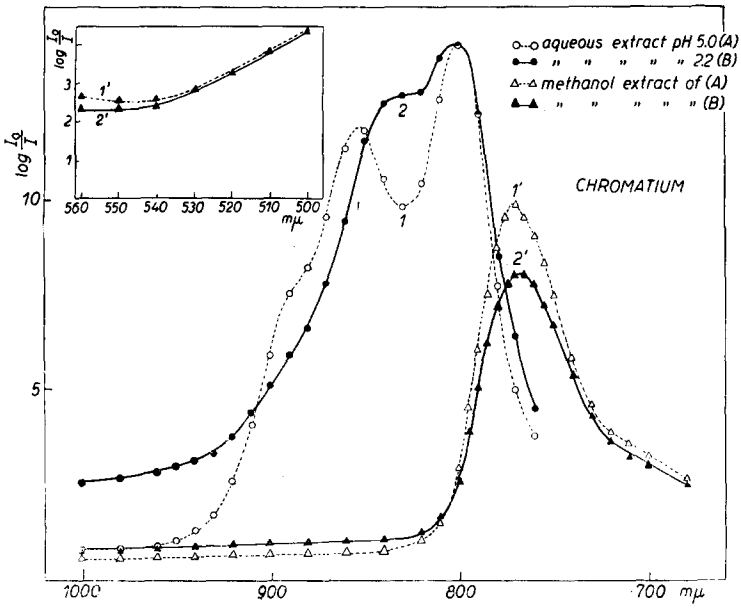

Fig. 4. Evidence that, in an aqueous *Chromatium* extract, the pH-induced 830 mμ absorption band is not due to some irreversible change in the bacteriochlorophyll molecule.

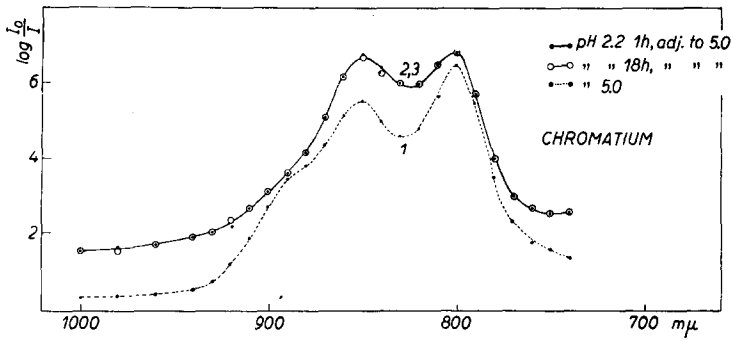

Fig. 5. Irreversible pH-induced disappearance of B 890 in an aqueous *Chromatium* extract.

were extracted with methanol. The spectra of these extracts are shown in graphs 1′ and 2′. They closely resemble each other; no absorption band of a deterioration product is present. The insert shows that, at 530 mμ, no increased absorption occurred and, thus, no pheophytin was formed. From these experiments it can be concluded that the pigment in its natural state is not irreversibly affected by pH 2.2.

Nevertheless, a second experiment showed that, within the pigment-bearer

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complex some irreversible reaction did occur. This was shown as follows. Two samples of the same *Chromatium* extract were adjusted to pH 5 and 2.2 respectively. One hour afterwards part of the most acid sample was adjusted to pH 5. The same was done with the remaining part of this sample after 18 hours. The resulting spectra are shown in Fig. 5. The additional absorption maximum at about 830 mμ which is formed at low pH, *cf.* Fig. 4 graph 2, disappeared instantaneously on adjusting the pH to 5.0. Thus, the formation of this peak is a reversible process. However, the 890 mμ band is not restored.

Quite unexpectedly, the spectra 2 and 3 of Fig. 5 coincide. The maintenance of a low pH for 18 hours did not affect the spectrum to a greater extent than this pH did after one hour. Consequently it can be stated that for *Chromatium*, in contrast to the B 890 type, the B 850 and B 800 types are very resistant to pH 2.2.

DISCUSSION

In discussing the results we may start from the assumption that the bacteriochlorophyll of the organisms studied is the same.

First, the results may be surveyed briefly. The immediate effect of pH on the height of the absorption maxima is different for the studied species, *cf.* Fig. 2. On lowering the pH of a *Chromatium* extract, the 800 mμ absorption decreases. However, below pH 3 it increases. From Figs. 3 *a* and *b* it seems most likely that this rise should be ascribed to the influence of a newly formed band at about 830 mμ. Thus it may well be that the concentration of the original B 800 material keeps on decreasing at decreasing pH values below 3. In any case it can be concluded that, for the pH range investigated, the three bacteriochlorophyll types from *Chromatium* are sensitive to a short exposure to low pH while those of *Rhodopseudomonas* are not. In *Rhodospirillum rubrum* only the 800 mμ band is affected.

Prolonged exposure to low pH yields a somewhat different picture. While the B 890 type is considerably affected in *Chromatium* and *Rhodopseudomonas*, it is only slightly influenced in *Rhodospirillum rubrum*. The B 850 type is most sensitive in *Chromatium*. In *Rhodopseudomonas* it is rather stable. The B 800 type seems only weakly affected in *Chromatium* while it is strongly reduced in *Rhodopseudomonas* and *Rhodospirillum rubrum*.

From the different reactions of each of the absorption bands studied in the organisms studied it can be concluded that, in some way, the nature of the mentioned bacteriochlorophyll types differs in different bacteria. With *Chromatium* it was shown that considerable pH-induced changes of the spectrum may occur without apparent changes within the pigment molecule. This observation suggests that the differences mentioned are due to physical factors. The most obvious factors are: interaction between the molecules of the pigment, or action of the bearer on the pigment, or a combination of these phenomena.

As to the pigment–pigment interaction, various authors, *cf.*[10,11,12], have shown that the absorption spectra of chlorophyllous pigments are considerably influenced by factors such as association and orientation. These studies are certainly of great importance for a better understanding of the spectra in question. It might, for instance, be possible to explain these spectra in terms of the occurrence of the chlorophyll in micellar or crystalline states.

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However, the above experiments showed that identical bands are differently affected by low pH in the studied organisms. If the occurrence and location of absorption bands depended merely on the presence of certain pigment micelles or crystals identical absorption bands should be affected in the same way in different bacteria. Thus, the present results render it highly probable that, in some way or another, interaction between pigment and bearer occurs. Again, this interaction may be of a physical or a chemical nature. In the first case, the pH influence may be thought to be based on *e.g.* changes in the intermolecular distances of the pigment due to "distortions" of the bearer molecule. In the second case, the pigment-protein bond may be pH-sensitive, while some change in this bond may affect the electron configuration of the pigment. It may as well be that part of the bearer molecule participates in this process. However, as was remarked by KOMEN[13], this interaction is probably restricted to the immediate neighbourhood of the bond in question.

So far, the interpretation of the results has been based on the assumption that one and the same kind of bacteriochlorophyll occurs both in the bacteria studied and in the different pigment "types". This assumption seems justified by the coincidence of the infrared absorption spectra of organic extracts from different species of bacteria. It is supported by chromotagraphic results, KOMEN[13]. Moreover, if each of the maxima were due to a chemically different kind of pigment molecule, the behaviour of a single "type" towards the effect of low pH could be expected to be the same for the bacteria studied. This is shown not to be true. These data do not necessarily rule out the possibility of the occurrence of slightly different bacteriochlorophyll molecules. However, they render it extremely doubtful that the location of the absorption bands is due to these differences.

It is still too early to decide on the mechanism which determines location of the absorption bands. However, the present results seem to imply a warning that, when studying spectra of photosynthetic pigment-bearer complexes, a pigment-bearer interaction cannot be left out of consideration.

SUMMARY

The pH dependence of the infrared absorption spectra of aqueous extracts from *Chromatium* strain D, *Rhodopseudomonas spheroides*, and *Rhodospirillum rubrum* strain 4 was studied.

Experiments on immediate as well as on long term influence of low pH on these spectra are described.

In the discussion it is concluded that an action of the bearer on the pigment is most probably an important factor in the causes which determine the location of the infrared absorption bands.

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