Chapter 16

Separation of the Two Pigment Systems

The two photochemical systems that we were led to postulate in the preceding chapters, must be closely integrated structurally and functionally with each other, and with at least three enzymatic "conveyor belts" operating in photosynthesis. Nevertheless, one can try to disintegrate photosynthetic organelles (chloroplasts) by mechanical (or chemical) means, in the hope of separating the two postulated systems, at least partially, from each other. Such fractionations have been, in fact, attempted with promising results. Their success can be judged by two criteria: *analytical*—that is, observing differences in chemical composition of the several fractions; and *functional*—by studying differences in their biochemical or photochemical activity.

Among early relevant observations is the finding that phycobilins (which form the bulk of pigment system II in red and blue-green alga) can be extracted into distilled water, leaving chlorophyll *a* in the cells.

Another early observation, by A. A Krasnovsky (in Moscow), and C. S. French (at Stanford), was that in green cells, a minor Chl component is preferentially bleached by strong red light. That a Chl component (Chl *a* 695) is preferentially damaged during ultrasonic disintegration of a suspension of *Chlorella* cells, particularly in an acid, aerobic medium, was already mentioned in Chapter 15.

Jan B. Thomas (at Utrecht, Holland) and Carl Cederstrand (in our

laboratory) noted that extracting chloroplasts with aqueous methanol or acetone of different strength, leads to fractions with different absorption and fluorescence spectra, suggesting preferential extraction of one of the two pigment systems. Extracts made with 10% methanol show similarity with unextracted chloroplasts—the red absorption band is located at about 675 nm and the fluorescence yield is about 3%. As the concentration of methanol goes up from 10 to 20 and 30%, the fluorescence maxima in the extracts are shifted towards the shorter waves, and the width of the fluorescence band increases. The greatest changes are shown by the fluorescence spectra at low temperature (77°K); for example, extracts in 10% methanol strongly emit the 698 nm band (tentatively attributed to the "trap" in pigment system II; see Chapter 15); while extracts in 20% methanol emit, at 77°K, about equally intense bands at 685 nm (system II), 695 nm (system II) and 710 nm (system I). The emission spectra of the extract in 30% methanol has a main peak at 670 nm (due to dissolved Chl *a*), another at 685 nm (bulk of Chl *a* in PSII) and some emission at 695 nm (trap in PSII) and 710 nm (PSI). Photochemically, the extracts in 10–20% solvent are as active in the Hill reaction (dye reduction in light) as the chloroplasts themselves. Provisional conclusion from these observations is that pigments belonging to PSII are extracted more easily, at lower solvent concentrations, than pigments contained in PSI.

Several attempts have been made to separate the pigment systems by physical means. One of the earliest ones, by Mary Belle Allen, involved repeated freezing and grinding, followed by sonication and a so-called "density gradient centrifugation." In this way, she obtained particles enriched in chlorophyll *b* and Chl *a* 670 (that is, in PSII). More successful have been recent studies by N. K. Boardman and Jan Anderson in Australia. They disintegrated spinach leaves in a blender, filtered the juice through muslin, and precipitated the material suspended in the filtrate by centrifugation at 100 × g.[1] After resuspension of the pellet, they added a detergent, digitonin, let it act for 30 minutes at 0°C, and fractionated the suspension by increasingly strong centrifugation—from 16 minutes at 1000 × g to 60 minutes at 144,000 × g.

The two extreme fractions—the "heavy" one (HF) precipitated already at 10,000 × g and the "light" one (LF) precipitated only at 144,000 × g,

[1] One g means centrifugal force equal to gravity force on the surface of the earth.

showed significant differences in composition (see Table 16.1). For example, the concentration ratio [Chl *a*] : [Chl *b*] was 2.3 in HF, as against 5.3 in LF. (We have concluded in earlier chapters that Chl *b* is relatively more abundant in PSII.) LF was richer than HF in P700 (the "PSI trap"). The concentration of cytochrome *b* was three times higher in HF, while the concentration of cytochrome *f* was somewhat higher in LF. (In scheme 14.4, we placed cyt b_3 close to PSII and cyt *f* close to PSI.) A significant difference in the ratio [carotenol] : [carotene] also was noted—3.3 in HF and 1.9 in LF—indicating that different carotenoids may be preferentially associated with the two systems. The content of iron was 1.5 times higher in LF than in HF. (PSI is supposed to be associated with the iron-containing enzymes ferredoxin, cytochrome b_6, and cytochrome *f*; while PSII is associated with only one such enzyme, cytochrome b_3.) Finally, the manganese content of HF was 5 times that of LF. Manganese is supposed to participate in the oxygen-liberating enzymatic reaction, sensitized by PSII. All this suggested preferential accumulation of PSI in LF and of PSII in HF.

Functional tests revealed a capacity of the heavy fraction to reduce the dyes di- or trichlorophenol indophenol in light, but little capacity for reducing pyridine nucleotide ($NADP^+$) with H_2O as the electron donor; the light fraction did reduce $NADP^+$ if reduced dichlorophenol indophenol was supplied as reductant, but did not reduce the oxidized form of dichlorophenol indophenol. This difference agrees with the assumption that HF contained more PSII and LF more PSI.

The *fluorescence yield* of HF was five times that of LF—and we recall that most chlorophyll fluoresence is ascribed to PSII. We found that the fluorescence of LF, excited by polarized light, to be more strongly polarized than that of HF (5.4% versus 2.7%); and we have seen in Chapter 15 that polarization is strongest in the long-wave emission band F720, probably originating in PSI.

We—as well as Boardman and co-workers—found that at 77°K, the LF emitted more strongly at 720 nm than at 695 nm, whereas the HF emission spectrum contained a strong 695 nm band, again in agreement with preferential assignment of PSII to HF, and of PSI to LF.

All these findings—summarized in Table 16.1—suggested that Boardman and Anderson's precedure does, in fact, lead to at least partial separation of PSI and PSII. The "light" fraction may contain mostly PSI, while the "heavy" one is significantly enriched in PSII (perhaps,

TABLE 16.1 Fractionation of Chloroplast Material

Property	Light Fraction	Heavy Fraction
Chl *a*/Chl *b* ratio	5.3	2.3
Relative concentration of cytochrome *b*	1	3
Carotenol/carotene ratio	1.9	3.3
Relative Fe content	1–5	1
Relative Mn content	1	5
Capacity for reduction of TCPIP (or DCPIP)	Low	High
Capacity for reduction of $NADP^+$ at the cost of reduced DCPIP	High	Low
Percent of polarization of fluorescence excited with polarized light	5.4	2.7
Quantum yield of fluorescence	Low	High
Emission spectra at 77°K	more F720	more F696

to about 70% of the total). Recently, a great deal of other detergents have been tried; one of the more successful ones was Triton X-100, used in L. P. Vernon's laboratory at Yellow Springs, Ohio. In one case, partial separation of the pigment systems was obtained even without the use of detergents.

Jean-Marie Briantais (at Gif-Sur-Yvette) was able to obtain partial reconstruction of the properties of the original chloroplast material when the separated particles of the two types were mixed together.