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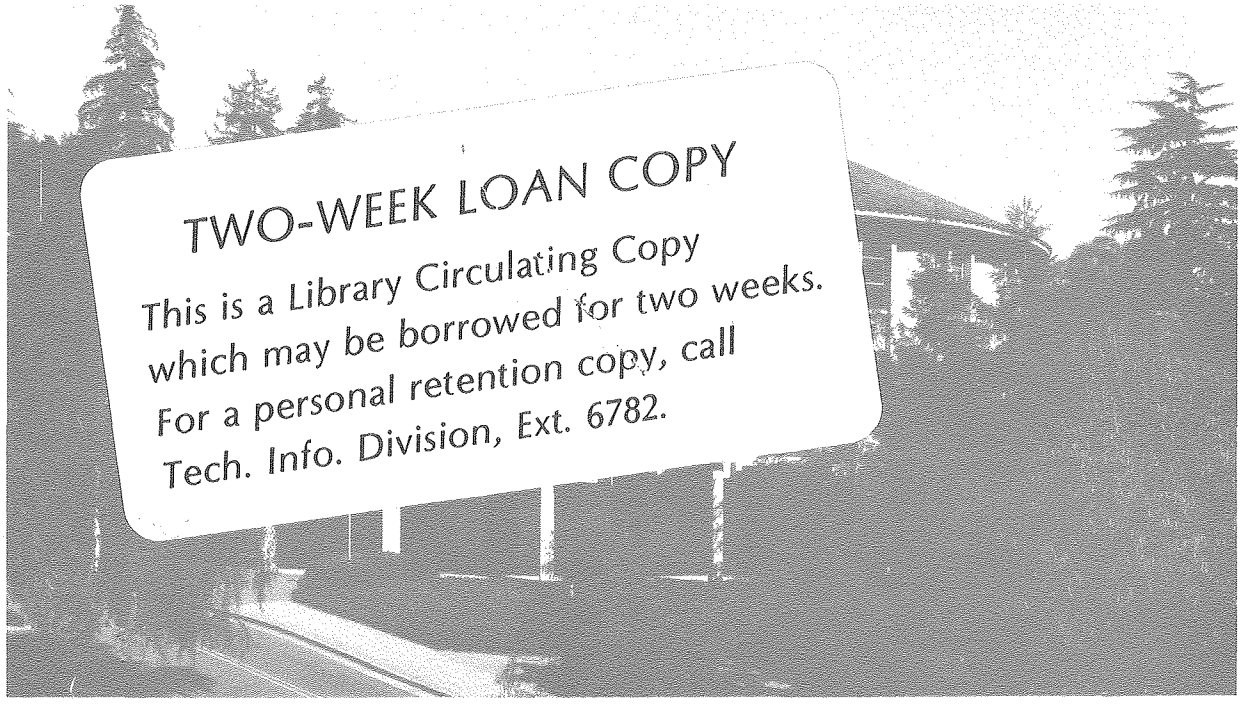

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DISCLAIMER

LINEAR AND CIRCULAR DICHROISM OF MEMBRANES FROM *Rhodopseudomonas capsulata*

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SUMMARY

Absorption, linear dichroism and circular dichroism spectra of Rhodopseudomonas capsulata (wild-type - St. Louis strain, mutant Y5 and mutant Ala^+) are particularly sensitive to the nature of the light-harvesting bacteriochlorophyll-carotenoid-protein complexes. Evidence for exciton-type interactions is seen near 855 nm in the membranes from the wild-type and from mutant Y5, as well as in an isolated B800+850 light-harvesting complex from mutant Y5. The strong circular dichroism that reflects these interactions is attenuated more than 10-fold in membranes from the Ala^+ mutant, which lacks both B800+850 and colored carotenoids and contains only the B875 light-harvesting complex. These results lead to the conclusion that these two light-harvesting complexes have significantly different chromophore arrangements or local environments.

Bacteriochlorophyll (BChl) and carotenoids (Car) in the intracytoplasmic membranes of photosynthetic bacteria are incorporated into several types of distinct complexes with protein [1-3]. In addition to those containing the reaction centers where the photoinduced electron transfer reactions are initiated, at least two light-harvesting complexes, B800+850 and B875, have been characterized in purple, non-sulfur bacteria. These complexes are readily distinguished in vivo by characteristic features in their absorption spectra in the long wavelength region [3], and it has been known since the work of Aagaard and Sistrom [4] that there is a close correlation between B875 and the reaction center complex. Recent reports of the isolation and characterization of the light-harvesting complex from

Rhodopseudomonas capsulata have helped to define their pigment composition, BChl/carotenoid ratios, pigment/protein ratios and peptide composition [1,5-8].

Spectroscopic techniques using polarized light, such as linear dichroism [9] and circular dichroism [10,11], provide evidence for the interactive associations of the pigment molecules and their orientations in the membranes and complexes. We have used these techniques to characterize the light harvesting BChl in intracytoplasmic membranes from Rps. capsulata, wild-type, mutant Ala^+, and mutant Y5 and in an isolated BChl/Car/protein complex from the Y5 mutant [1]. The most dramatic differences from the spectral properties of the wild-type occur in the Ala^+ mutant, which is missing both the B800+850 complex and colored carotenoids. The CD, in particular, is an order of magnitude weaker in the mutant relative to the absorption. This supports the view, in agreement with observations on Rps. sphaeroides [10], that the CD in the long wavelength region of the wild-type organism is dominated by contributions from the B800+850 light-harvesting complex.

MATERIALS AND METHODS

The growth of Rps. capsulata, wild-type (St. Louis strain) and mutant strains Y-5 (RC^-, $B875^-$, $B800+850^+$, Car^+ altered, derived from parent St. Louis strain) and Ala^+ (RC^+, $B875^+$, $B800+850^-$, Car^-, derived from parent strain 37b4) were described by Feick and Drews [1], as was the preparation of intracytoplasmic membranes. The isolation of the B800+850 light-harvesting complex follows that of Shiozawa, et al. [8]. The membrane samples were suspended in 10 mM tris-Cl (pH 7.6). After the last step of purification the pigment-protein complex was dialyzed

overnight against 10 mM tris-Cl (pH 7.6) to remove excess detergent.

Absorption spectra were measured using a Cary 14R recording spectrophotometer. Circular dichroism spectra were measured using a specially constructed instrument [12] and following procedures described previously [10,11]. For the linear dichroism measurements, the samples were embedded in polyvinyl alcohol films which were then stretched according to procedures that are described elsewhere [9]. All films were stretched to two times their original length.

RESULTS

The absorption, CD and LD spectra for intracytoplasmic membranes from Rps. capsulata, wild-type, mutant Y5 and mutant Ala^+ are shown in Figs. 1-3, respectively. The absorption and CD spectra of the wild-type (Fig. 1) are quite similar to those reported previously for Rps. sphaeroides [10,13]. The strong double CD centered near the absorbance maximum at 855 nm, the much weaker CD associated with the sharp 800 nm absorption band, the positive CD at the 590 nm transition of BChl and the complex shape of the CD in the region of the carotenoid absorption (430-530 nm) are remarkably similar for the two organisms. In the linear dichroism spectra, the absorption oscillators lie more parallel to the stretch direction in the long wavelength region and more perpendicular in the visible. Although the dichroism decreases progressively from 900 to 750 nm, there is only a small change in passing across the 855 nm band, where two exciton components are expected based on the CD spectrum. The other noteworthy LD feature is the pronounced decreasing dichroic ratio in the red edge of the Q_X transition near 600 nm.

The Y-5 mutant of Rps. capsulata, which lacks reaction centers and the B875 light-harvesting complex [14], shows features which are similar, but with minor modifications, to those of the wild-type organism. The ratio of absorbances at 855 and 800 nm appears to be decreased in the mutant, but the CD in this region is almost identical between the two. (The absorbance ratio A855/A802 is normally 1.55 in the spectra of intracytoplasmic membranes from the Y5 mutant as well as in the B800+850 complex isolated from the membranes; see Fig. 4. The reason why this ratio is low in Fig. 2 is not known, but it may reflect some loss in this sample of the B800 component, which is known to be relatively labile [5,10]). The LD is somewhat greater at long wavelength and there is a stronger decrease approaching the 855 nm absorbance maximum in the mutant than in the wild-type organism. Again in the mutant, the carotenoid absorption is broader and shows no fine structure; similar behavior is seen in the CD. This reflects, in part, the altered path of carotenoid biosynthesis in this mutant. The B800+850 complex isolated from the Y-5 mutant exhibits quite similar spectral properties (Fig. 4) to those of the intracytoplasmic membranes. Some evidence of residual structure in the carotenoid absorption region is seen in the isolated protein complex. The LD values are closer to unity, probably reflecting greater difficulty in orienting the small protein complexes in the stretched PVA films. The wavelength dependence (magnitude relative to $A_{\parallel}/A_{\perp} = 1.0$) is the same for the membranes and the isolated protein complex.

The Ala^{+} mutant of Rps. capsulata lacks both colored carotenoids and the B800+850 complex [1]. Dramatic effects are seen in all of its spectra (Fig. 3). The sharp absorbance peak at 800 nm is absent, as is

the carotenoid absorption. The long wavelength absorbance maximum occurs at 870 nm. The features in the CD spectra are not only markedly different, but are also uniformly smaller in amplitude by a factor of about 10, compared with the wild-type or the Y-5 mutant. Some of the CD features, such as the double CD at 800 nm and the more structured CD near 600 nm, may result from the reaction centers, which are relatively exposed in the CD spectrum of the Ala^+ mutant. Similar features are seen for the isolated reaction centers of several other photosynthetic bacteria [11]. The LD spectra in the long wavelength region show less positive values than those of the wild type or the Y-5 mutant and no decrease from long to short wavelength. In the region of the Q_x transition near 590 nm, the dichroic ratio ($A_{\parallel}/A_{\perp}$) is both smaller and more constant across the absorption band for the Ala^+ mutant than for the wild-type membranes.

DISCUSSION

On the basis of the spectroscopic results presented here and in previous publications, it is easy to distinguish the contributions of the reaction centers, the B875 and the B800+850 complexes from non-sulfur purple bacteria (e.g., Rps. capsulata, Rps. sphaeroides). Some examples include: reaction centers exhibit positive CD for the long wavelength bleachable absorption band (P870) and double CD (-,+) at 800 nm, positive CD at 605 nm crossing to negative at longer wavelengths; B875 exhibits an absorbance maximum at 870-880 nm, shows relatively weak CD that is perhaps double, but probably mixed with reaction center contributions (Fig. 3); B800+850 exhibits two sharp absorbance maxima at 800 and 855 nm, strong double CD (-,+) at 855, weak negative CD near 800 nm but shifted to shorter wavelengths than

the absorbance maximum, and a single positive CD centered on the Q_x transition at 590 nm. At least some of these features are identifiable in the less closely related species Rhodospirillum rubrum and Rps. viridis.

Because these spectroscopic properties are so distinctive for the different components, they are useful in characterizing differences in the pigment associations within the different complexes as well as similarities and differences among various organisms. For example, the pronounced differences in the absorption and CD specta of the B875 and B800+850 complexes point to rather profound differences in their relative chromophore geometries. Unfortunately, the state of our knowledge of these complexes is not sufficient to say what these differences are.

The smallest stoichiometric unit of the B800+850 complex isolated from Rps. capsulata consists of three polypeptides (12.0, 9.3 and 5.1 kd) in an equimolar ratio, three moles of BChl and one mole of Car. The 850 nm peak is due to two BChl molecules associated with the 9.3 kd polypeptide, and the BChl associated with the 800 nm peak has been attributed to one mole of BChl associated with the 5.1 kd polypeptide [1,2,5,6; J.A. Shiozawa, W.Welte, N. Hodapp and G.Drews, unpublished results]. The Car may be associated with the small polypeptide [6; R. van Grondelle and C.P.R. Rijgersberg, private communication]. The isolated B800+850 complex of Rps. capsulata appears to exist as an aggregate of three or four stoichiometric units. In membranes, the complex is organized into larger aggregates consisting of six to eight stoichiometric units [Shiozawa, et al., unpublished results].

The 855 nm absorption and double CD are interpreted to result from moderate exciton coupling between two of the BChl, and the 800 nm absorption arises from the third BChl. The expected splitting is not seen in the absorption band at 855 nm; however, it has been reported from 4th derivative spectra [15]. Similar splittings in the 4th derivative spectra were not seen for either the wild-type or the Y5 mutant of Rps. capsulata, however [16]. This latter study did detect a splitting in the 870 nm region of the spectrum of membranes from the Ala^{+} mutant, which would be consistent with assigning the small double CD in that region (Fig. 3) to exciton interaction in the B875 complex.

In other comparisons, it is noteworthy that the light-harvesting complex from the R-26 (carotenoidless) mutant of Rps. sphaeroides has a strong double CD at long wavelength like that of the B800+850 complex of the wild-type, despite missing the 800 nm absorption band. A strong double CD is also seen in Rds. rubrum [17]. In terms of their CD spectra, the light-harvesting absorption of Rds. rubrum resembles B800+850 more closely, although it has generally been attributed to B875 on the basis of its longer wavelength and correlation with the reaction center complex. It remains to be seen how comprehensive these pigment-protein classifications will prove to be.

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REFERENCES

[1] Feick, R. and Drews, G. (1978) Biochim. Biophys. Acta 501, 499-513.

[2] Cogdell, R.J. and Thornber, J.P. (1979) in: Chlorophyll Organization and Energy Transfer in Photosynthesis, Ciba Foundation Symposium 61, 61-73.

[3] Drews, G. and Oelze, J. (1980) Adv. Microb. Physiol. 22, in press.

[4] Aagaard, J. and Sistrom, W.R. (1972) Photochem. Photobiol. 15, 209-225.

[5] Feick, R. and Drews, G. (1979) Z. Naturforsch. 34c, 196-199.

[6] Webster, G.D., Cogdell, R.J. and Lindsay, G.J. (1980) FEBS Lett. 111, 391-394.

[7] Cogdell, R.J., Lindsay, J.G., Reid, G.P. and Webster, G.D. (1980) Biochim. Biophys. Acta 591, 312-320.

[8] Shiozawa, J.A., Cuendet, P.A., Drews, G. and Zuber, H. (1980) Eur. J. Biochem., in press.

[9] Bolt, J. and Sauèr, K. (1979) Biochim. Biophys. Acta 546, 54-63.

[10] Sauer, K. and Austin, L.A. (1978) Biochemistry 17, 2011-2019.

[11] Philipson, K.D. and Sauer, K. (1973) Biochemistry 12, 535-539.

[12] Sutherland, J.C., Vickery, L.E. and Klein, M.P. (1974) Rev. Sci. Instrum. 45, 1089-1094.

[13] Sauer, K. (1972) Methods Enzymol. 24, 206-217.

[14] Drews, G., Dierstein, R. and Schumacher, A. (1976) FEBS Lett. 68, 132-136.

[15] Cogdell, R.J. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 409-416.

[16] Talsky, G., Rijgersberg, C.P., van Grondelle, R., Feick, R. and Drews, G. (1980) Z. Naturforsch, in press.

[17] Dratz, E.A., Schultz, A.J. and Sauer, K. (1967) Brookhaven Symp. Biol. 19, 303-318.

FIGURE CAPTIONS

FIG. 1. Absorption, CD and LD spectra of intracytoplasmic membrane from Rhodopseudomonas capsulata, wild type (St. Louis strain). The CD spectrum is shown at the bottom and the LD measurements (solid circles) are shown calculated as $A_{\parallel} / A_{\perp}$, where parallel refers to the stretch direction of the polyvinyl alcohol film in which the membranes were suspended. All measurements at room temperature; absorbance and CD, 1 cm path.

Fig. 2. Absorption, CD and LD spectra of intracytoplasmic membranes from Rps. capsulata, mutant Y5. Conditions as in Fig. 1.

Fig. 3. Absorption, CD and LD spectra of intracytoplasmic membranes from Rps. capsulata, mutant Ala^{+}. Conditions as in Fig. 1.

Fig. 4. Absorption, CD and LD spectra of a light-harvesting BChl-protein complex B800+850 isolated from Rps. capsulata, mutant Y5. Complex suspended in 0.1% dodecyldimethylamine oxide. Other conditions as in Fig. 1.

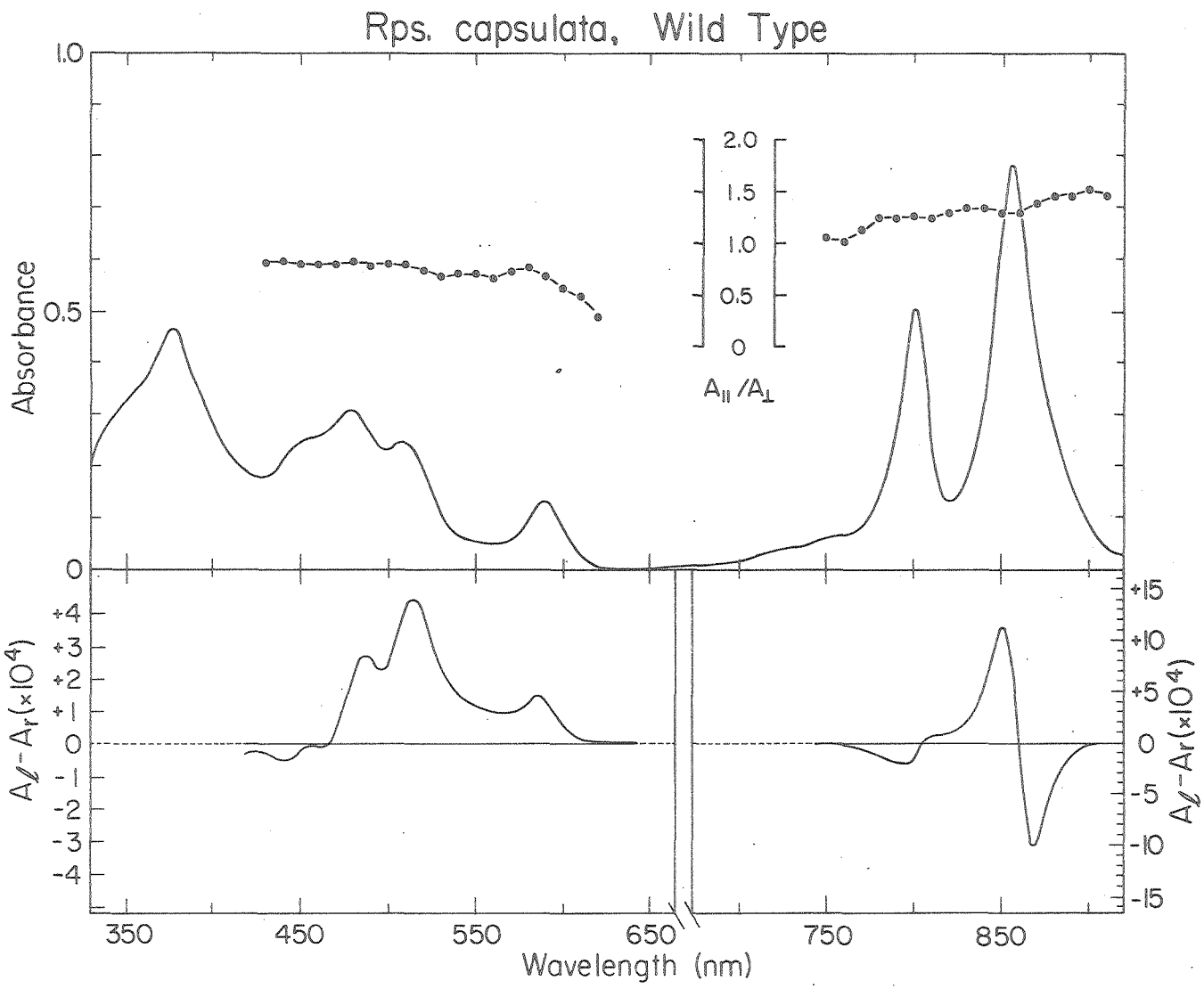

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Fig 1

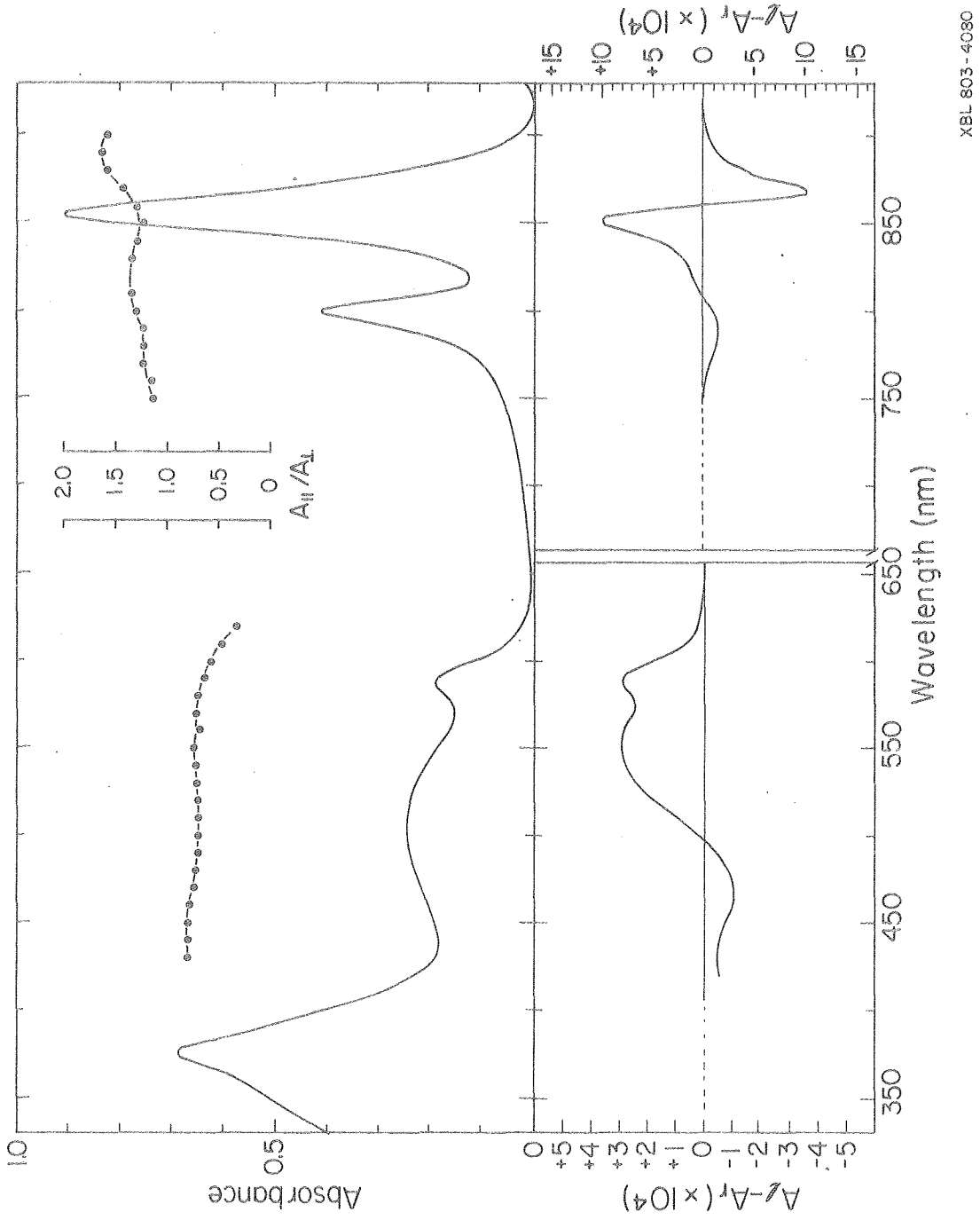

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Fig 2

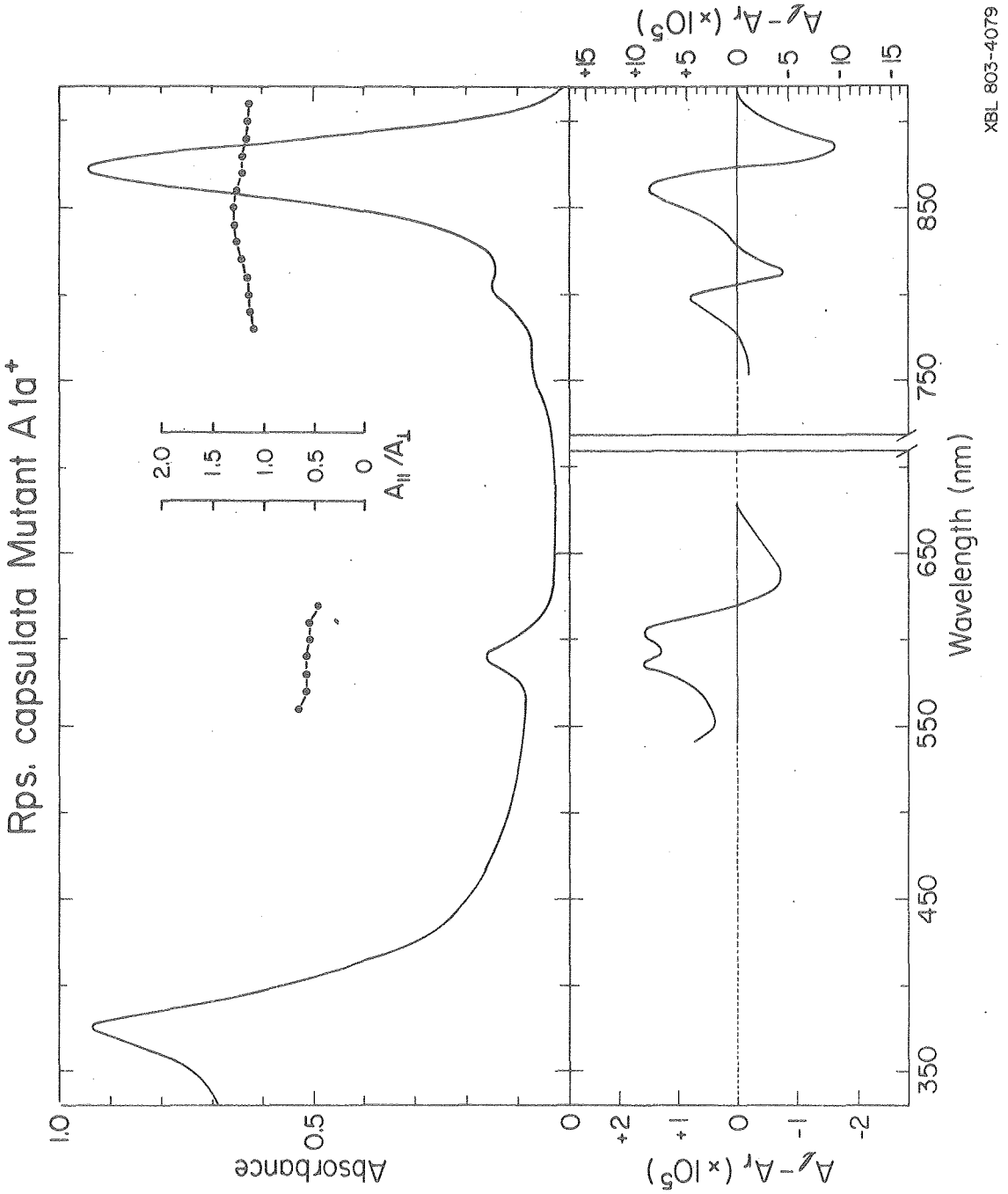

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Fig 3

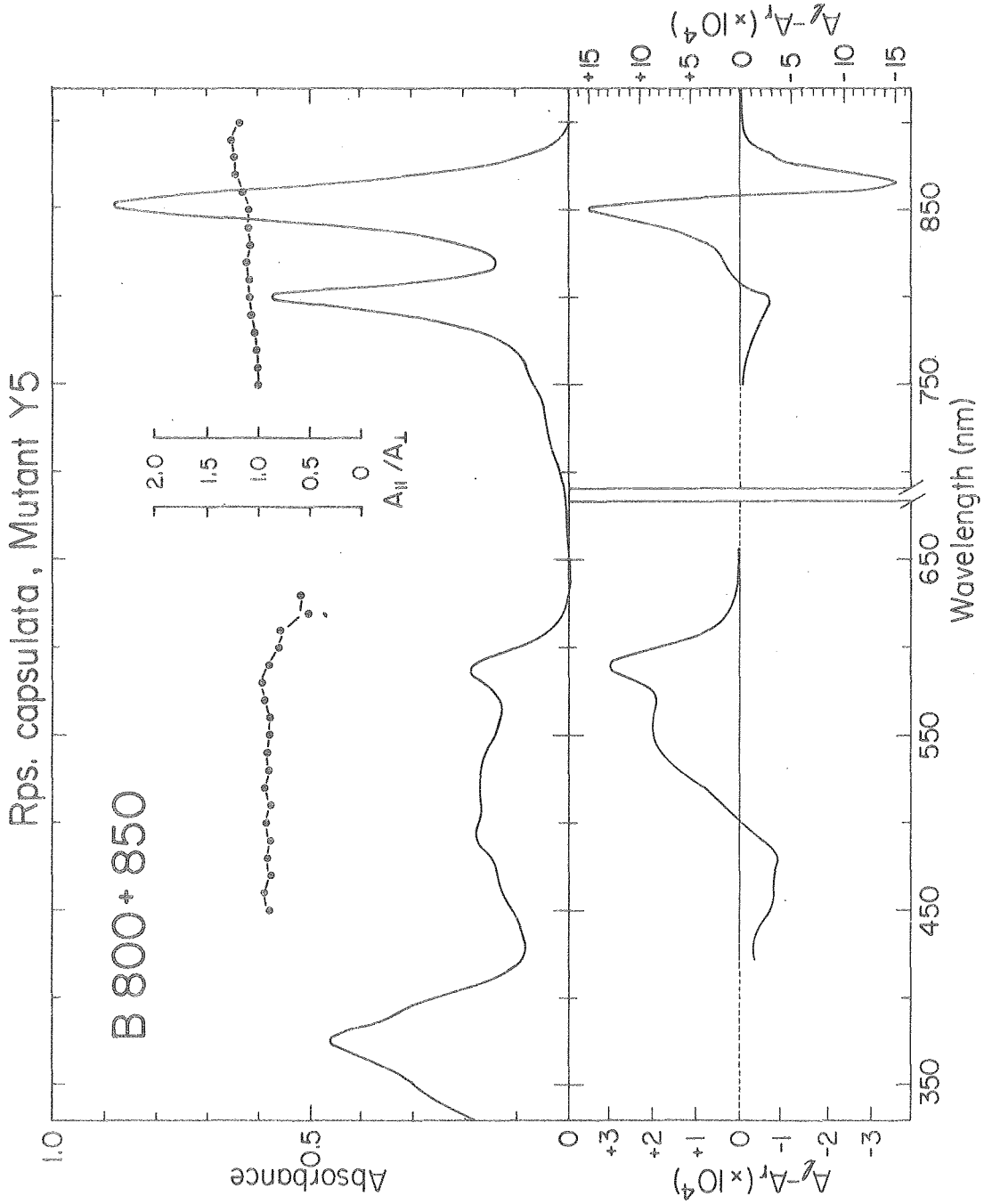

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Fig. 4