

## The Crystal and Molecular Structure of Ethyl Chlorophyllide $a \cdot 2H_2O$ and Its Relationship to the Structure and Aggregation of Chlorophyll $a^*$

(exciton migration/photosynthesis)

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**ABSTRACT** Determination of the crystal structure of ethyl chlorophyllide  $a \cdot 2H_2O$  by single crystal x-ray diffraction techniques has provided the first detailed molecular structural data available for a magnesium-containing derivative of chlorophyll. At the same time the aggregation observed in this structure serves as the basis for a model of chlorophyll aggregation *in vitro* and *in vivo*. The magnesium atom in ethyl chlorophyllide is displaced 0.4 Å from the plane of the chlorin ring in the same direction as the methyl ester substituent. A water molecule occupies the fifth coordination site. A second water molecule is hydrogen bonded to the coordinated water molecule and also to the carbonyl oxygen atom of the methyl ester. Hydrogen bonds between the water molecules and carbonyl oxygen atoms link the chlorophyllide molecules into a two-dimensional aggregate of crosslinked one-dimensional polymers. The relationship of this aggregate to aggregates of chlorophyll both *in vitro* and *in vivo* is discussed, as is the efficiency of exciton transport within the polymer.

Prompted by the key role of chlorophylls in photosynthetic processes, numerous investigators have studied the structure and function of these pigments both *in vivo* and *in vitro*. The complete synthesis of chlorophyll  $a$  by Woodward (1) crowned an early sequence of structural studies. A vast amount of information has been provided by the application of modern physical techniques. Infrared and magnetic resonance measurements (2, 3) have been used to great advantage and x-ray structural investigations (4, 5) have been carried out on several degradation products. These studies have not, however, provided an unambiguous model for the detailed molecular structure of chlorophyll or any magnesium-containing derivative.

As a result of various studies designed to determine the nature and environment of chlorophyll in photosynthetic systems, it is now widely accepted (6-11) that chlorophyll aggregates play important roles in photosynthesis. An early model of the chloroplast lamellae (7) envisioned a two-dimensional aggregate of chlorophyll molecules at the interface between protein and lipid layers. This model was based primarily on the similarity of the position of the red absorption maximum in the visible spectrum of chlorophyll *in vivo* to that observed for chlorophyll aggregates *in vitro*. While chlorophyll *in vivo* may occur in a variety of environments and the shifts of the red absorption band may arise from combinations of chlorophyll-protein and chlorophyll-chlorophyll interactions, recent investigations (8-13) have provided

convincing evidence for the existence of chlorophyll aggregates in several types of photosynthetic material. It is clear that an understanding of the nature of chlorophyll aggregation is of prime importance in an understanding of the molecular processes involved in the initial stages of photosynthesis.

In most respects the alkyl chlorophyllides† serve as excellent models of chlorophyll. Visible absorption spectra, nuclear magnetic resonance spectra and infrared spectra of the chlorophyllides *in vitro* differ little from those of the parent chlorophyll (2, 14). As might be expected the most profound effect of the absence of the phytol group is the reduced solubility of the chlorophyllides in hydrocarbon solvents. Chlorophyllide itself occurs *in vivo* as the immediate biosynthetic precursor of chlorophyll (15). Schultz and Sauer (11) have presented evidence that aggregated chlorophyllide is present in greening leaves.

The x-ray structural determination of ethyl chlorophyllide  $a \cdot 2H_2O$  reported herein provides the first detailed structural data for a magnesium-containing chlorophyll derivative. In addition, the nature of the aggregate observed in this crystalline derivative provides a useful model of chlorophyll aggregates, both *in vivo* and *in vitro*.

### MATERIALS AND METHODS

Ethyl chlorophyllide  $a$  was prepared by the method of Holt and Jacobs (16), and crystals were grown by the slow addition of water to an acetone solution of the chlorophyllide. The crystals obtained were morphologically similar to those described by Holt and Jacobs and x-ray measurements revealed the same space group and approximately the same unit cell dimensions observed by Hanson (17) for mixed crystals of ethyl chlorophyllides  $a$  and  $b$ . From elemental analysis Holt and Jacobs determined that these crystals contain 1.5-2.0 molecules of water per pigment molecule.

*X-Ray Data and Structural Solution.* A dark blue-green crystal of ethyl chlorophyllide  $a \cdot 2H_2O$  in the form of a triangular plate (edge length of 0.20 mm and thickness of 0.05 mm) was mounted with the 3-fold axis roughly parallel to the goniometer head axis. The crystals possess trigonal symmetry, space group  $C_3^2 - P3_1$ , or  $C_3^3 - P3_2$ . Unit cell dimensions are  $a = 8.847(2)$  Å and  $c = 38.00(1)$  Å (based on  $MoK\alpha = 0.7107$  Å, temperature = 22°), observed density = 1.28(3) g/cm<sup>3</sup> by flotation in zinc chloride solution; calculated density = 1.31 g/cm<sup>3</sup> for  $z = 3$ . Data were collected

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† Chlorophyll  $a$  is the phytol ester of chlorophyllide  $a$ . Phytol is a twenty-carbon alcohol with a double bond in the 2 position.

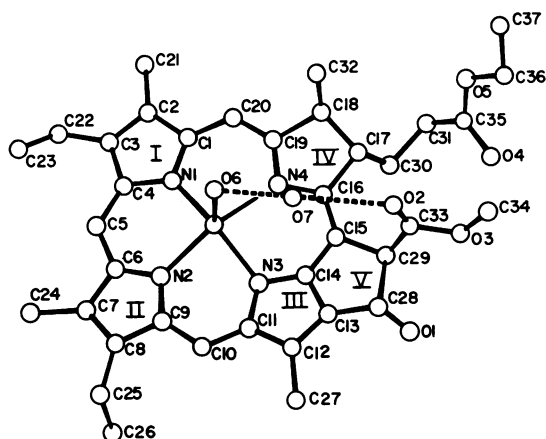


Fig. 1. The molecular structure of ethyl chlorophyllide  $a \cdot 2H_2O$ . The numbering system follows that used for methyl pheophorbide (5).

on a Syntex PI diffractometer with crystal-monochromatized  $MoK\alpha$  radiation. All independent reflections with  $2\theta \leq 45.0^\circ$  were measured. Of these, 781 reflections with observed intensities greater than three times their estimated standard deviations were used in subsequent solution and refinement of the structure.

The orientation of the molecular plane in the unit cell was deduced from the distribution of intense reflections [compare Hanson (17) (1939)]. The orientation about the axis perpendicular to the molecular plane and the position with respect to the 3-fold screw axis were determined by a trial-and-error technique. Models of the rigid portion of the chlorophyllide molecule based on the known structure of methyl pheophorbide $\ddagger$  (5) were systematically rotated and translated throughout the independent portion of the unit cell, and an R value based on 103 low-angle reflections was calculated. The minimum R obtained by this technique was 0.33. Subsequent Fourier and least-squares refinement of this configuration revealed that the correct orientation required a rotation of  $180^\circ$  about the axis perpendicular to the molecular plane. Successive least squares refinement and electron density difference syntheses yielded the positions of all nonhydrogen atoms. Full matrix least-squares refinement of positional parameters and isotropic temperature factors for all nonhydrogen atoms converged to  $R_1 = [\sum ||F_o| - |F_c|| / \sum |F_o|] = 0.127$  and  $R_2 = [\sum w||F_o| - |F_c||^2 / \sum w|F_o|^2]^{1/2} = 0.098$ , where  $w = 1/\sigma(F_o)^2$ . Refinement of anisotropic thermal parameters was not justified because of the low data-to-parameter ratio. Final refinement awaits collection of a more complete data set, but the structure obtained at this stage reveals the coordination geometry about the central magnesium, and provides the first unambiguous picture of a chlorophyllide-water aggregate.

The molecular structure of ethyl chlorophyllide  $a$  is shown in Fig. 1 §, and selected structural parameters are presented in Table 1. The magnesium atom is displaced  $0.4 \text{ \AA}$  from the

‡ A pheophorbide is a chlorophyllide from which the magnesium atom has been removed.

§ The preliminary data is not of sufficient accuracy to determine the absolute configuration and therefore the configuration proposed by Fleming (18) has been adopted in the figures. This configuration is consistent with the space group  $P3_1$ .

TABLE 1. Selected structural parameters\*

Distances and angles about the water molecules	
O6-Mg = 2.06(3) Å	O7-O6-O1 = 107(1)°
O6-O7 = 2.79(4) Å	O7-O6-Mg = 119(1)°
O7-O2 = 2.94(5) Å	O1-O6-Mg = 114(1)°
O6-O1 <sup>a</sup> = 2.60(4) Å	O6-O7-O2 = 125(1)°
O7-O4 <sup>r</sup> = 3.08(5) Å	O6-O7-O4 = 113(1)°
	O2-O7-O4 = 108(1)°

#### Short intermolecular distances

C1-O1 <sup>a</sup> = 3.43 Å
C1-C28 <sup>a</sup> = 3.47 Å
C5-C27 <sup>a</sup> = 3.50 Å
C20-O1 <sup>a</sup> = 3.51 Å
C23-C10 <sup>a</sup> = 3.43 Å
C23-C11 <sup>a</sup> = 3.55 Å

\* A detailed description of the structure will be published elsewhere upon completion of the final refinement of a more complete data set. Figures in parentheses represent estimated standard deviations in the least significant figure.

<sup>a</sup> ( $x - 1.0, y, z$ )

<sup>r</sup> ( $x - 1.0, y - 1.0, z$ )

plane of the four nitrogen atoms in the same direction as the methyl ester. A water molecule occupies the fifth coordination site. A second water molecule is hydrogen bonded to the coordinated water molecule and also to the carbonyl oxygen atom of the methyl ester. Two intermolecular hydrogen bonds link the structure into a two-dimensional array. The ketone oxygen of one chlorophyllide is hydrogen bonded to the coordinated water molecule of another to form a one dimensional chain. These chains are crosslinked by hydrogen bonding between the carbonyl of the ethyl ester and the noncoordinated water molecule (Fig. 2). The chlorin portion of the molecule is similar to that observed in methyl pheophorbide (5) but differs in the orientation of the vinyl substituent. The configuration of this group may be of some importance in the ease of exciton transport in chlorophyll aggregates (*vide infra*).

## DISCUSSION

Visible absorption spectra (7) of chlorophyll monolayers and colloidal suspensions have distinguished two different types of chlorophyll-water aggregates. If chlorophyll dissolved in an organic solvent is allowed to spread without restriction on a water surface, evaporation of the solvent leaves an "amorphous monolayer" with an absorption maximum at 675 nm. If the amorphous monolayer is compressed it undergoes a distinct transition to a so-called "crystalline monolayer" whose absorption maximum occurs at 735 nm. Similar aggregates are observed if water is added to an acetone solution of chlorophyll in which the red absorption maximum occurs at 660 nm. Initial aggregation results in the formation of colloidal particles, whose absorption maximum is shifted to 670-675 nm. Formation of larger particles is accompanied by a further red shift to 735 nm. It has been suggested (7) that the amorphous monolayers and colloidal chlorophyll may be good models for chlorophyll aggregates *in vivo*, where chlorophyll absorbs in the range 662-705 nm (19). On the other hand, crystals and crystalline monolayers of chlorophyll  $a$  and ethyl chlorophyllide  $a$  all show absorption maxima in the 730- to 735-nm range (20, 21).

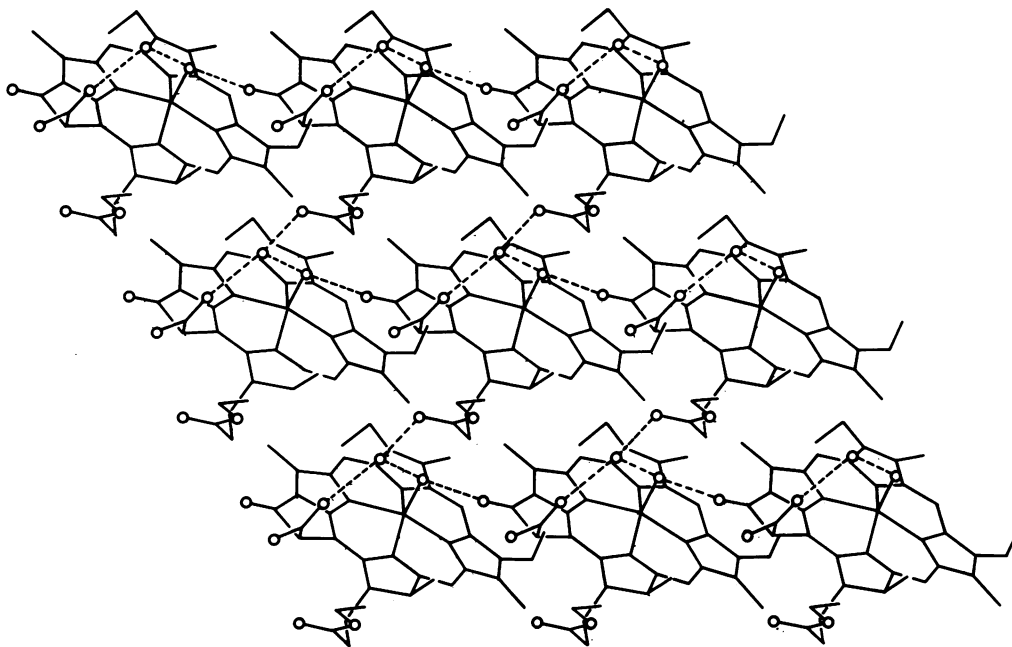


FIG. 2. The packing of one layer in the structure of ethyl chlorophyllide  $a \cdot 2H_2O$  viewed down the  $3_1$  axis.

While the type of aggregation that occurs in crystalline ethyl chlorophyllide  $a \cdot 2H_2O$  is not necessarily universal to all chlorophyll-water aggregates, it is probably closely related if not identical to that in so-called "crystalline monolayers." In addition, the nature of this aggregate suggests a plausible structure for the "amorphous monolayers" and "colloidal chlorophyll." These less highly ordered species may contain one-dimensional polymers of the type shown in Fig. 3 that are randomly arranged and perhaps randomly crosslinked by hydrogen bonds. Similar one-dimensional polymers could exist *in vivo* in the form of chlorophyll-protein complexes. The overlap between the  $\pi$  electron systems of adjacent molecules in an aggregate of this type is apparent in Fig. 3. The involvement of the ketone and vinyl functions in this overlap are of particular interest. This arrangement may provide a very efficient pathway for exciton migration within chlorophyll aggregates. Such a migration is an essential part of current theories of photosynthesis, which require rapid transport of photoexcitation from the light-harvesting chlorophyll to the reaction center.

From a structural point of view it is at least possible that chlorophyll *a*, chlorophyll *b*, and bacteriochlorophyll form aqueous aggregates similar to that of ethyl chlorophyllide *a*. The disposition of the phytol ester in such an aggregate serves to localize the hydrophobic portion on one side of the polymer. The replacement of the methyl group of chlorophyll *a* with the formyl group of chlorophyll *b* would not be expected to have a profound effect. Likewise, the saturation of ring II in bacteriochlorophyll would not necessarily alter the nature of the aggregate. Substitution of the vinyl group of chlorophyll by the acetyl group of bacteriochlorophyll could also be accommodated with retention of the substantial overlap between the conjugated systems. By the same reasoning, however, substitution of the vinyl group of chlorophyll *a* by the  $\alpha$ -hydroxyethyl group of chlorobium chlorophyll (22), present in some photosynthetic bacteria, is likely to result in a change in the nature of the aggregate.

While the presence of the magnesium is required for formation of aggregates in solution (3), the ring I-ring III overlap observed in the ethyl chlorophyllide  $a \cdot 2H_2O$  is also present in

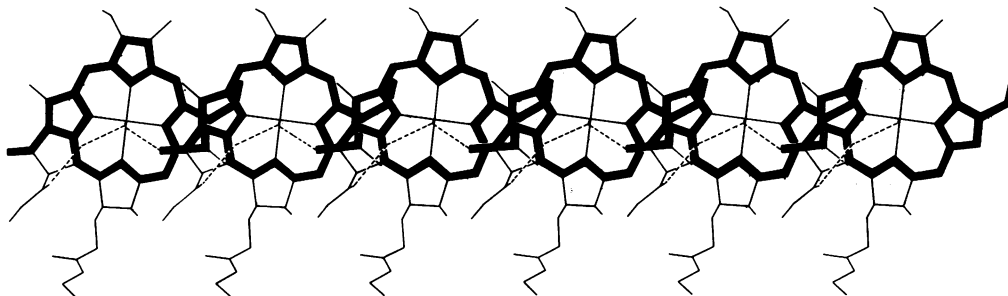


FIG. 3. A fragment of the chlorophyllide polymer found in crystalline ethyl chlorophyllide  $a \cdot 2H_2O$  viewed perpendicularly to ring III. The heavy lines indicate the conjugated portion of each molecule. Dashed lines indicate hydrogen bonds.

crystals of methyl pheophorbide (5). Since the  $\pi$ - $\pi$  overlap provides only a very weak bonding interaction, this configuration is not retained in solutions of the pheophorbide.

In a comprehensive study of chlorophyll-chlorophyll and chlorophyll-water interactions *in vitro*, Ballschmiter and Katz (3) pointed out the difference between the chlorophyll aggregates observed in the presence and absence of water. Anhydrous solutions of chlorophyll in noncoordinating solvents were shown to contain polymers (predominately dimers) in which the ketone oxygen of one molecule is coordinated to the magnesium atom of another. It is unlikely that this type of aggregation occurs in a photosynthetic organism.

The structure of the chlorophyll-water aggregate or micelle proposed by Ballschmiter and Katz has much in common with the ethyl chlorophyllide  $a \cdot 2H_2O$  aggregate. This proposed structure is based on an infrared study of a 1:1 chlorophyll *a*-water adduct in a hexadecane mull that shows a visible absorption maximum at 740-745 nm indicative of a highly ordered crystalline aggregate. Their model differs from that in Fig. 3 in that the magnesium atom and coordinated water molecule are on the opposite side of the chlorin ring from the methyl ester, with the water molecule hydrogen bonded to both the ketone and the methyl ester of an adjacent chlorophyll. Their infrared data can be equally well interpreted in terms of an aggregate similar to that found in ethyl chlorophyllide  $a \cdot 2H_2O$ . It is reasonable to assume that the chlorophyll *a* monohydrate contains chains that differ from those in Fig. 3 only by removal of the uncoordinated and presumably loosely held water molecule. The crosslinking between such chains is necessarily different from that observed in crystalline ethyl chlorophyllide  $a \cdot 2H_2O$ .

A different model for a linear chlorophyll-water polymer was recently proposed (5) in which adjacent chlorophyll molecules are related by  $2_1$  symmetry operations. In the construction of this model, however, the possibility that adjacent molecules could be related by simple translations was discounted because of "molecular crowding." The observed structure of ethyl chlorophyllide *a* refutes this argument.

Numerous recent investigations have provided direct evidence for the existence of chlorophyll aggregates *in vivo*. Most of these investigations have been directed toward a determination of the nature of the chlorophyll present in photosynthetic reaction centers. Reaction center preparations that contain no light-harvesting chlorophyll have been obtained by several investigators (23). Circular dichroism (8-11) and electron paramagnetic resonance measurements (12, 13) indicate the presence of at least two strongly coupled bacteriochlorophyll molecules in reaction centers isolated from purple bacteria. Circular dichroism difference spectra (11) of the P700 chlorophyll preparations enriched in photosystem I reaction centers indicate that at least two chlorophyll molecules coupled by exciton interaction are present in this reaction center of higher plants.

Evidence for the existence of chlorophyll aggregates *in vivo* is not limited to reaction centers. A water-soluble bacteriochlorophyll-protein complex has been isolated (23) from the green photosynthetic bacteria *Chloropseudomonas ethylica*, each subunit of which contains several bacteriochlorophyll molecules. This complex is assumed to transfer excitation from the bulk chlorobium chlorophyll to the reaction center bacteriochlorophyll. Absorption and circular dichroism measurements (8) indicate a strong exciton interaction between the bacteriochlorophyll molecules in each subunit.

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