Technical Papers

Absorption Spectra of Chlorophylls in Solutions at Low Temperatures—Equilibria between Isomers[1]

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The refinement in spectra that accompany a reduction in temperature prompted us to measure the absorption spectra of solutions of chlorophyll *a*, chlorophyll *b*, and chlorophyll *b'*[2] over a range of temperatures from room temperature (300° K) to that of liquid nitrogen (75° K), somewhat below the boiling point.

The spectra of the solutions of chlorophylls *a* and *b* at room temperature were the same as have been accepted in the literature (*1*). That of chlorophyll *b'* had been found (*2*) to be practically indistinguishable from the spectrum of chlorophyll *b*, and with this description our spectra at room temperature are in agreement except that in the spectrum of chlorophyll *b'* 4 weak bands in the ultraviolet region are superimposed on a greater general absorption than is exhibited in this region by chlorophyll *b*.[3]

The solvent employed for all the chlorophylls at low temperatures[4] consisted of about 20% by volume di-*n*-propyl ether, 40% propane, and 40% propene, and the solvent for temperatures above 230° K consisted of 20% of the ether and 80% *n*-hexane.

In general, it may be observed from Figs. 1 and 2 that the spectra of the solutions are shifted toward longer wavelengths as the temperature is reduced and concurrently new band maxima make their appearance or become more noticeable. In each spectrum the strong absorption peak in the blue region and the neighboring structure toward shorter wavelengths are most responsive to changes in temperature. The weak peaks of chlorophyll *a* on the short wavelength side of the prominent red absorption are also temperature-sensitive. Although at first glance the spectra of the chlorophylls at 75° K differ from their spectra at 230° K chiefly by a shift in wavelength, most of this shift was found to be apparent only, since at intermediate temperatures one spectrum disappears and at its expense a similar spectrum makes its appearance—toward longer wavelengths as the temperature is reduced. Figs. 3 and 4 show the change of the blue bands on an enlarged wavelength scale.

Since the structures of the spectra of the high and low temperature modifications are closely similar, they may be ascribed to isomers that appear to be in equilibrium. The same spectra were observed at a given temperature with both falling and rising temperatures.

However, the changes in the form of the spectra of chlorophyll *a* are not so sharply differentiated as are those of chlorophylls *b* and *b'*. The latter two, barely distinguishable at room temperature and also at our lowest temperature, 75° K, show large differences at intermediate temperatures because the energies of the isomeric transformations have proved to be substantially unequal. The isomers of chlorophyll *b* coexist in equal amounts at 180° K (Fig. 3), whereas those of chlorophyll *b'* reach equality at 230° K (Fig. 4). The isomers of chlorophyll *a* are present in equal concentrations at about 180° K.

Several definite structural features in the spectra of chlorophyll *b* varied with different preparations, made in supposedly the same way (Fig. 2). Especially marked were the extent and sharpness of the shoulder on the long wavelength side of the blue peak—at 4,815 A (77° K). Accompanying this was a small band on the short wavelength side of the red peak—at 6,300 A (77° K). Along with these features the shape of the peak at 4,500 A varied with preparation.

Experiments were undertaken to discover whether such differences could possibly be experimental artifacts. For example, the thermal treatment of the solution was varied from sudden quenching to extremely slow cooling from room temperature to 77° K. On the possibility that in the evaporation of the original solvent the ethyl ether may not have been completely removed, quantities of di-ethyl ether were added to the di-*n*-propyl ether. All our trials ended with solutions that continued to give identical spectra. The spectrum of pheophytin was also examined to eliminate it as a

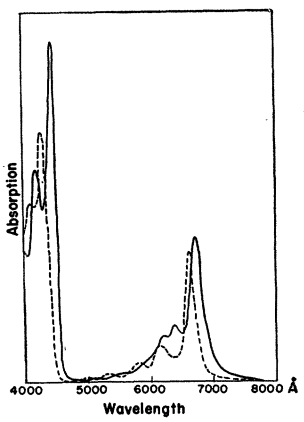

FIG. 1. Visible absorption spectra of solutions of chlorophyll *a* at 230° K (- - - - - - - -) and at 75° K (————). Concentrations are different at the two temperatures.

[1] Work was performed under the auspices of the Atomic Energy Commission.

[2] The chlorophylls were prepared and purified by Robert Livingston and his associates at the University of Minnesota (ONR Project N 60 ri-212 Task Order I). We are deeply grateful to them for their assistance and cooperation throughout the progress of this work.

[3] We learned from a private communication that Robert Livingston and his associates had previously observed some difference in the ultraviolet spectra at room temperature.

[4] For method of preparing solutions, see S. Freed and C. J. Hochanadel, *J. Chem. Phys.*, **17**, 664 (1949).

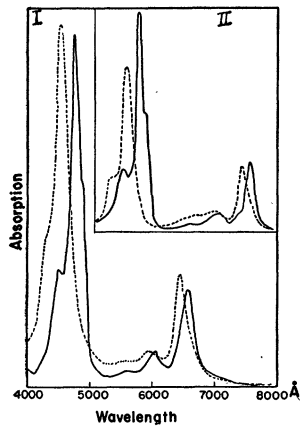

FIG. 2. Visible absorption spectra of solutions of chlorophyll *b* at 230° K (- - - - - - - -) and at 75° K (————) for preparations I and II. Concentrations are different at the two temperatures.

possible impurity. Finally, with the cooperation of Professor Livingston, the purified chlorophyll *b* on the chromatographic adsorption column was removed in 3 fractions. The spectra of these fractions succeeded only in showing that the lowest fraction on the column consisted of considerably more chlorophyll *b'* than had been suspected, whereas the other 2 fractions gave the same spectra at each temperature. Neither of them furnished the shoulders and associate features as sharply and definitely as had been exhibited by a preparation of chlorophyll *b* made several months earlier. We are forced to conclude that there exists at least one other component in chlorophyll *b*, with a probably similar spectrum that has hitherto not been isolated by this process.

Insufficient work has been done to determine the nature of these isomers. The accepted chemical structures of the chlorophylls allow for a number of possible isomers: first, in the enol-keto forms and, second, in the different mutual configurations about the 3 asymmetric carbon atoms. The rate of transformation of one isomer of chlorophyll *b* into the other was rapid even at the lowest temperature 150° K at which the new isomer was first detected as the temperature was raised. At any fixed temperature no increase in its concentration was noted with time. However, a rise in temperature brought in at once an increase in the intensity of the spectrum of the high temperature form.

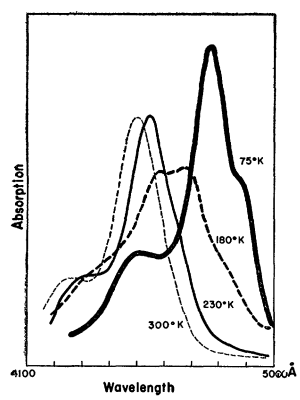

FIG. 3. Changes with temperature of the absorption spectra of the isomers of chlorophyll *b* in solution.

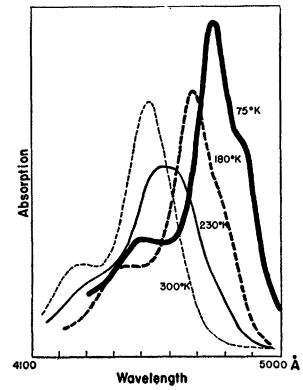

FIG. 4. Changes with temperature of the absorption spectra of the isomers of chlorophyll *b'* in solution.

As the spectrum of the low temperature form begins to grow in, it modifies the over-all spectrum in a way that suggests the differences in appearance at room temperature of the spectra of chlorophylls in different solvents, especially in the 4,100 A to 4,300 A region (*3*). It is to be expected that the character of the solvent affects the energies of the isomeric transformations, especially if they are of the enol-keto type, and hence we are led to ask whether the difference in the spectra of the chlorophylls in different solvents may not be largely due to the change in the relative concentrations of the isomers present. Similarly, the well-known change in color of chlorophyll when it is adsorbed may to an appreciable degree be a shift in the equilibrium between the isomers induced by the adsorption process.

References

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The Pulmonary Circulation as a Source of Leucocytes and Platelets in Man

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The intravenous administration of epinephrine in man is followed by an immediate leucocytosis and thrombocytosis, the source of which has been variously attributed to the spleen and/or the bone marrow

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