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Abstract

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ON THE STATE OF WATER IN THE CHLOROPHYLL MOLECULE

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The question of the form in which water enters the chlorophyll molecule is of great interest for understanding the mechanism of participation of this pigment as a sensitizer in the process of photosynthesis. It is considered that the chlorophyll molecule is hydrated and, in the case of component *a*, has the composition $C_{55}H_{72}O_5N_4Mg \cdot {}^1/_2H_2O$ (^{1}).

The hypothesis of the direct interaction of chlorophyll with water in photosynthesis is associated with the idea of the hygroscopicity of the pigment. It is known that the hygroscopicity of chlorophyll preparations depends to a considerable extent on the presence of contaminants of a lipoid character. The possibility of obtaining dry chlorophyll has been indicated (^{2}). At the same time, there also existed the opinion that chlorophyll possesses zeolitic properties, i.e., the ability reversibly to attach and give up water (^{3}).

To what extent pure chlorophyll preparations are hygroscopic and whether water is present in stoichiometric proportion—these questions could be answered only on the basis of new experimental data. The methodological error of this kind of experiment must be less than one percent, since the presence of ${}^1/_2M$ H_2O per 1 mole of chlorophyll corresponds to 1% by weight.

Comparatively recently, one of us showed (^{4}) that thoroughly purified and dried chlorophyll preparations contain no more than 0.6% moisture. Data on the isotopic exchange of hydrogen atoms of chlorophyll with D_2O showed that the process of deuterium exchange proceeds slowly (^{5,6}): in 24 hours at 25° the degree of exchange was 20%, calculated per one exchangeable hydrogen atom. If one assumes that the observed exchange occurs at the expense of the hydrogen atoms of water stoichiometrically bound to the chlorophyll molecule, then in our case (^{6}) the percentage of exchange would correspond to 10^{-2}–10^{-3} mole of exchangeable water.

	In light	In darkness
Isotopic exchange, %	6.7	0.7

Figure 1

Figure 1: Figure 1

	In light	In darkness
Number of moles of D_2O per 1 mole of chlorophyll	$3.3 \cdot 10^{-2}$	$3.6 \cdot 10^{-3}$

Thus, the isotopic-exchange data indicated either the absence of water in the chlorophyll molecule (more than 10^{-2} mole), or the absence of labile bound water. It could then be assumed that the amount of firmly bound water by weight is less than 0.6%. Refinement of the method of quantitative analysis of chlorophyll by determining magnesium from a weighed portion of chlorophyll with Trilon B reduced, for our preparations, the possible percentage of water entering the chlorophyll molecule to 0.4%.

In this connection, the possibility of determining the forms of water in chlorophyll by studying the infrared absorption spectra of the pigment was of great interest. The sensitivity of the IR method with respect to water absorption is $\sim 0.1\%$.

For this work we used preparations of chlorophyll *a*, *a* + *b*, and *b*, isolated from nettle (*Urtica urens*) by the method described previously (^{7}). The purity of the chlorophyll preparations used, with respect to magnesium and nitrogen, was $99.9 \pm 0.4\%$.

The infrared absorption spectra of solid films of chlorophylls *a*, *a* + *b*, and *b*, obtained by evaporating their ethereal solutions on silvinite plates, as well as of potassium bromide tablets with pressed-in

with pigment were measured on a UR-10 two-beam spectrophotometer (Zeiss, Jena) in the region 3700-700 cm^{-1}.

At present it is very difficult to give a complete interpretation of the complex vibrational absorption spectrum of chlorophyll and its derivatives. Nevertheless, among the reliably interpreted vibrational bands one should include the absorption bands lying in the region 3650-3200 cm^{-1} (the region of stretching vibrations of the OH group), and also in the region 1800-1600 cm^{-1} (stretching vibrations of C=O and C=C bonds) (^{8}).

Fig. 1. Absorption spectra of a solid film of chlorophyll a: **I** —before vacuum pumping; **II** —after vacuum pumping to $5 \cdot 10^{-6}$-10^{-5} mm Hg and heating at 55-60°; **III** —10 h after admission of air.

In the spectra of solid films of chlorophylls a, a + b, b in the region 3600-3200 cm^{-1} there is observed a broad asymmetric absorption band, whose intensity

Figure 2

Figure 2: Figure 2

increases from chlorophyll a to chlorophyll b. It was previously suggested ([9]) that this band may be attributed to absorption by liquid water, bound by a hydrogen bond to the nitrogen atoms of the pyrrole nuclei of the porphyrin ring. In this connection it was of interest to determine the strength of this interaction, proceeding from the ease of removal of the water bound by the pigment. Solid films of the pigments under study were placed in a vacuum cell, pumped down to high vacuum ($P = 5 \cdot 10^{-6}$-10^{-5} mm Hg), both at room temperature and with continuous heating to 55-60° for one and a half hours. Vacuum treatment of a chlorophyll-a film at room temperature led to partial removal of bound water, which was reflected in a decrease in the intensity of absorption at 3500-3200 cm^{-1} and the appearance of a slight structuring in this region (Fig. 1). Further pumping with heating of the same film caused an almost complete disappearance of absorption in the region 3500-3200 cm^{-1}, which corresponds to the complete disappearance of water, and the appearance of a distinct narrow band with a frequency of 3495 cm^{-1}, which can be interpreted as absorption of the OH-enol form of the cyclopentanone ring, linked by an intramolecular hydrogen bond with the keto group at C_{10} (Fig. 1). Admission of air into the cell immediately caused a slight broadening of the 3495 cm^{-1} band on the low-frequency side, while prolonged exposure of the pigment to air (up to 10 h) led to a practically reversible restoration of the initial absorption intensity in the spectral region under consideration.

Fig. 2. Absorption spectra of a solid film of chlorophyll b. **I** —before vacuum pumping; **II** —after vacuum pumping to $5 \cdot 10^{-6}$-10^{-5} mm Hg and heating at 55-60°.

Analogous experiments carried out with a film of chlorophyll b did not reveal a noticeable change in absorption (Fig. 2).

The result of vacuum treatment of a chlorophyll a + b film was only a small decrease in absorption intensity with the appearance in the region of 3500 cm^{-1} of a noticeable peak. Consequently, in chlorophyll b water is bound considerably more strongly to the pigment molecule. Similar reversible changes—

Changes in absorption also occurred in the region 1800-1600 cm^{-1}. In the spectrum of solid films of chlorophylls a and $a+b$, four absorption bands appear, interpreted as follows [9,10]:

1735 cm^{-1}—stretching vibration	$(C{=}O)$ at C_7 and C_{10}
1700 cm^{-1}—""	$(C{=}O)$ at C_9
1675—1665 cm^{-1} ""	$(C{=}O \ldots H{-}O{-})$
1610 cm^{-1}—""	$(C{=}C)$ of the enol form

In the spectrum of chlorophyll b, in addition, one more band is observed, with a frequency of 1690 cm^{-1}, apparently belonging to the ($C{=}O$) of an aldehyde group.

Vacuum conditioning of pigment films causes the following changes in the spectral region under consideration. Pumping down to $5 \cdot 10^{-6}$ mm Hg at room temperature for one and a half hours leads to a slight decrease in the intensity of the bands with frequencies of 1665 cm^{-1} ($C{=}O \ldots H{-}O{-}$) for chlorophyll a and 1675 cm^{-1} ($C{=}O \ldots H{-}O{-}$) for chlorophyll $a + b$. Pumping with heating at 55-60° leads to a further decrease in the intensity of these bands, as well as to a slight increase in absorption in the region of 1700 cm^{-1} ($C{=}O$ at C_9) (Fig. 1). Admission of air eliminates the effect of changes in the intensities of the bands.

Vacuum conditioning of a chlorophyll b film does not cause noticeable changes in this region of the spectrum (Fig. 2).

Thus, the reversible changes in absorption in the regions 3600-3200 cm^{-1} and 1700-1600 cm^{-1}, as well as the appearance of a narrow band with a frequency of 3495 cm^{-1}, indicate the following: 1) chlorophyll a and chlorophyll b partially exist in the enol form (bands 3495 cm^{-1}$-\nu$(OH) and 1610 cm^{-1}$-\nu(C{=}C)$), with the intensity of the band at 1610 cm^{-1} being greater for chlorophyll a than for b; 2) for chlorophyll a, the possible site of adsorption of water by the pigment is, apparently, the oxygen atoms of the cyclopentanone ring (reversible change in the intensity of the absorption bands with frequencies of 1665 cm^{-1} for ($C{=}O \ldots H{-}O{-}$) and 1690 cm^{-1} for ($C{=}O$) at C_9); 3) the smaller effect in the case of chlorophyll $a+b$ is due to the presence of the b component, for which no changes in absorption were detected; 4) we suppose that the water molecule is bound to chlorophyll b more strongly, and possibly also has a different type of interaction, which may be caused by the presence of the aldehyde group in the molecule of this pigment.

It is likely that one cannot speak of a stoichiometric inclusion of 1/2 mole of H_2O in chlorophyll.

In connection with the established fact that the forms of water differ in chlorophylls a and b, it is of interest to note their different ability to form microcrystals when the pigments are isolated from aqueous solutions [11,12]. As is known, amorphous preparations are isolated from anhydrous solutions, which was confirmed by X-ray examination of our preparations of chlorophyll a and $a + b$.

When water is added and aqueous solutions are evaporated, chlorophyll a with a crystalline structure is obtained, which is not observed for chlorophyll b. Whether water enters into the crystalline structure of chlorophyll a remains an open question for the present.

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