Photosynthesis Research 16: 285–289 (1988)

Historical corner

The discovery of chlorophyll-protein complex by Emil L. Smith during 1937–1941

After Emil L. Smith finished his PhD thesis in Photosynthesis (see Smith 1937) under Professor Selig Hecht, the founder of modern Biophysics in the U.S.A., in 1936, he turned to the question: is chlorophyll in plants associated with proteins? By that time, it was known that enzymes were proteins, rhodopsin was a protein and antibodies were proteins. Thus, this question was a natural one for him to ask (see Smith 1982). He knew that the aqueous extracts of leaves yielded only particulate suspensions, not solutions of chlorophyll. Working in a laboratory where behavior of rhodopsin was being studied daily, he noticed the similarity of the two problems and thus pioneered the use of the detergent digitonin (used for rhodopsin extraction) for extracting chlorophyll from ground-up leaves. To his delight, he obtained soluble preparations of chlorophyll that gave spectra resembling that of leaves, not solutions of chlorophyll in organic solvents (see the discovery paper: Smith 1938; appendix 1). This was the first demonstration that chlorophyll may be bound to a protein – the previous suggestions by others that chlorophyll might be bound to protein had been ignored until then and the material had never been characterized before (also see Rabinowitch 1945, 1951). Later, on a Guggenheim fellowship at Molteno Institute and working in the laboratory of David Keilin (Cambridge University, U.K.) (who is famous for his discoveries on cytochromes), Smith obtained firm evidence that chlorophyll was indeed associated with proteins (see Smith 1941). He, however, writes, 'my impression is that few took the results seriously and that this premature work had only little impact on the field' (see Smith 1982).

After returning to the U.S.A. in 1939, Smith collaborated with E.G. Pickels, who was then at the Rockefeller University and had an ultracentrifuge. It was the paper of Smith and Pickels (1941) that provided the final information that the chlorophyll-protein complex, studied thus far, had the size expected of a protein; they suggested that the protein contained 3 chlorophyll *a*, 1 chlorophyll *b* and some carotenoid molecules. It appears that they must have discovered the light-harvesting chlorophyll *a*/chlorophyll *b* complex. We now know how important these chlorophyll-protein complexes are for light-harvesting in photosynthesis (see Thornber 1986). It took almost fifty years before Nanba and Satoh (1987) isolated the reaction center chlorophyll-protein complex of the so-called Photosystem II form plants – their preparation contains cytochrome *b*-559 and polypep-

tides labeled D_1, and D_2, 4-6 chlorophyll *a* and 2 pheophytin molecules. And, this chlorophyll-protein complex is indeed capable of doing primary photochemistry, i.e., conversion of light energy into chemical energy.

University of Illinois GOVINDJEE
Urbana, IL

University of Illinois at Urbana-Champaign
Department of Plant Biology
289 Morrill Hall
505 S. Goodwin Avenue
Urbana, IL 61801 (USA)

Appendix 1. E.L. Smith (1938) Science 88: 170–171.

Solutions of chlorophyll-protein compounds (phyllochlorins) extracted from spinach

The differences in solubility, fluorescence and absorption spectrum between the green pigments in the leaf and the chlorophylls extracted in solvents such as alcohol have been ascribed either to dispersion of the green pigments in the leaf, or to adsorption or combination of the chlorophyll with lipoid or protein.[1] We have prepared aqueous solutions of the green pigments which show characteristic protein properties and which resemble the pigments in the leaf. To distinguish them from the chlorophylls we have adopted the name *phyllochlorin* for these chromoproteins, as suggested by Mestre.

Our extracts have been prepared using dilute aqueous digitalin, a solvent currently used for the photosensitive retinal pigments.[2] Ordinary leaf press juice or distilled water extracts show the green pigments not in true solution,[3] but in a fine suspension whose particles are visible under the microscope and can be retained on a fine filter.

About 100 gm of fresh spinach is thoroughly ground with fine sand, water is added to make 100 ml, and the suspension filtered through a coarse fluted filter. The moist cake is reground and again extracted. To the combined extracts is added 5 gm of Filter-Cel.[4] per 100 cc, and the whole is filtered through a thin layer of Filter Cel. on a Buchner funnel. The deep yellow-brown filtrate is discarded. The cake is washed in distilled water several times until the filtrate shows no trace of yellow color. It is then extracted with 25 ml of 1 or 2 per cent aqueous digitalin;[5] the result is a dark green solution which shows no trace of suspended material under an oil immersion

lens. Similar preparations can be made with 4 per cent purified bile salts. More dilute extracts are obtained in concentrated (40–50 per cent) urea solutions. Digitalin solutions of the phyllochlorin kept for some weeks in the cold room (5°C) show a little precipitated pigment which does not redissolve.

The absorption bands of the phyllochlorin (Fig. 1), measured with Shaler's spectrophotometer,[6] are like those of the leaf and are shifted towards the longer wave-lengths as compared with the natural mixtures of chlorophyll *a* and *b*.[7] The three bands are at 437, 470 and 675 mμ. The 420 and 660 mμ maxima of chlorophyll have about the same height, while for phyllochlorin solutions the 437 mμ maximum is always 60 per cent higher than the 674 band. This suggests the presence of carotenoids associated with phyllochlorin, such as French[8] found for the chromoprotein solutioins from photosynthetic purple bacteria.

Boiling a neutral digitalin extract shifts the red absorption band towards the shorter wave-lengths. When a solution is made strongly acid or weakly acid and boiled, the solutions turn yellow, corresponding to the formation of phaeophytins. A digitalin extract saturated with solid ammonium sulfate precipitates the phyllochlorin only after several days, but when boiled forms a bright green viscous mess. No pigment is lost on prolonged dialysis (about two weeks) of a digitalin extract, and only a part of the pigment precipitates. This precipitate, separated by centrifuging, does not readily redissolve in digitalin solution. The pigment which remains in solution is now readily

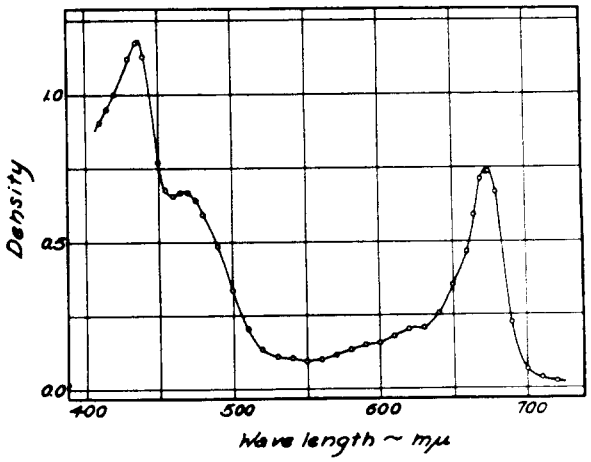

Fig. 1. The absorption spectrum of a phyllochlorin solution prepared with 2 per cent digitalin and diluted 1 to 10 with distilled water. The density values are for a 5 mm depth of solution.

precipitated with high concentrations of ammonium sulfate. Such precipitates are easily redissolved in digitalin solution but not in water. It is likely that the solvent action of the digitalin and the bile salts is due to the formation of coordination compounds which are not taken up even on prolonged dialysis. Phyllochlorin is precipitated and the chlorophyll extracted by strong alcohol, methyl alcohol or acetone but not by petroleum ether in agreement with the effects of these solvents on the leaf. Phyllochlorin solutions show a positive Biuret reaction.

In agreement with observations of the green leaf, phyllochlorin solutions show little or no red fluorescence when irradiated with blue light (436 mμ). This is in contrast with the strong red fluorescence of alcoholic chlorophyll solutions. Phyllochlorin solutions are quite stable to visible light.

The behavior of phyllochlorin solutions in strong centrifugal fields is being investigated in collaboration with Dr. E. G. Pickels[9] using an air-driven ultracentrifuge.[10] Preliminary studies show that the phyllochlorin when subjected to a force of 160,000 gravity can be sedimented completely through a 10 mm column of the liquid medium within three hours, leaving no color in the supernatant fluid. Our best preparation showed two sedimentation boundaries which correspond to particles of high molecular weight, i.e., above 70,000. The two boundaries retained their identity with respect to their sedimentation rates when studied by the light absorption method in the red and blue regions corresponding to the absorption maxima of phyllochlorin in the visible, and in the ultra-violet region characteristically absorbed by proteins. One boundary sedimented almost twice as fast as the other; these more rapidly moving and presumably heavier particles showed a greater total absorption in each of the two regions of the visible spectrum than did the smaller particles.

It is tempting to assume that these two proteins correspond with phyllochlorins *a* and *b*. The similarity of sedimentation properties throughout the spectrum indicates that the additional blue absorption is characteristic of the phyllochlorins and not of some other component.

It now appears that the classical organic chemical studies of the chlorophylls and carotenoids were concerned with the prosthetic groups of extremely complex specific catalysts, perhaps analogous to the hemoglobins and enzymes such as cytochrome, catalase and the yellow respiratory enzyme. Presumably there are many additional components concerned in photosynthesis, since phyllochlorin does not carry on photosynthesis in vitro.

Laboratory of Biophysics,
Columbia University

Emil L. Smith

References

Nanba O and Satoh K (1987) Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome b-559. Proc Natl Acad Sci USA 84: 109–112

Rabinowitch E (1945) Photosynthesis and related processes. Volume I. New York: Interscience Publishers, Inc

Rabinowitch E (1951) Photosynthesis and related processes. Volume II, Part I. New York and London: Interscience Publishers, Inc

Smith E L (1937) The influence of light and carbon dioxide on photosynthesis. J Gen Physiol 20: 807–830

Smith E L (1938) Solution of chlorophyll-protein compounds (Phyllochloris) extracted from spinach. Science 88: 170–171

Smith E L (1941) The chlorophyll-protein compound of the green leaf. J Gen Physiol 24: 565–582

Smith E L (1982) The evolution of a biochemist. In: G. Semenza, *Of Oxygen, Fuels, and Living Matter*, Part 2, pp. 361–445. Chichester, New York, Brisbane, Toronto, Singapore: John Wiley and Sons

Smith E L and Pickels E G (1941) The effect of detergents on the chlorophyll-protein compound of spinach as studied in the ultracentrifuge. J Gen Physiol 24: 753–764

Thornber J P (1986) Biochemical characterization and structure of pigment-proteins of photosynthetic organism. Encyclopedia of Plant Physiology New Series 19: 98–142

Notes

1. Reviewed by H. Mestre in 'The Investigation of the Pigments of the Living Photosynthetic Cell', in: Contributions to Marine Biology, Stanford University Press, 1930
2. e.g., K. Tansley, *Jour Physiol* 71: 442, 1931; A. M. Chase and C. Haig, *Jour Gen Physiol* 21: 111, 1938; G. Wald, *Nature* 140: 545, 1937
3. T.B. Osborne and A.J. Wakeman, *Jour Biol Chem* 42: 1, 1920. Such suspensions have also been studied by V. Lubimenko, *Rev Gen de Bot* 39: 547, 1927; and B. Hubert, *Rec trav bot néerl* 32: 324, 1935
4. Filter-Cel., Johns-Manville, New York
5. Crystalline digitalin, Eimer and Amend, New York
6. S. Shaler, *Jour Opt Soc America* 28: 18, 1938
7. c.f. E. Rabinowitch and J. Weiss, *Proc Roy Soc London* A: 162: 251, 1937
8. C.S. French, Abstract in the Proceedings of the American Society of Biological Chemists, Baltimore meeting, March 30–April 2, 1938
9. Of the Laboratories of the International Health Division, The Rockefeller Foundation, New York
10. J.H. Bauer and E.G. Pickels, *Jour Exp Med* 65: 565, 1937