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Fractionation of spinach chloroplasts with sodium deoxycholate

After dissociation of spinach chloroplasts with recrystallized digitonin, followed by differential[1] or density gradient[2] centrifugation, fractions are obtained that differ in C_a/C_b ratios as well as in the various spectroscopically different forms of chlorophyll *a*. Moreover, these fractions show significant differences in low-temperature emission spectra[3] and photochemical activities[4] considered to be characteristic for the two photochemical systems of photosynthesis. Attempts in this laboratory to fractionate spinach chloroplasts according to this procedure led to variable results, which is the reason we have looked for other agents to disintegrate the chloroplasts. Hydrolytic enzymes proved to be unsuccessful in this respect[5], whereas the detergents Triton X-100 and sodium dodecyl sulfate were found to cause considerable destruction of pigment complexes. Good and reproducible results were obtained with the detergent deoxycholate. These results will be reported here and compared with those published for digitonin.

Suspensions of chloroplast fragments were prepared according to THOMAS, VAN LIEROP AND TEN HAM[6] in 0.05 M Tris buffer (pH 8.0) which was used throughout. Low-temperature absorption spectra were recorded with a Cary Model 14R spectrophotometer. Except for a slight modification, fluorescence spectra at 77°K were measured with the apparatus described by GOEDHEER[7]. Instead of using the chloroplast extracts on filter paper, they were made 60% with respect to glycerol (final concentration of chlorophyll approx. 10 μg/ml) and put into 1-mm perspex cuvettes, closely fitting the window of the three-walled Dewar vessel and fully immersed in liquid nitrogen. A removable side of these cuvettes, which allowed convenient cleaning, was refixed with silicone grease. This procedure yielded clear and crack-free glasses of 1-mm path length. C_a/C_b ratios were determined with the use of the nomogram given by KIRK[8]. Sodium deoxycholate was obtained from British Drug Houses and used without further purification. At pH values below 8.0, sodium deoxycholate solutions become more and more viscous and may solidify into a gel.

The action of sodium deoxycholate, in contrast to that of digitonin, is dependent on sodium deoxycholate concentration, sodium deoxycholate/chlorophyll ratio and ionic strength. After investigation of the effect of these variables to establish optimal conditions, the following standard procedure was adopted. A buffered sodium deoxycholate–KCl solution is added to a suspension of unwashed chloroplast fragments in the cold to give a final concentration of 0.4% sodium deoxycholate, 0.15 M KCl and approx. 100 μg chlorophyll per ml. An instantaneous clarification of the chloroplast suspension occurs, and no incubation is necessary. The mixture is subsequently centrifuged for 30 min at 10000 $\times$ *g*, 30 min at 100000 $\times$ *g* and finally for 60 min at 144000 $\times$ *g*, leaving a light-green supernatant containing solubilized chlorophyll (see below).

The low-temperature fluorescence spectra of the 10000 $\times$ *g* and 144000 $\times$ *g* sediments shown in Fig. 1 demonstrate that a good fractionation with sodium deoxycholate can be achieved, which under these controlled conditions is fully reproducible. Of the three emission bands of untreated spinach chloroplasts at 685, 695 and 735 nm, the heavy fraction mainly shows the two short-wave bands, whereas the 735-nm band is emitted almost exclusively by the light fraction. These results are similar to those

reported for digitonin. A change in either the sodium deoxycholate or the KCl concentration results in a less satisfactory separation, as demonstrated in Fig. 2 which depicts the emission spectra of 10 000 × *g* fractions from the same sample of chloroplast fragments obtained with different sodium deoxycholate concentrations at a fixed concentration of KCl. A similar set of spectra can be obtained by varying KCl concentrations at a fixed concentration of sodium deoxycholate.

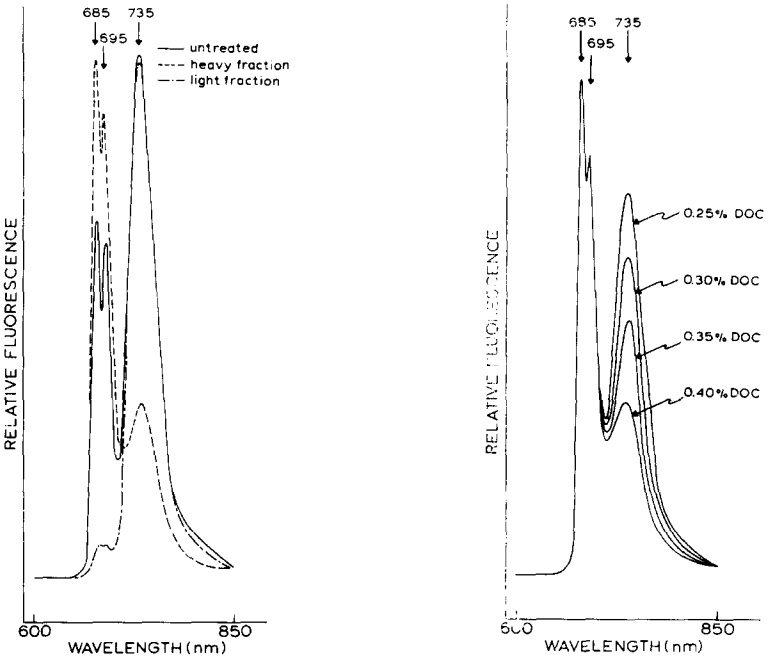

Fig. 1. Fluorescence spectra at 77°K of untreated chloroplast fragments (———), heavy fraction (— — —) and light fraction (— · — · —).

Fig. 2. Fluorescence spectra at 77°K of 10000 × *g* fractions from the same sample of chloroplast fragments obtained with different sodium deoxycholate (DOC) concentrations as indicated at a fixed concentration of 0.15 M KCl. The curves have been adjusted to give the same fluorescence intensity at 685 nm.

Upon addition of the detergent to the chloroplast suspension under the conditions described, an intensification and broadening of the 685-nm band occurs. This phenomenon, which we also observed with digitonin, is likely to be caused by some chlorophyll, solubilized by the detergent, fluorescing with a maximum at 676 nm. As mentioned before, this "free" chlorophyll remains in the final supernatant after centrifugation. Solutions of detergent-solubilized chlorophyll with this characteristic emission band are readily prepared artificially by adding a few drops of the pigment in acetone to a solution of any detergent (*cf.* ref. 9).

The heavy fraction is enriched in chlorophyll *b*. Six fractionations yielded mean values of 1.9 and 6.6 for the C_a/C_b ratios of the heavy and light fractions, respectively. However, differences in C_a/C_b ratios by themselves are no criterion for a separation in photochemical systems[5]. Evidence that an actual separation of these systems had

been achieved was obtained by the measurement of P_{700} bleaching, which could be readily demonstrated in the 144000 × *g* fraction. The magnitude of the absorbance change was increased in the presence of ascorbate, followed by a more rapid decay of the signal in the dark. No P_{700} bleaching could be measured in the heavy fraction (Fig. 3). On the other hand, Hill reaction activity measured as 2,6-dichlorophenol-indophenol reduction was found to be abolished by sodium deoxycholate and, contrary to published data, also by digitonin. These negative results might be due to the fact that these experiments were carried out with winter-grown spinach, notably low in Hill activity.

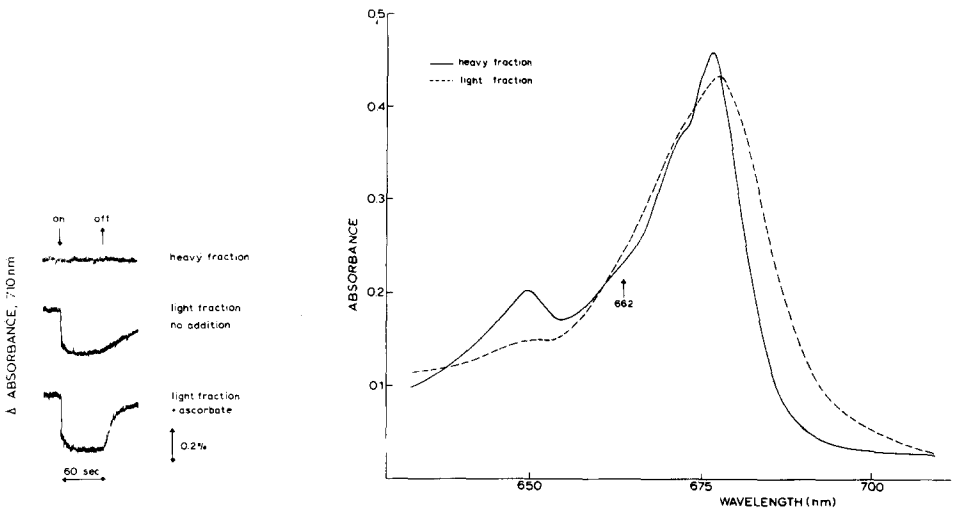

Fig. 3. P_{700} bleaching of heavy and light fractions. Intensity of actinic blue light: $0.15 \cdot 10^4$ erg · cm^{-2} · sec^{-1}.

Fig. 4. Absorption spectra at 77°K of heavy (———) and light (— — —) fractions.

Absorption spectra of both fractions measured at 77°K have a composite structure of the chlorophyll *a* band, as shown in Fig. 4. The light fraction absorbs more strongly at longer wavelengths, although C_a670 is clearly present, while its absorption maximum is shifted 2–3 nm towards longer wavelengths relative to that of the heavy fraction. An interesting feature of the absorption spectrum of the 10000 × *g* fraction, being narrower and showing more details, is the presence of a distinct shoulder at about 662 nm, which might represent a C_a665 type observed earlier[10,11].

It is concluded that both sodium deoxycholate and digitonin dissociate spinach chloroplast membranes in a non-random fashion allowing a partial separation of the two photochemical systems of photosynthesis. Considering the influence of ionic strength, the mechanism of action of sodium deoxycholate is apparently dependent on the physical state of the detergent micelles (for a discussion, see ref. 12). As also with digitonin, the physical state of the membranes appears to be of importance[13], as we have found that fractionation is less satisfactory with washed chloroplast fragments.

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Stabilization of cardiac microsomal calcium uptake by hypertonic sucrose solutions

During storage in isotonic sucrose cardiac microsomes rapidly lose their ability to take up calcium, and most investigators have found almost complete loss of this activity after only a few days[1–4]. Sucrose density gradients have been used to obtain cardiac microsomes of greater stability, which has been attributed to removal of contaminants on the density gradients[5,6]. This interpretation may be incorrect, however, because the ability to take up calcium can be preserved simply by storage of the microsomes in hypertonic sucrose solutions such as those in which microsomes were recovered from the sucrose density gradients used in earlier studies.

Microsomes were prepared from canine ventricles by a slight modification of the standard method of this laboratory[6]. The muscle, obtained from dogs anaesthetized with pentobarbital, was homogenized for 60 sec at top speed in a Waring blender in 3–4 vol. of ice cold 10 % sucrose (w/v) containing 6 mM $MgCl_2$, and buffered with 20 mM Tris chloride at pH 8.0. After centrifugation for 5 min at 800 × *g*, the supernatant was filtered through 6 layers of gauze and centrifuged for 30 min at 15000 × *g*. The supernatant was again filtered through gauze and centrifuged for 60 min at