

Chlorophyll-Water Interactions in the Solid State

A MARKED, reversible effect of water on the absorption spectrum of crystalline chlorophyll *a* in 'Nujol' mull has recently been observed¹. Thin films of such mulls, dried by prolonged evacuation or standing over phosphorus pentoxide, absorb at 672 nm, but this absorption shifts to about 743 nm when the film is exposed to water, or if the chlorophyll contains water of crystallization. The infrared spectra of the wet and dry films are correspondingly different¹. The 743 form shows only a single carbonyl absorption at 1,647 cm^{-1} , similar to the band assigned to an "associated" carbonyl at the C(9) position in dissolved chlorophyll², while the 672 species exhibits an additional infra-red peak at 1,698 cm^{-1} , which has been assigned to a "free" carbonyl. The intensity of this band is about two-thirds that of the 1,647 cm^{-1} peak. This suggests² that the 672 form contains dimers or other stoichiometrically associated forms. The differences in spectrum (both red and Soret bands) between the 672 species and the chlorophyll *a* dimer definitely identified in solution³ may be attributable to effects of the medium or to exciton interactions in the solid phase. It is remarkable, however, that dimers in non-polar solvents are disaggregated by addition of small concentrations of Lewis bases (ethanol, pyridine, water)^{2,4}, whereas in these chlorophyll crystal mulls, moisture increases the extent of dye interaction, as indicated by the bathochromic shift of the optical spectrum and, presumably, by the disappearance of the "free" carbonyl band. The water effect in solid chlorophyll has therefore been studied further, particularly to determine whether the 672-743 transition is sharp or whether intermediate forms can be obtained. Such intermediate forms are reported here.

Chlorophyll *a*, prepared from fresh spinach⁵, was brought into crystalline form by evaporation from solution in an ether layer on water. The material consisted of brilliant, purple microcrystals, and gave very sharp X-ray patterns agreeing with those of Donnay⁶. 'Nujol' mulls, spread evenly in thin films on 'Pyrex' plates, were equilibrated in absorption cells at various vapour pressures of water, controlled by connexion to water reservoirs which were maintained at temperatures lower than the films. The cell-reservoir assembly could be evacuated on the vacuum line and the cells closed off and removed for determination of the spectrum.

The position of the main red absorption band is found to depend on the water vapour pressure, the temperature of the film and equilibration time. The conditions in the previous work, in which the film was placed over water in a closed desiccator, were such that liquid water condensed on the film surface, thereby completely shifting the absorption from 672 to 743 nm. With more careful control of

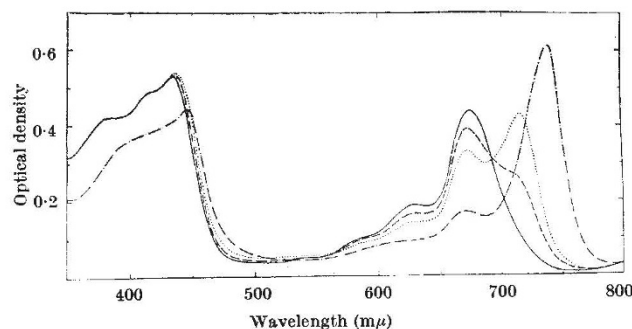


Fig. 1. Visible spectra of films of chlorophyll *a* suspended in paraffin oil: —, film in the presence of excess water; - - -, film evacuated with high vacuum for 2 h; ····, film exposed to vapour pressure of 8-9 mm for 2 h, at about 20° C; — · — ·, film exposed to vapour pressure of 8-9 mm for total of 43 h, at about 20° C.

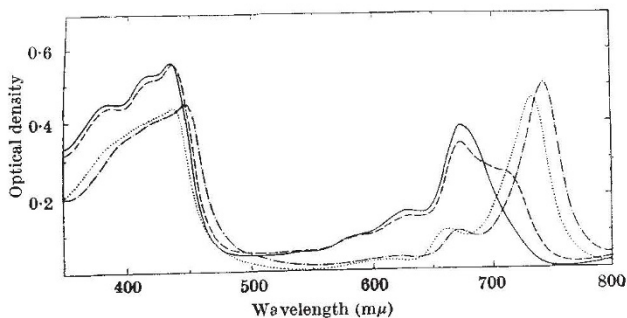


Fig. 2. Visible spectra of films of chlorophyll *a* suspended in paraffin oil: —, film dried by evacuation for 2 h; - - -, film exposed to vapour pressure of 15.5 mm for 4 h, at 27° C; ····, film exposed to vapour pressure of 15.5 mm for total of 26 h, at 27° C; — · — ·, film exposed to vapour pressure of 15.5 mm for total of 50 h, at 27° C.

hydration of the dry film, new absorption peaks have been observed at 716 and 733 nm (Figs. 1 and 2). In other experiments in which diffusion was much slower, peaks at 722 and 736 nm have been found. While the complicated vapour pressure, temperature and time dependence of the spectral shift have not yet been fully established, it is evident that a continuous shift in the visible spectrum between the extreme limits of 672 (dry) and 743 (wet) may be obtained. The spectrum can always be reversed back to 672 nm, from any stage of hydration, by drying on the vacuum line, or over phosphorus pentoxide.

The spectrum of the water-saturated 743 form of chlorophyll *a* is practically identical with the limiting spectrum of ethyl chlorophyllide *a* microcrystals of increasing size, prepared by dilution of acetone solutions with water⁷. The correlation between the magnitude of the spectral shift and the size of the chlorophyllide microcrystals⁸ suggests that, in the chlorophyll-'Nujol' mulls, hydration controls the spectral shift (Figs. 1 and 2) in the same way, by controlling the size of the aggregate in which effective intermolecular dye interactions can occur, or by influencing the degree of coupling between the dye molecules. Either effect could result from a "tightening" of the crystal structure by added water. The mechanism may resemble the large red-shift in chlorophyll *a* "monolayers", produced by the presence of calcium ions⁹. The involvement of water in the crystallization of chlorophyll has also been noted earlier⁵. The manner in which hydration leads to disappearance of the "free" carbonyl absorption is still not clear.

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