SPECTRAL ABSORPTION OF PRODIGIOSIN IN INTACT CELLS

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The pigment of Serratia marcescens has usually been studied after its extraction from the cells using acid, alkali, or organic solvents. Williams et al. (1956) refer to earlier methods and describe a simple acetone extraction process which they used in studying the spectral absorption of prodigiosin and 4 fractions separated by paper chromatography. It is possible, however, to study the spectral absorption of the pigment while it is still in the cells. The absorption spectra of the extract and the whole cells should agree if the extraction process does not affect the pigment; the native pigment is not shielded or altered by its association with other cellular components; and the method used with whole cells truly eliminates the effect of scattering. Bateman and Monk (1955) described a new method of measuring the true absorption of turbid suspensions using diffuse light and referred to several earlier methods reported in the literature. The method used in this work also employs the principles of diffuse light spectrophotometry. It consists of adding the absorbing system to a heavy suspension of a scattering substance in a wide cell and measuring the transmission of the diffuse slab in a double beam spectrophotometer. The added scattering due to the sample is insignificant and its absorption alone determines the transmission of the slab relative to a similar slab without absorber in the reference beam.

EXPERIMENTAL METHODS

Serratia marcescens Detrick strain 8UK was harvested with distilled water after 24 hr growth at 31°C on tryptose agar. It was then washed and resuspended in distilled water at a concentration of about 10¹¹ cells/ml and refrigerated until used.

A Cary double-beam recording spectrophotometer was used to measure the absorption in cells 1 cm long, 5 cm wide and 5 cm high. About 0.05 ml of the stock suspension was added to 20 ml of Dow polystyrene latex (PSL) at a concentration of 1.55 mg/ml and containing a mixture of particles 0.31 and 0.86 μ in diameter. The method, cells, and PSL have been previously used in this laboratory. Due to the absorption of PSL, the method was satisfactory only above 340 μ. The transmission of the PSL slabs was low, of course, and the slit width was automatically adjusted to the values given in table 1.

Extracts were prepared simply by vibrating the cells with glass beads for 10 min in the Mickle disintegrator followed by clarification at about 10,000 × G for 30 min.

RESULTS AND DISCUSSION

The absorption spectra of the stock suspension, pH 5.5, is shown in figure 1 along with curves for similar aliquots adjusted to pH 11.6 with NaOH and pH 2.2 with HCl. Corresponding curves are given in figure 2 for the mechanically prepared crude extract measured in standard 1 cm cells without PSL. The region of most interest is above 400 μ where there are 3 absorbing components at about 470, 495, and 535 μ as pointed out by Williams et al. (1956). They failed to resolve all 3 components at any one hydrogen ion concentration. The first component is represented only by a shoulder in the basic curves of figures 1 and 2 but is clearly shown in later figures. The extracts show high absorption in the near ultraviolet as expected, but, with the whole cells,

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<tr>
<th>Wave Length (μm)</th>
<th>Slit Width (mm)</th>
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<tr>
<td>350</td>
<td>1.8</td>
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<tr>
<td>400</td>
<td>0.7</td>
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<td>600</td>
<td>0.5</td>
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<td>650</td>
<td>1.2</td>
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Figure 1. Spectral absorption of Serratia marcescens in normal, acidic, and alkaline suspensions measured by the diffuse slab method.

This region is not available for study because of the PSL absorption. The use of MgO as the scattering material might extend the useful range of the method.

These curves indicate that the pigment in intact cells has essentially the same absorption pattern as the crude extract separated from the cells. A critical quantitative comparison cannot be made, however, since, as found during the course of our work, the pigment, particularly its broad orange component (470 m), is quite unstable. This is illustrated in figure 3 for an extract that was initially alkaline. The lower curve (1) represents the initial orange color when basic. Curves 2, 3, and 4 were taken at subsequent 10 min intervals and each was plotted 0.1 OD unit higher than the previous one for clarity. It is obvious that the strong 470 m component decreases and the 535 m component increases quickly with time.

In order to show that a similar effect takes place with pigment in whole cells, young (24 hr) orange colonies were washed off nutrient agar with distilled water and studied by the diffuse light method. The results are shown in figure 4; here again the lower curve shows the basic condition and later spectra have been displaced upward.

Figure 2. Spectral absorption of mechanically prepared and clarified extracts of Serratia marcescens by the standard method in a 1 cm cell.
SPECTRAL ABSORPTION OF PRODIGIOSIN

Figure 3. The change in spectral properties over a half-hour of a mechanically prepared extract. The initial alkaline spectrum is numbered 1 and later ones have each been displaced 0.1 optical density unit above the previous ones.

The initial alkaline spectrum is numbered 1 and later ones have each been displaced 0.1 optical density unit above the previous ones.

0.1 OD unit. The transition from 470 to 535 mµ is apparent. This instability was observed at all pH values although it was greatest under alkaline conditions. The terminal spectra was always similar to that for acidified fresh cells and was not affected by subsequent changes in pH. The effect of air on the orange component is unknown, but oxidizing agents did not alter the pattern in any striking manner.

In spite of the complication due to instability, it is obvious from the spectra given that it is possible to get a much greater 475 mµ component with extracts than with whole cells. In order to see if this was an artifact of the extraction process or if this 475 mµ component is normally shielded in the cell, an additional experiment was carried out with a culture in the early stages of pigmentation grown in tryptose broth. After 24 hr, it showed turbidity and a slight pinkish color. The cells were concentrated by centrifugation and the spectra of the whole cells and unclarified extract studied by the diffuse slab method. The results are shown in figure 5 in which the normal and alkaline spectra for the extract are compared above those for the whole cells. Even in the early stages of pigmentation, all 3 components are present, and the absorption at about 400 mµ seems to be relatively more important at this stage of growth. It cannot be considered a part of the normal visible pigment.

Rough measurements of the ratio of the optical densities at 475 and 535 mµ indicate that about 30 per cent more of the 475 mµ component is available in extracts than in whole cells under

Figure 4. The change in the spectral properties of whole cells with time. The initial alkaline curve is numbered 1 and later ones have each been displaced 0.1 optical density unit above the previous ones.
cells is qualitatively identical with mechanically prepared extracts and with acetone extracts described by Williams et al. (1956). Of the 3 absorbing components, the orange one at 475 m\(\mu\) is very unstable and appears to convert to a form absorbing at 535 m\(\mu\). The 500 m\(\mu\) component apparently is not affected by pH changes, but in alkaline conditions the 535 m\(\mu\) peak is shifted to 475 m\(\mu\). The shift is more or less reversible, but, due to the instability of the latter, a stable terminal spectra is eventually reached with the highest peak at 535 m\(\mu\), one at 500 m\(\mu\) about 50–75 per cent as high and the 475 m\(\mu\) component absent.

While the method used in this research is simple and can be adapted for use with other pigments and cells, one must carefully evaluate in each case the possible importance of such factors as ultraviolet absorption of the PSL, adsorption of materials on the PSL, and the location of the chromophore within the cell.

**SUMMARY**

The spectrum of pigment in whole cells of *Serratia marcescens* is qualitatively the same as that in mechanically prepared extracts and agrees with the spectrum of acetone extracted prodigiosin reported by other workers except that all 3 components at 475, 500 and 535 m\(\mu\) can be resolved. The 475 m\(\mu\) component is very unstable at room temperature and is altered in HCl so as to absorb at 535 m\(\mu\). This shift is partially reversible, but the instability of the 475 m\(\mu\) component eventually produces a stable terminal spectra similar to that of the acidified fresh pigment or whole cells and independent of pH. All components are present in the early stages of pigmentation. Relatively more of the orange component is present in extracts than in whole cells implying that it may be more shielded inside the cell than are the other components.

**REFERENCES**


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*Figure 5.* Spectral properties of normal (solid line) and alkaline (dashed line) suspensions of whole cells (bottom) and their unclarified extract (top) displaced 0.3 optical density unit. Both were measured by the diffuse slab method using polystyrene latex.

either normal or alkaline conditions. The inability to resolve the orange component makes a quantitative comparison difficult at this early stage of pigmentation, but these curves suggest that it may be shielded in the whole cell to a greater extent than either of the other two components, and all must be considered part of prodigiosin. It may be that the orange fraction is created inside the cell but on reaching another site, perhaps the wall, it is converted into more stable forms, absorbing at 535 and, perhaps, 500 m\(\mu\). This orange component may be important to cellular metabolism and the terminal color only incidental.

The results indicate that the pigment in whole