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Fig. 1

Figure 1: Fig. 1

Abstract

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MOLECULAR COMPOUNDS OF CHLOROPHYLL WITH CERTAIN SALTS

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There are a number of data indicating the ability of chlorophyll to form molecular complexes with polar compounds [1–6]. Some authors assume [4] that the cyclopentanone ring, which behaves actively in a whole series of chemical reactions, is responsible for the formation of these molecular compounds. Others believe [1–3] that the attachment of polar molecules takes place at the central magnesium atom. It must be thought that the nitrogen atoms of the pyrrole rings, which are centers of localization of electron density in the chlorophyll molecule, should also play a major role in the formation of addition products. This is evidenced by data recently obtained in our laboratory [7–8], from which it is seen that chlorophyll *a* forms molecular complexes with such electrophilic agents as ferric chloride and stannous chloride.

Fig. 1. Changes in the absorption spectrum of an acetone solution of chlorophyll *a* upon its interaction with $AgClO_4$. **1** –spectrum of the initial solution of chlorophyll *a*; **2** –the same after introduction of $AgClO_4$; **3** –spectrum of the molecular complex of chlorophyll *a* with $AgClO_4$ in the region λ 440–490 mμ; **4** –spectrum of the molecular complex of pheophytin *a* with $AgClO_4$ in the region λ 440–490 mμ.

As follows from what is set forth in the present article, analogous compounds with chlorophyll (and pheophytin) are also formed by other chlorides–$HgCl_2$, $BeCl_2$, $AlCl_3$, $BiCl_3$, and $NbCl_5$, as well as by silver perchlorate. These compounds can be isolated in the solid state. Direct analysis, carried out for the chlorophyll complex with ferric chloride isolated in this way, showed that it has a composition of 1:1.

The interaction of all the salts listed with chlorophyll leads to deformations of the absorption spectrum of the pigment. In Fig. 1 these spectral changes are illustrated by the example of the interaction of chlorophyll *a* with $AgClO_4$ in acetone. As can be seen from Fig. 1, the resulting spectrum preserves, in the main, the structure characteristic of dihydroporphyrins. The number of bands and their mutual arrangement are the same as in chlorophyll; however, their

Fig. 2. Reversibility curves of the intermolecular interaction of pheophytin a with water. 1 —absorption curve of the initial solution of pheophytin a; 2 —the same after introduction of $SnCl_2$; 3 —the same after an additional addition of 2% water

Figure 2: Fig. 2. Reversibility curves of the intermolecular interaction of pheophytin a with water. 1 —absorption curve of the initial solution of pheophytin a; 2 —the same after introduction of $SnCl_2$; 3 —the same after an additional addition of 2% water

positions are different. Especially strongly shifted toward the short-wavelength side are the two most intense bands of chlorophyll. The sharp decrease in the intensity of the red band and the strong increase in the intensity of the Soret band are striking.

The spectra of molecular complexes with the above-listed chlorides, within the limits of measurement error, differ little from one another and from the spectrum shown in Fig. 1 (curve 2). A characteristic feature of the observed spectral changes is that they are detected in the presence of small amounts of salts in the solutions. Thus, when silver perchlorate acts on chlorophyll a (at a concentration on the order of 10^{-5} mole/l), at a concentration not less than $1 \cdot 10^{-4}$ mole/l the equilibrium is completely shifted toward formation of the molecular complex. At lower salt concentrations the solution contains both chlorophyll and the molecular complex. This made it possible to determine the absorption coefficients for the maxima in the spectra of the molecular compounds we studied. In doing so, we assumed that each molecule of the complex contains one molecule of chlorophyll*. Table 1 gives the corresponding data for the molecular compounds of chlorophyll with silver perchlorate and stannous chloride. As is seen from Table 1, the absorption coefficients of these two compounds differ little from one another. It should be noted that these figures were highly reproducible in a series of repeated measurements.

Fig. 2. Reversibility curves of the intermolecular interaction of pheophytin a with water. **1** —absorption curve of the initial solution of pheophytin a; **2** —the same after introduction of $SnCl_2$; **3** —the same after an additional addition of 2% water.

Table 1

	I	I $\varepsilon \cdot$	II	II $\varepsilon \cdot$	III	III $\varepsilon \cdot$	IV	IV $\varepsilon \cdot$	V	V $\varepsilon \cdot$
	λ, m	10^{-4}	λ, m	10^{-4}	λ, m	10^{-4}	λ, m	10^{-4}	λ, m	10^{-4}
Chlorophyll a	663	13.9	617	2.76	580	1.59	535	0.92	430	17.3
$AgClO_4$ · 3Chl	553	7.85	600	1.61	565	1.51	532	1.66	418	32.0
$SnCl_2$ · 6Chl	553	7.06	600	1.82	565	1.80	532	1.94	418	30.0

The absorption spectra of the products of the interaction of pheophytin with the corresponding salts are also very close to those considered. It should, however, be noted that the "chlorophyll product" has stronger absorption in comparison with the "pheophytin product" in the 440–500 mμ region of the spectrum, as shown by the curves in the upper left corner of Fig. 1. This seemingly small difference in their spectra is quite distinct, however, in the color of the solutions of the corresponding products: the "chlorophyll product" is green, while the "pheophytin product" has a pale bluish tint.

In chlorophyll b and pheophytin b, changes in the spectra are also observed upon addition of the same salts (Table 2).

* This assumption was subsequently confirmed by data from direct analysis.

The changes described above in the spectra of chlorophyll and pheophytin are observed not only in acetone, but also in other solvents (methanol, ethanol, benzene, benzyl alcohol, etc.). The fact that, upon considerable dilution, no deviations from the Lambert–Beer law are observed indicates that the complexes formed are sufficiently stable. It is characteristic, however, that in the presence of a small amount of moisture the complexes decompose, with regeneration of the original pigment. Such a reverse process is observed especially clearly when traces of water are added to the corresponding solution (Fig. 2). The high sensitivity of the interaction reaction to traces of moisture imposes rather stringent requirements on the conditions for carrying out the experiments: the need to use anhydrous salts, to make the solvents as absolute as possible, and to carry out the experiments under conditions excluding the penetration of atmospheric moisture into the reaction volume. Most of the experiments were carried out under vacuum conditions according to the method of (7).

Table 2

Positions of the absorption bands of pheophytin b and of its complex with $HgCl_2$ in acetone

	Wavelengths at absorption maxima, mμ	Wavelengths at absorption maxima, mμ	Wavelengths at absorption maxima, mμ	Wavelengths at absorption maxima, mμ	Wavelengths at absorption maxima, mμ
	I	II	III	IV	V
Pheophytin b	660	605	–	530	428

Molecular complex of pheophytin b with $HgCl_2$	651	590	555	525	423

If the experiments described on the action of moisture testify to the reversibility of the processes of interaction of chlorophyll with salts, then the behavior of the solutions under consideration during prolonged storage under vacuum conditions indicates the slow occurrence in them of irreversible processes of replacement of the central atom in the pigment molecule by the corresponding metals. Fig. 3 illustrates a process of this type using as an example the interaction of chlorophyll a with $AgClO_4$*. Analogous processes of transformation of complexes of the type under consideration into pheophytins of the corresponding metals were observed previously in the interaction of chlorophyll a with ferric chloride and stannous chloride (7, 8). Thus, the previously expressed idea is confirmed that the formation of molecular complexes of chlorophyll with salts constitutes an intermediate, pre-reaction stage of the processes of replacement of the central atom in the pigment molecule. In general form the process under consideration may be written as follows: $MgPh + MCl_2 \quad MgPh \cdot MCl_2 \rightarrow MPh + MgCl_2$.

The first stage of the process proceeds rapidly, immediately after mixing the components. The rate of the second depends on the nature of the reacting salts and of the solvent. Thus, replacement of Mg by Hg or Be takes place within several minutes. For Fe, Sn, and Ag this process takes days. It has not been possible to establish the introduction of Bi and Al ions into the chlorophyll nucleus at all. The introduction processes proceed faster in methanol than in acetone, and are not observed at all in benzene solutions. According to the data for the second stage of the process, an essential role is played by the electrical properties of the solvent and the magnitude of the effective radii of the corresponding ions.

The interaction of chlorophyll with salts in benzene solution, as noted above, is accompanied by the same deformations of the spectrum that are characteristic of acetone solutions. However, in this nonpolar solvent the solubility of the primary products of interaction that are formed is very low, and they rapidly precipitate completely. This feature of benzene solutions was used to isolate the molecular compounds in pure form and to determine their qualitative and quantitative composition. Although the isolation of complexes in the solid state was observed by us in the interaction of the pigment with most of the salts studied, obtaining a product suitable for analysis proved possible

* The spectrum of the final product (curve 3 in Fig. 3) was identified with the spectrum of silver pheophytin previously obtained in our laboratory by M. S. Ashkinazi and V. E. Karpitskaya.

Figure 3

Figure 3: Figure 3

only in the reaction of chlorophyll (and pheophytin) with $FeCl_3$. In the other cases the task was complicated by the difficulty of washing the products free of excess salts.

The thoroughly purified product of the interaction of the pigments with $FeCl_3$ was a powdery blue substance. It was completely insoluble in benzene, ether, and acetone, in which the free pigment is readily soluble. However, it is fairly well soluble in slightly acidified acetone and in a mixture of benzene with acetone (1 : 5).

Determinations of the composition of the complex in four parallel experiments gave the following molar ratios: Chl : $FeCl_3$ = 1 : 1.02; 1 : 1.5; 1 : 1.1; 1 : 1.13. For the pheophytin–$FeCl_3$ complex, the following ratios were obtained, respectively: 1 : 0.94; 1 : 0.88 (in three experiments); 1 : 0.85. On the basis of these data we assume that the molecular ratio of the pigments and $FeCl_3$ in the products formed by the interaction is equal to 1 : 1.

Fig. 3. Changes in the spectrum of a methanolic solution of chlorophyll *a* during its reaction with $AgClO_4$.
1 —absorption curve of the initial solution; 2 —the same immediately after addition of $AgClO_4$; 3 —the same after storage of the solution in the presence of $AgClO_4$ for 3 days.

On the question of the nature of the bond between metal salts and chlorophyll in the molecular complexes under consideration, only preliminary judgments can be expressed. It is most probable that the salt molecule (an electrophilic agent) attaches to chlorophyll at one of the nitrogen atoms of the pyrrole rings, with one of the unshared electron pairs being used for the bond. This is indicated, in particular, by the fact that in the compound formed the bond between Mg and the nitrogen atoms is weakened, which is the reason for the easy replacement of the magnesium atom by the metal of the attached salt. Attachment of the salt molecule to chlorophyll must inevitably lead, as it were, to a "pumping" of an electron within the molecular compound and, consequently, to a redistribution of the electron density in the conjugated π-bond system of the porphyrin skeleton. The changes observed experimentally in the spectrum upon formation of the molecular complex are connected with this. The attachment of the salt molecule affects the conjugated system not directly, but inductively through the nitrogen atoms, which, it should be assumed, explains why the observed deformations of the spectra are small and why the spectra of complexes with different metals in the visible region are close in structure.

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Note: Figure translations are in progress. See original paper for figures.