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SOME FACTORS INFLUENCING THE SHAPE OF THE NEAR-INFRARED ABSORPTION SPECTRUM OF *CHROMATIUM*, STRAIN D

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1. Introduction

During a considerable period of time, several investigators have been engaged in the problem of the multi-peak character of the absorption spectra of purple bacteria. In Athiorhodaceae mainly two, and in Thiorhodaceae mainly three absorption maxima exist (1, 2). Different suggestions were made to explain the existence of several peaks in the *in vivo* spectra of purple bacteria, since only one bacteriochlorophyll maximum was found in organic extracts.

Pigments, active in light absorption in photosynthesis all seem to be closely related to proteins (3, 4). Some reasons to believe in this relation may be mentioned, viz.,

1. Suspensions, containing the chlorophyll or bacteriochlorophyll pigments may be obtained artificially, e.g., by ultrasonic disintegration of cells, by thorough grinding of cells and subsequent aqueous extraction or with the aid of detergents. In aqueous suspensions, amphoteric motion in an electric field has been observed (1).
2. Purple bacteria (probably dead cells) sometimes show spontaneous extrusion of a bacteriochlorophyll and carotenoids containing pigment complex similar to that obtained by grinding the cells.
3. Practically all pigments known to play a part in photosynthesis show a shift of their absorption bands by extraction.

As already remarked, in several strains of purple bacteria, bacteriochlorophyll *in vivo* has a complicated absorption spectrum in the near-infrared which differs with the species (1, 2). In organic solvents, however, bacteriochlorophyll, regardless of the species from which it has been extracted, shows only one absorption peak, the two or three original maxima have all shifted to the same

shorter wavelength, the exact location depending on the solvent. Further observations of this type in purple bacteria also give support to the supposition of a pigment-protein bond *in vivo*, e.g., the demonstration that near-infrared absorption maxima of bacteriochlorophyll *in vivo* in harvested cells show different sensitivity towards heat (1), pH (5, 6), photoxidation, and extraction with organic solvents (7). Furthermore, for one and the same species, the relative heights of the maxima may vary according to culture conditions (2, 8). In most cases, analysis of the absorption spectra yields symmetrical single maxima (2). The justification of this analysis is supported by the fact that absorption bands which stand free, very nearly approximate a symmetric shape, which fact suggests the existence of separate bacteriochlorophyll complexes in the living cell (2), probably (lipo)-protein-pigment complexes of somewhat different properties. The special type of complex formation between the pigment and its carrier, evidently determines the position of its absorption maximum in the near-infrared. WASSINK, KATZ and DORRESTEIN (2) concluded to the existence of different pigment-protein complexes in mutually variable amounts, presumably present in each cell.

It should be emphasized that, aside of the assumption of a pigment protein bond, other possibilities have been set forward. For instance the suggestion that the different maxima in the near-infrared absorption spectrum of purple (sulphur) bacteria are due to different states of aggregation of bacteriochlorophyll molecules (9, 10), or the existence of isomeric or tautomeric forms, and small differences in chemical composition or in the reduction level of bacteriochlorophyll (11). In order to avoid any specification with regard to the nature of the molecular state of the pigments, DUYSENS suggested the term 'types' for the various states of bacteriochlorophyll rather than 'protein complexes' (12). He suggests that the difference in aspect of the peaks in the absorption spectrum of bacteriochlorophyll in different species of bacteria is due to differences in the relation of bacteriochlorophyll to different carrier molecules.

The suggestion made by BERGERON and FULLER (13) that the complexity of the near-infrared spectrum is related to the interaction of carotenoid and bacteriochlorophyll systems, was based on the observation of a strong reduction of absorption in the long wavelength region in *Chromatium*, strain D accompanying the great reduction of the absorption of carotenoids after growth on diphenylamine (DPA), causing carotenoid deficiency. However, it is possible to obtain the normal type of absorption spectrum under growth on DPA (14, 15). The effect of carotenoid deficiency in *Chromatium*, strain D, found by BERGERON and FULLER was due to changes in culture conditions other than the addition of DPA (21). There is no evidence, so far, that carotenoids affect the near-infrared part of the spectrum.

Attempts at separation of the different pigment-protein complexes in purple bacteria, carried out by several investigators (16, 17, 18, 19, 20), were not completely successful. Aside of this, the present author tried to find other methods to further characterization of the different complexes in *Chromatium*, strain D. A method which seemed to be promising was changing the cultural conditions,

and examining the alterations in the absorption spectrum owing to these changes.

It has been known for a long time that the shape of the near-infrared absorption spectrum of *Chromatium* is liable to differences. The first factor definitely influencing the shape of the spectrum found was the light intensity during growth. Decrease of light intensity caused the origination of the so called stair-case type of absorption spectrum, in which the 850 nm peak is well below the 800 nm one (2). The exact reason of this phenomenon is still obscure. FULLER *et al* (21) undertook electron microcopy of cells exhibiting spectral and chemical differences, to ascertain whether the changes in the spectrum and in the chlorophyll-protein ratios found at different light intensities were reflected in the structure of the photochemical apparatus. At low light intensities, they found relatively small vesicular structures, first designated as chromatophores by STANIER and his coworkers (22), throughout the cell, in accordance with the observations of other investigators. At high light intensities, however, the chromatophores were still present, but an intracytoplasmic membrane system was also observed. The pigment and lipid contents of the cell increased when low light intensities were used for growth.

In the present investigation changes in factors which may influence the shape of the absorption spectrum were introduced, e.g., in light intensity and/or temperature, while also changes in the composition of the culture medium have been studied.

2. MATERIAL AND METHODS

Chromatium cultures were obtained from Professor J. B. THOMAS, Utrecht, Netherlands and Professor R. C. FULLER, Dartmouth, N.H., U.S.A. Moreover, a strain, called 'Wageningen culture', was reisolated from our original culture.

Chromatium, strain D of different origin was grown in 100 ml sterile, completely filled, glass-stoppered culture bottles in the light cabinet under incandescent light. These cultures were used as starting material for the experiments. Growth in the experiments took place either in malate-thiosulphate or in bicarbonate/malate-thiosulphate medium, in the absence and in the presence of diphenylamine (DPA). The malate-thiosulphate medium consisted of 2.0% NaCl (partly pure, partly in crude form; 1% of each was used), 0.05% K_2HPO_4, 0.02% $MgSO_4$, 0.015% Na_2S, 0.15% $Na_2S_2O_3$, 0.1% $(NH_4)_2SO_4$ and 0.38% L(–) malic acid. The bicarbonate medium contained always L(–) malic acid in a concentration of at least 0.02% and differed from the malate medium by the presence of sodium bicarbonate in concentrations up to 3.7%. Finally, pH of the media was adjusted at 7.6 by addition of NaOH or HCl.

The L(-) malic acid used was from Nutritional Biochemicals Corporation, Cleveland, U.S.A., and the other chemicals from MERCK Aktiengesellschaft, Darmstadt, W.-Germany.

Since it was not possible to maintain the temperature in the light cabinet sufficiently constant, experiments were performed in a thermostatic waterbath

(P.M. TAMSON N.V., Holland, type TVZB 45–50), in which the bacteria were grown in paraffin-stoppered glass tubes of 20 ml contents and a diameter of 15 mm, placed at different distances from the windows at the long sides of the bath. Light from four 200 Watt PHILIPS Argenta incandescent lamps placed in a square was admitted through both windows, and variation of light intensity was effected by use of copper gauze screens and by variation of the distance between tubes and light sources. Decrease of light intensity during growth also took place by the screening effect of heavy grown cultures standing in front of tubes at greater distance from the window.

Light intensity at the beginning was estimated with a light meter developed by 'Stichting Technische en Fysische Dienst voor de Landbouw' (TFDL) at Wageningen, containing a PHILIPS ORP 63 detector. In the measurements the spot of the tube in the tubeholder, and the absorption by cultures in front of the tube under consideration were taken into account.

Growth experiments in 100 ml glass-stoppered culture bottles were carried out in a home-made thermostatic waterbath with an opal plexiglass (RÖHM und HAAS, Darmstadt, W-Germany) window. Light from three 200 Watt PHILIPS Argenta incandescent lamps placed side by side was admitted through the plexiglass window. Decrease of light intensity was realized by use of copper gauze screens and increase of the distance of the bottles in the bath from the window. No differences in temperature between heavily grown, light absorbing cultures and the surrounding water were observed.

Measurements of the absorption spectra were made with a CARY model 14 spectrophotometer (Applied Physics Corporation, Monrovia, U.S.A.), equipped with a scattered transmission accessory. As a quantitative characterization of the effects, the ratio in height of the 850 and 800 nm maxima (E850/E800) was taken. The scattering in this region was subtracted by drawing a straight, somewhat inclining line from the extinction reading at 960 to that at 720 nm, and measuring the heights of the maxima at 800 and 850 nm above this line; the same procedure as applied in (2). When the 850 nm peak decreases, it shows an apparent shift to somewhat shorter wavelengths. The maximum extinction value after correction for scattering of this shifted peak, was taken as '850 nm' value in determining the E850/E800 ratio. When the so called stair-case spectrum had developed, and the 850 nm peak was reduced to a shoulder of the 800 nm maximum, the corrected value at 840 nm was – arbitrarily – chosen for the determination of the E850/E800 ratio. For the matter of simplicity, the corrected E850/E800 ratio is abbreviated as E/E ratio. Measurements of growth were made by centrifugation during ten minutes at 3000 rpm of samples of the cell suspensions in TROMMSDORFF tubes, and expressed as mm^3 cells from 10 ml sample. In most cases measurements were made with bacterial cultures virtually after completion of growth.

3. Experiments

a. Effect of light intensity during growth

According to earlier findings (2), using normal light intensities, other conditions being suitable, the near-infrared absorption spectrum of *Chromatium* was of the two peak type, the 850 nm peak being at least as high as the 800 nm one or even higher, with an additional shoulder at about 890 nm. Thus, the value of the E/E ratio equalled or surpassed unity; in the case of the stair-case type of absorption spectrum this value was 0.60–0.80.

The E/E ratio of cells grown under certain constant conditions on malate medium, seemed to reach very fast the final value, as observed under these conditions after growth was completed. Even at very low Trommsdorff values, e.g., at the end of the lag phase of growth, the ratio was already considerably different from that of the inoculum (fig. 1). Sometimes, conditions were such that the final ratio of the two main absorption peaks was reached, passing higher values. It seemed possible that light intensity played an important role in the beginning, in still thinly populated cultures. Light intensity then was much higher than in more heavily grown cultures, so that possibly the E/E ratio first increased. Later on, as density increased, the E/E values reached a lower level, in which the temperature during growth seemed of great importance.

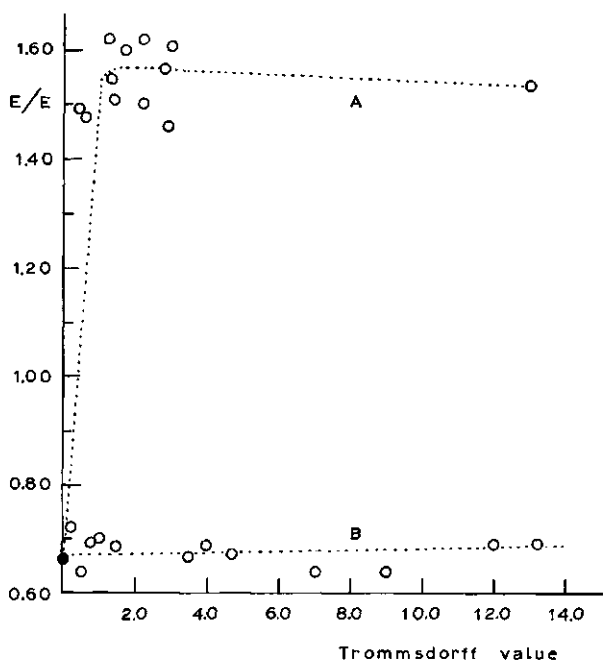

Fig. 1. Course of the E/E ratio during the initial phase of growth. Samples taken at different times from different cultures under the same condition of growth, viz., high light intensity, 38 °C (A), and high light intensity, 30 °C (B). In both cases the same inoculum was used. Malate-thiosulphate medium.

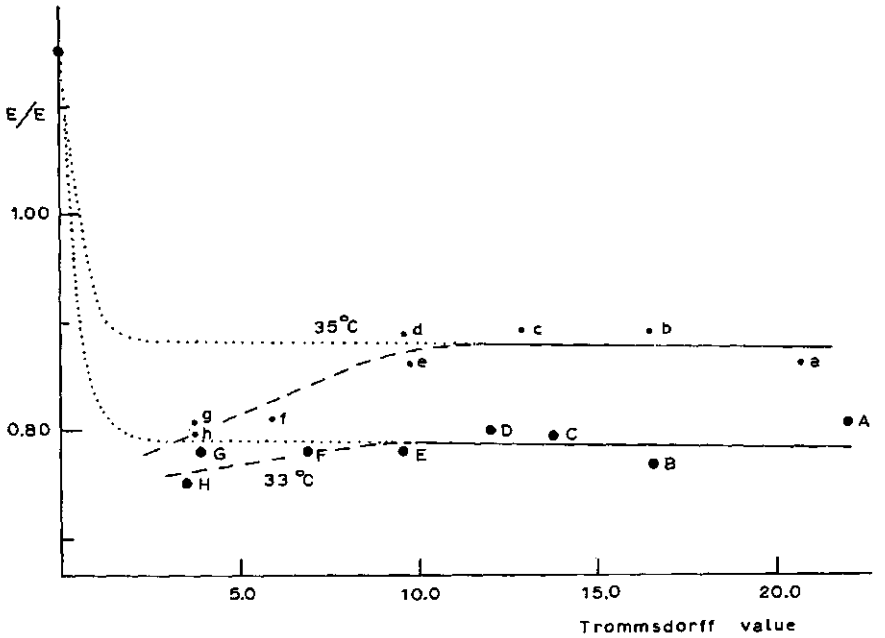

FIG. 2. E/E ratios at final TROMMSDORFF values. Wageningen culture, started from the same inoculum, grown at 8 different light intensities, covering a range from $25 - 3 \times 10^3$ ergs/cm²sec, numbered a to h (35 °C) and A to H (33 °C), in decreasing order of intensities. Estimated course of E/E ratio towards its final value for the highest light intensities is indicated by ···——. Decrease of E/E ratio and TROMMSDORFF value with decrease of light intensity is indicated by ————. Malate-thiosulphate medium.

At constant temperature, decrease in light intensity, in all strains tested, resulted in a decrease of the E/E ratio while also much lower final TROMMSDORFF values were reached. In a growth experiment at 35 °C with the Wageningen culture in a range of light intensities from 3000 to 25000 ergs/cm²sec, values of the E/E ratio from 0.80 to 0.90 were found in the stationary phase of growth, the lowest light intensity having the lowest ratio. At 33 °C the same tendency was found, whereby all values were lower (fig. 2).

In another experiment, at 37 °C, using the FULLER strain, the influence of light intensity was also present, which gave rise to a range of ratios from 0.95 to 1.40, while at 34 °C all values were lower and extended from 0.70 to 1.05 (fig. 3). From figures 2 and 3 the influence of light intensity during growth on the shape of the near-infrared absorption spectrum of *Chromatium*, strain D, and on the final TROMMSDORFF value will be clear. This influence seems to be somewhat stronger for the FULLER strain, while the influence of the temperature has already been indicated.

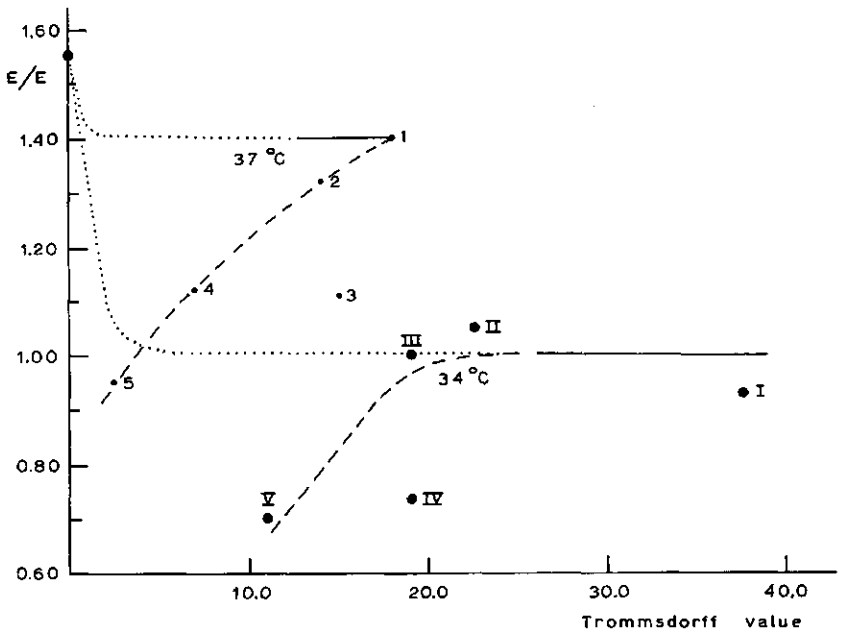

FIG. 3. E/E ratios at final TROMMSDORFF values. FULLER culture, started from the same inoculum, grown at 5 different light intensities, covering a range from 25–3 × 10^3 ergs/cm² sec, numbered 1 to 5 and I to V respectively at 37° and 34 °C in decreasing order of intensities. Estimated course of E/E ratio towards its final value for the highest light intensities is indicated by · · · ——. Decrease of E/E ratio and TROMMSDORFF value with decrease of light intensity is indicated by – – – – –. Malate-thiosulphate medium.

b. Effect of temperature during growth

In earlier experiments in the light cabinet (15), the exact control of temperature during growth was neither carried out, nor expected to be very important. But when, in spite of light intensities normally used, sometimes a low E/E ratio appeared and in the opposite case at lower light intensities the ratio sometimes did not decrease, a more serious control of temperature during growth seemed to be indicated. Even a rough control of temperature, simply by means of a thermometer in the culture bottle just before harvest, presented a possibility to 'predict' the shape of the absorption spectrum. Since it was hard to obtain constant temperatures throughout the whole cabinet, only preculturing was maintained in the light cabinet, while growth experiments were carried out in the waterbath.

The average results of a number of experiments at different temperatures and light intensities were collected, and the average values for five ranges of temperature and for two ranges of light intensity determined (table 1).

In several experiments interim changes in conditions were performed. In table 2, a result of an experiment of this type is given.

TABLE 1. Effect of temperature and light intensity on E/E ratio and TROMMSDORFF value. Malate-thiosulphate medium.

Temperature range (°C)	Light intensity range (10^3 ergs/cm²sec)	Number of exp.	E850/E800	TROMMSDORFF (mm³/10 ml)
30.0–33.0	40–50	15	0.88	22.4
34.5–35.5	40–50	4	1.03	23.6
36.0–37.0	40–50	7	1.19	28.4
36.0–37.0	2– 5	13	1.10	17.8
38.0	2– 5	4	1.54	10.8

TABLE 2. Effect of changes in culture condition (cf. text) on the E/E ratio and the TROMMSDORFF value.

	Sample 1				Sample 2		
Starting condition	Hours from start	E/E	TR	Replaced to condition	Hours from start	E/E	TR
A	–	–	–	A	117	0.72	32.8
A	67	0.71	2.8	B	160	0.75	16.5
A	67	0.71	3.0	C	141	1.36	46.5
A	67	0.71	2.8	D	285	0.97	22.0
B	–	–	–	B	285	0.67	18.0
B	67	0.68	0.5	A	141	0.72	39.0
B	67	0.69	1.3	C	117	1.31	36.5
B	67	0.68	0.5	D	285	1.03	24.0
C	–	–	–	C	117	1.27	24.6
C	67	1.50	25.7	A	285	0.99	51.0
C	67	1.50	20.5	B	285	1.18	32.0
C	67	1.54	17.5	D	141	1.38	23.5
D	–	–	–	D	141	1.10	28.0
D	67	1.21	5.8	A	285	0.88	42.5
D	67	1.27	7.5	B	285	0.95	31.0
D	67	1.21	7.0	C	141	1.30	36.0

Combinations of light intensity and temperature: A: 50×10^3 ergs/cm²sec and 30 °C, B: 4×10^3 ergs/cm²sec and 30 °C, C: 50×10^3 ergs/cm²sec and 37 °C, D: 4×10^3 ergs/cm²sec and 37 °C. E/E ratio of the inoculum (Wageningen strain) 0.66. Malate-thiosulphate medium.

After growth during some time under different initial conditions, a 5 ml sample was taken from the cultures, and the E/E ratio and the TROMMSDORFF value (TR) determined. After complete refilling of the culture bottles with fresh sterile medium, growth was continued both under the same and under different conditions. Samples were taken again after some time.

When cultures were still in the exponential phase of growth, it was possible to change the E/E ratio by changing the culture conditions. A culture in which total cell volume is reached does not alter its characteristics very much without

refreshment of the medium, even when important changes in the conditions e.g. increase in light intensity are brought about. Data from experiments with the other *Chromatium* strains showed the same tendency. The results obtained until now seem to demonstrate the influence of both light intensity and temperature. At a certain light intensity, the shape of the spectrum could be forced into the direction of the stair-case type by using lower temperatures during growth. At a certain temperature, increase of the E/E ratio could be obtained by applying a higher licht intensity (fig. 4). In this figure, data from 24 experiments in the TAMSON bath at different constant temperatures were collected. In each experiment several culture tubes were used. The average value of the E/E ratios of cultures which had grown at different temperatures (ranges of about 1.5 °C taken together), was determined for nine ranges of light intensity, numbered 1 to 9, viz. of 0–2, 2–4, 4–6, 6–9, 9–20, 20–30, 30–45, 45–80, and 80–105 $\times$ 10^3 ergs/cm^{2}sec respectively. In this way temperature curves at different levels were obtained for the E/E values at the different light intensities. Some bleaching of the 850 nm peak in the case of the highest light intensity, resulting in lower E/E ratios, seemed to occur. The experiments carried out at different temperatures under different light intensities, show the tendency of less influence of the light intensity at low temperatures (about 30 °C), all E/E ratios being low. In order to obtain an increase of the ratio the temperature had to be chosen higher, while the highest temperatures induced higher ratios even at relatively low light intensities. Under these circumstances, the appearance of

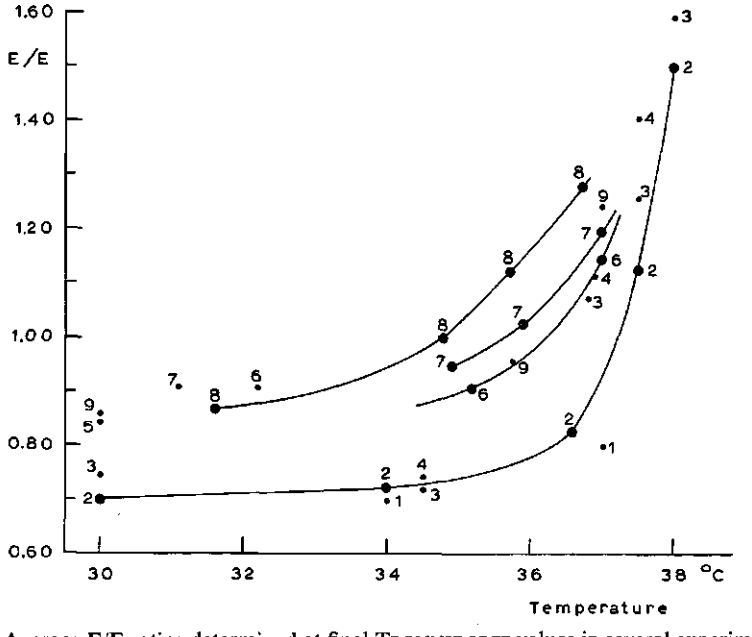

FIG. 4. Average E/E ratios determined at final TROMMSDORFF values in several experiments at different temperatures and different light intensities, the latter numbered from 1 to 9, increasing in this direction. Malate-thiosulphate medium.

the stair-case spectrum is rare. After growth under the conditions mentioned before, the cultures were still able to grow further after refreshment of the culture medium, under the same or other conditions. In fact, growth even at 44 °C was observed, giving rise to high E/E ratios (about 1.60). The measurement of the physiological activity of cells grown under these and other conditions is planned for the near future.

Results with carotenoid-deficient *Chromatium*, strain D, obtained after growth experiments in the presence of diphenylamine and having practically the same near-infrared spectrum as normal cells (14), showed similar influences of temperature and light intensity as described above (23).

c. Effect of an inorganic carbon source

As described before (15, 21), differences exist between the absorption spectra of *Chromatium* cultures grown on malate and those of cultures grown on bicarbonate under the same conditions. Results of further research on this subject under controlled conditions are collected in table 3. The average E/E ratios and TROMMSDORFF values of several experiments at 2 temperatures, viz., 33 ° and 37 °C, and a range of light intensities viz., from $45 - 80 \times 10^3$, and from $3 - 4 \times 10^3$ ergs/cm^{2}sec respectively, are presented.

Addition of bicarbonate to a constant malate concentration in general resulted in an increase of the E/E ratio. These values were higher than those in media having the same total carbon concentration, supplied as malate alone. Addition of too much carbon as bicarbonate again led to decrease of the ratio. This decrease seemed to shift to higher bicarbonate concentrations with higher initial malate concentrations, and the increase of the ratio continued onto higher bicarbonate concentrations at low light intensities. Furthermore, all E/E values obtained at 37 °C, were higher than those at 33 °C, even when lower light intensities were applied. As compared with pure malate media, TROMMSDORFF values were lower when carbon was present in both forms at the same overall concentration. Too high concentrations of bicarbonate gave rise to some decrease in TROMMSDORFF value, while increase of the malate concentration lead to increased TROMMSDORFF values, as expected. Furthermore, TROMMSDORFF values of cultures grown at low light intensities were lower than those of cultures grown at high light intensities.

The observed differences of the E/E ratios after completion of growth under different nutritional conditions, such as addition of bicarbonate, were not due to large differences in the thickness of the suspensions, in which case the influence of the light intensity in the suspensions might play an important part. Determination of the E/E ratio at low TROMMSDORFF values during growth excluded this possibility.

TABLE 3. Effect of malate and bicarbonate on E/E ratio and TROMMSDORFF value of *Chromatium*, strain D, with cells, grown under different conditions of temperature and light intensity.

meq C/l		E 850/E 800 ratio				TROMMSDORFF value			
		33 °C		37 °C		33 °C		37 °C	
mal.	bic.	high light int.		low light int.		high light int.		low light int.	
7.2		0.99		1.18		6.9		7.1	
7.2	3.6		1.02		1.18		4.1		4.0
10.8		1.01		1.26		7.2		6.6	
7.2	21.7		1.14		1.22		7.1		8.1
28.9		1.00		1.14		16.5		13.1	
7.2	72.4		1.33		1.38		5.1		5.8
79.6		1.03		1.23		17.1		12.7	
7.2	105.9		1.24		1.37		5.6		5.5
113.1		0.99		1.12		29.4		21.5	
7.2	216.0		1.07		1.28		4.2		3.9
223.2		1.00		1.11		34.1		23.7	
28.9		1.00		1.14		16.5		13.1	
28.9	14.4		1.00		1.24		13.3		6.9
43.3		0.92		1.21		15.5		8.6	
28.9	28.9		1.14		1.36		11.8		6.4
57.8		1.02		1.21		16.0		5.0	
28.9	84.2		1.31		1.38		12.8		9.9
113.1		0.99		1.12		29.4		21.5	
28.9	289.0		0.91		1.49		3.8		1.3
317.9		0.99		1.19		27.5		11.9	
113.1		0.99		1.12		29.4		21.5	
113.1	56.6		1.25		1.37		23.2		7.7
169.7		1.07		1.25		30.4		22.6	
113.1	113.1		1.21		1.39		21.1		17.6
226.2		1.00		1.11		34.1		23.7	
113.1	339.3		0.94		1.40		16.7		15.7
452.4		1.02		1.05		34.5		25.3	
226.2		1.00		1.11		34.1		23.7	
226.2	113.1		1.32		1.34		18.3		19.3
339.3		1.04		1.15		36.8		24.0	
226.2	226.2		1.03		1.35		21.1		19.4
452.4		1.02		1.05		34.5		25.3	

4. DISCUSSION

Until now, three types of differences have been observed in *Chromatium* cells, grown under low and high light intensities. Their connection so far is uncertain. First, spectral differences, as described, e.g., in the present paper. Furthermore, differences in electron micrographs and in the chemical composition of cells, as described by FULLER *et al.* (21). Circular vesicular chromatophores were always present throughout the cell. At high light intensities, also intracytoplasmic membrane systems were observed, especially in the peripheral areas. At low light intensities, the authors found the amount of bacteriochlorophyll per mg

protein doubled, and the lipid content (including carotenoids) considerably increased. This increase of lipids possibly represents structural lipids, associated with the pigment-bearing structures. As far as observed until now, the 850 nm peak is the most sensitive to different kinds of agents. Attempts at separation of the complexes suffered from this sensitivity, so that the initial absorption, of the 850 nm peak especially, in many cases had already changed before topographical separation could be attempted (20, 23). Both with photooxidation (7, 16, 20) and extraction of pigments by organic solvents (7, 20), the 850 nm peak was the first to disappear. Also variation in cultural conditions (2, 15, 23, this paper) seemed to affect especially the 850 nm peak. To some extent the 850 complex seems to be formed at the expence of the 800 one, so that in spectra with a low 850 nm peak, the 800 nm extinction appears increased. If the 800 nm peak were of constant height, increase of the 850 nm peak should also affect the height of the 800 nm one by its spur on the short wavelength side. However, as already mentioned, the 800 nm peak is decreased in this case, as compared with the peak in the stair-case spectrum.

It is still not understood in which way variation in light intensity or temperature influences biosynthesis of the complexes so that decrease of light intensity or temperature can decrease the 850 nm absorption considerably, while the increased amount of bacteriochlorophyll present under these conditions seems to contribute mainly to the 800 nm peak.

It remains to be seen whether differences in temperatures affect structural and chemical properties of the cells in the same way as reported for the light intensity. This may be expected if differences in the shape of the near-infrared spectrum are correlated with structural and chemical changes. Our experiments, namely, indicate a similar action of light intensity and temperature on the resulting spectrum. So far, experiments comparing structural and chemical properties of cells grown under low and high temperatures have not been made. Therefore, it remains to be seen whether a high 850 nm peak is correlated with the presence of lower amounts of bacteriochlorophyll and lipids than in cases in which this peak has almost disappeared.

Under less favourable conditions such as low light intensity or low temperature, when the physiological activity of the cells probably is reduced, storage of lipid material appears somewhat unexpected. Activity determinations and tests of these parts are planned for the near future. It is to be expected that lipids in general accumulate when there is excess photosynthesis over the possibility to incorporate primary products of photosynthesis into 'growth', i.e., primarily protein synthesis.

In the case of high 850 nm absorption, the presence of lower amounts of lipids is reported (21). It could be that bacteriochlorophyll, in this complex is less imbedded in lipids and so possibly more sensitive towards external influences.

Heating of harvested cells of *Chromatium* results in changes in the absorption spectrum (1, 20), due to the formation of bacteriopheophytin. Under certain conditions it is possible to obtain increase of absorption in the 850 nm region

as compared with the 800 nm absorption peak, instead of increase at 760 nm only (20). Until now, no increase in bacteriopheophytin is observed and the temperatures during growth were not as high as those used in the pheophytinization experiments. This is not in favour of the supposition that the increase of the 850 nm peak at higher temperatures is due to pheophytinization.

The carbon and nitrogen content of the culture medium may well influence the relative amounts of proteins, lipids and pigments. Results of preliminary experiments in this direction show the increase of the E/E ratio when low concentrations of the nitrogen and carbon sources (the latter provided as malate) are used, while considerably higher concentrations than those normally used, result in a strong decrease of the ratio, even at higher temperatures and light intensities (23), by which a stair-case type of absorption spectrum originates, like that of cells rich in lipid, grown at low light intensity (21). The fact that changes in the composition of the culture medium result in differences in the spectra, seems to favour the supposition of the existence of pigment-protein complexes in variable amounts, rather than suggesting differences in e.g. orientation, to explain the multi-peak character of the near-infrared spectrum of purple bacteria.

Earlier observations have shown that it is possible to obtain the extinction maximum at 890 nm, normally present as a shoulder of the 850 nm peak, as free-standing absorption band by bleaching the 850 nm peak (16, 20) as well as by growth at low light intensities and at low temperatures. Since the 890 nm peak is normally masked to a large extent by the 850 nm one, it is hard to say whether the 890 nm peak is influenced or not, and into what direction. When bicarbonate was present, the resulting spectrum was very similar to that of malate grown cells, mostly with a somewhat increased 890 nm shoulder. Our cultural conditions used seem to be different from those used by FULLER *et al.* (21). They found increase of the 850 nm peak, but also a strong increase of the 890 nm absorption, after growth at high light intensity. This effect was depressed to some extent by growth under heterotrophic conditions (malate, acetate or succinate). In the present experiments, at high light intensity, increase of the 890 nm shoulder so that it results in an absorption band which stands free, was only obtained in some exceptional cases with the cultures tested, and not preferently so in FULLER's strain. We may suggest that the different complexes are built up along partly different lines under auto- and heterotrophic conditions, in order to explain the differences in the spectrum after growth on these media under the same environmental conditions.

With *Chromatium*, strain D, under the conditions of our experiments the growth rate and the yield of cell material are increased under heterotrophic conditions as compared to autotrophic ones, while the optimum temperature for growth seems to be about 35–36 °C. In general, high light intensity favours growth but excessively high light intensities have some bleaching effect on the 850 nm maximum (fig. 4), and result in lower TROMMSDORFF values. At very low light intensities, growth is strongly inhibited, and the TROMMSDORFF value never reaches a high level, not even after very long periods of growth.

5. Summary

The present paper reports on effects of light intensity and temperature and of bicarbonate condition during the growth period on the shape of the near-infrared absorption spectra of *Chromatium*, strain D, in cultures from different origin.

The results obtained until now seem to demonstrate the influence of both light intensity and temperature.

At high temperature (37–38 °C), the 800 nm peak and that at 850 nm were found to be of comparable height.

At low temperature (30–33 °C), the 800 nm peak strongly predominated, and a 'stair-case' type spectrum resulted.

At intermediate temperatures, the E850/E800 ratio was affected by light intensity, so that high light intensities resulted in peaks of comparable height, low light intensities in the stair-case type of spectrum (with E850/E800 ratio below 1.0).

Addition of bicarbonate to the culture medium results in an increase of the 850 nm peak, as compared with the situation when carbon, in the same concentration, is present as malate only. Too high carbonate concentrations result in decrease of the 850 nm peak, which decrease is somewhat checked at lower light intensity.

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