Biochimica et Biophysica Acta, 764 (1984) 295–300
Elsevier

BBA 41460

MODE OF INSERTION OF CHLOROPHYLL *a* IN A LIPID LAYER

A CONFORMATIONAL APPROACH

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(Received July 25th, 1983)
(Revised manuscript received November 29th, 1983)

Key words: Model membrane; Chlorophyll a orientation; Lipid bilayer

A new procedure for conformational analysis is described to define the orientation of chlorophyll *a* in model membranes. The method allows to predict the configuration of assembled amphiphilic molecules. The minimal conformational energy is calculated as the sum of the contributions, resulting from the Van der Waals interactions, the torsional potentials, the electrostatic interactions and the transfer energy. In the calculated conformation, the porphyrin ring is orientated at an angle of 45° ± 5° to the membrane interface, with the phytol chain inserted into the lipid layer. This orientation of the porphyrin ring is in excellent agreement with the experimental value obtained with model membranes. The method could allow to define the orientation of other components in the thylakoid membrane.

Introduction

The inner membrane of chloroplasts of higher plants is responsible for the conversion of solar energy to chemical. Light is first absorbed by the chlorophylls, most of which act in collecting light and transferring energy to the reaction center of the two photosystems, where the quantum conversion takes place. Because of their key role in photosynthesis, a knowledge of their orientation and location within the thylakoid membrane is of prime importance. This orientation has been defined in artificial chlorophyll membranes. Weller and Ti Tien [1] obtained from photovoltage spectroscopy an angle of 45° ± 5° between the plane of the porphyrin ring and the plane of the membrane for chlorophyll inserted into black lipid membranes made of lipid chloroplasts extracts. Cherry et al. [2] found angles of 48° for chlorophyll *a*-egg phosphatidylcholine black lipid membranes; Steinemann et al. [3] angles of 44° ± 3°, 46° ± 3° and 49° ± 5° for chlorophyll *a*-phosphatidylethanolamine, chlorophyll *a*-dioleylphosphatidylcholine and chlorophyll *a*-phosphatidylserine membranes, respectively, from polarized absorption spectroscopy.

In this report, we propose a new conformational analysis procedure to define the orientation and position of chlorophyll in the lipid bilayer phase of the membrane.

Methods

The computational approach consisted in a two-steps procedure. First, the conformation of the isolated chlorophyll *a* molecule and its orientation at a simulated lipid-water interface were

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established by a method used elsewhere [4]. Briefly, the total conformational energy was calculated from the Van der Waals, torsional and electrostatic energies. This last was calculated for a dielectric constant of 16, a value intermediate to those currently used for the aqueous and hydrophobic phases at the simulated interface [4]. Selected conformers were then submitted to a simplex minimization procedure [5] and their orientation at the interface defined by calculations of the hydrophobic and hydrophilic gravity centers [6]. The hydrophilic gravity center (C_w) located in the porphyrin ring is defined by the following equation:

$$C_w = \frac{\sum_{i=1}^{n} \left[E^+ \text{transfer}_i \left(X_i^2 + Y_i^2 + Z_i^2 \right)^{1/2} \right]}{\sum_{i=1}^{n} E^+ \text{transfer}_i}$$

in which X_i, Y_i, Z_i are the coordinates of the ith atom. The hydrophobic gravity center located in the hydrocarbon domain (C_{HC}) is defined by the same equation, except that the negative transfer energies are taken into account [6]. The values for the transfer energies here used were identical to those determined experimentally by numerous authors and summarized elsewhere [7].

The interface position (I) is defined by the equation:

$$\frac{\sum_{i=1}^{n} E^+ \text{transfer}_i}{C_w - I} = \frac{\sum_{i=1}^{m} E^- \text{transfer}_i}{C_{HC} - I}$$

In the second step of the procedure, the assembly of molecules in the monolayer was computed as follows [8]. The position of a lipid molecule (B) relative to a chlorophyll molecule (A) was assessed. The five following parameters were successively modified: the distance between the hydrophilic centers of A and B (from 0.05 to 5 nm, by steps of 0.05 nm each), the rotation of molecule B around its own z-axis (by steps of 30° each), the gravitation of molecule B around molecule A (also by steps of 30° each), the up and down migration of molecule B along the z-axis perpendicular to the lipid-water interface (by steps of 0.05 nm each), and the oscillation of molecule B around its z-axis (by steps of 2°30′ each). In each case, the interaction between molecules A and B was calculated from the Van der Waals and electrostatic energies. The configuration of the A and B pair yielding the lowest energy was then used as reference to assess the position of a third molecule C. We limited for time-consuming reasons our approach to a number of lipid molecules, sufficient to surround the chlorophyll molecule. When the configuration of the cluster has been established, the mean molecular area was calculated from both the area occupied by each molecule and the intermolecular area, which were estimated after projection on the XY-plane, using a grid of square, each with a 0.1 nm

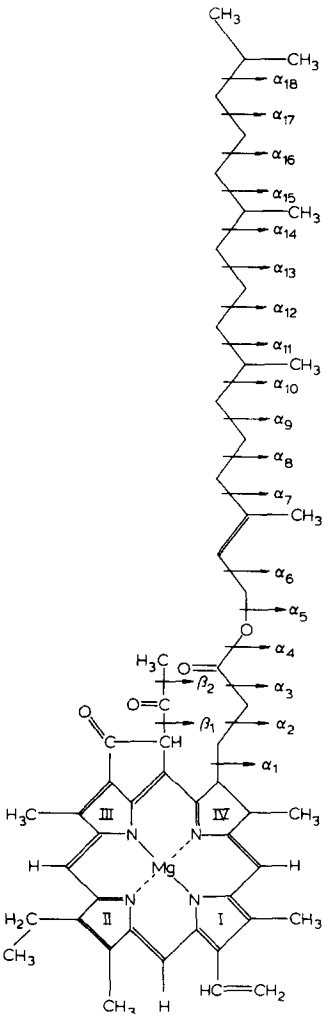

Fig. 1. Structural formula of chlorophyll *a* with numbering of the torsional angles.

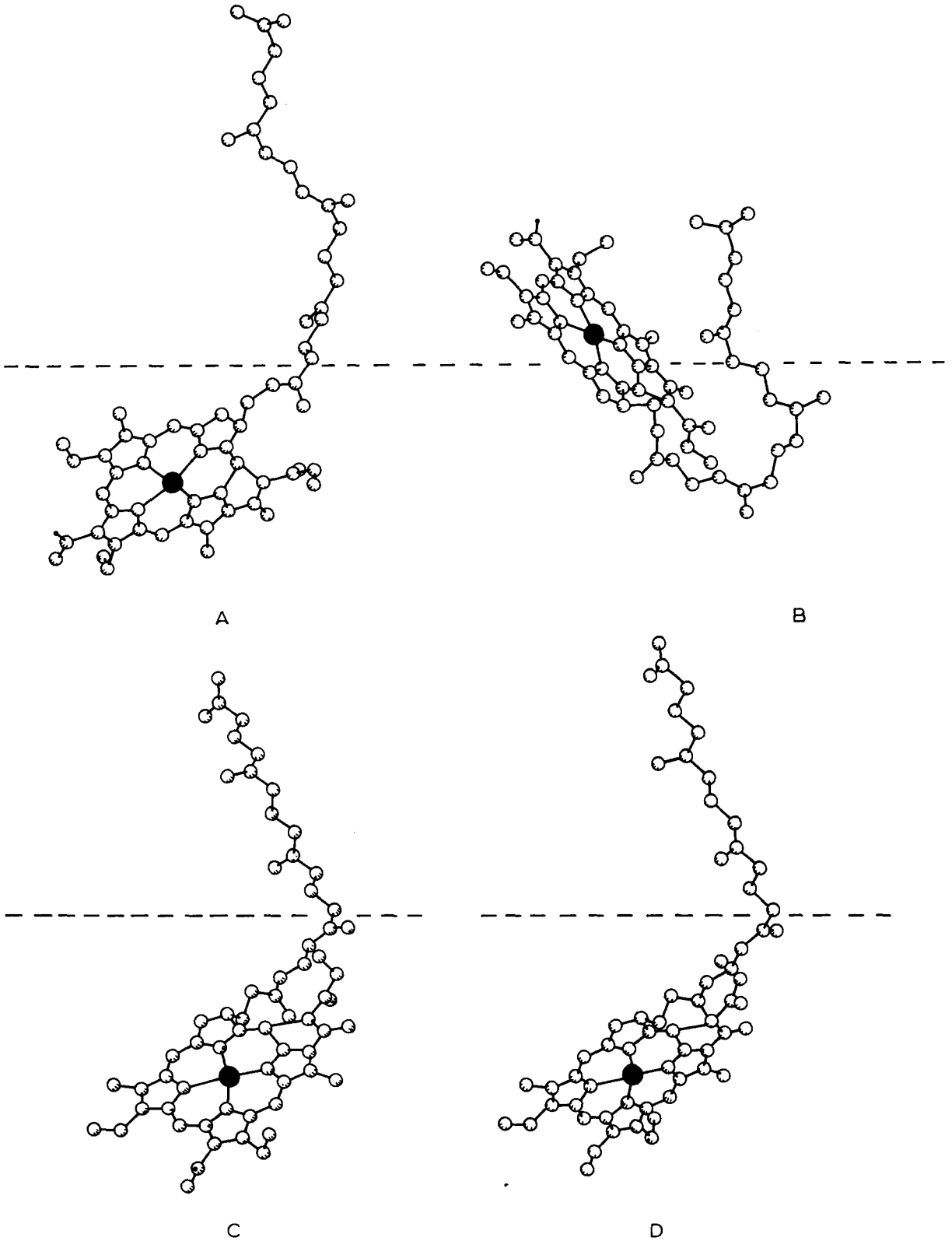

Fig. 2. Most probable conformers of isolated chlorophyll *a* at the interface. Dotted line delineates the hydrophobic and hydrophilic medium. Black circle represents the Mg atom.

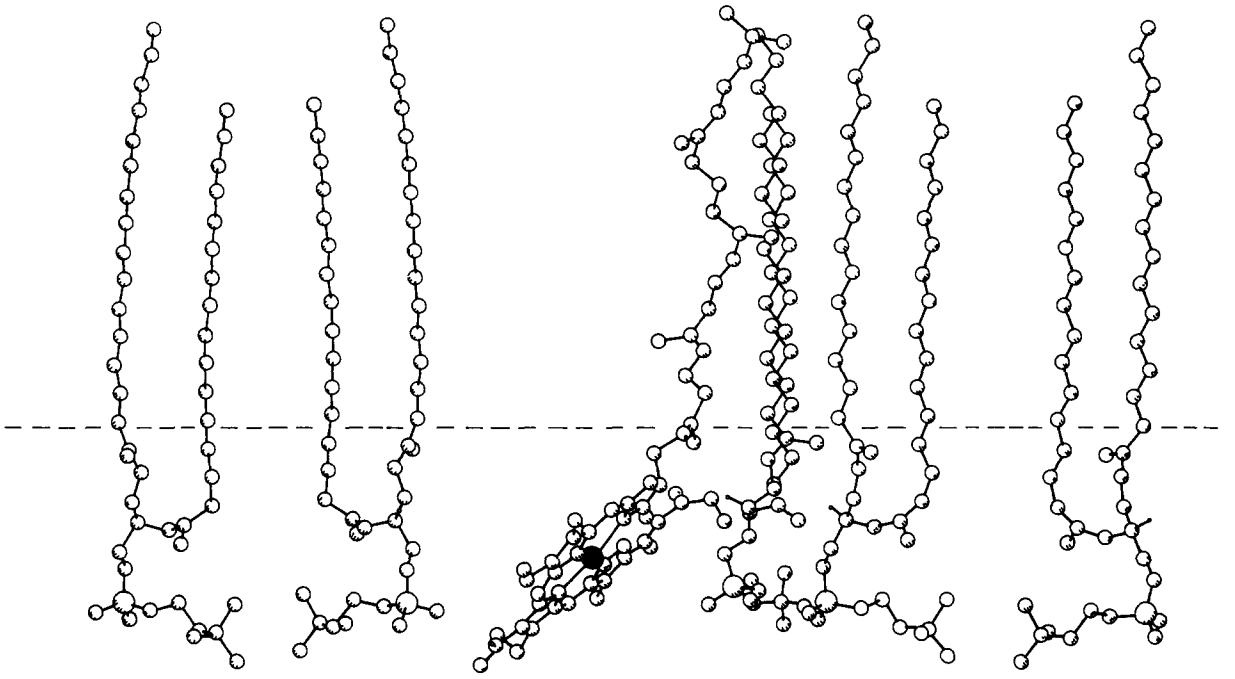

Fig. 3. Mode of insertion of chlorophyll *a* into the dipalmitoylphosphatidylcholine monolayer. The black circle represents the Mg atom.

side [9]. Calculations were made on a C.D.C.-Cyber Computer, coupled to a Benson drawing table.

Results and Discussion

Conformation and orientation of the isolated chlorophyll molecule

A first systematic study was performed by steps of 60° each on the eight torsional angles β_1, β_2, α_1, α_2, α_3, α_4, α_5 and α_6 (Fig. 1), yielding 6^8 or 1679616 conformers. Four conformers were selected with an individual probability of at least 5% (Table I). The characteristics of the four conformers, after orientation at the interface and application of the minimization procedure [5] bearing on all torsional angles, are listed in Table II. Fig. 2 gives the orientation of these four most probable conformers at the interface.

Conformation of the chlorophyll molecule inserted into the lipid monolayer

All probable conformers of chlorophyll a were inserted into a DL-α-dipalmitoylphosphatidylcholine monolayer, but only the assembling modes

TABLE I

CONFORMERS OF CHLOROPHYLL *a* AFTER SYSTEMATIC ANALYSIS

The torsional angles for α_i with $i > 6$ are 180°.

	Torsional angles (°)								Energy above minimal value (kcal/mol)	Probability (%)
	β_1	β_2	α_1	α_2	α_3	α_4	α_5	α_6		
A	0	180	300	300	300	180	180	180	–	30.13
B	0	240	300	300	300	180	180	180	0.274	18.97
C	0	180	240	120	120	180	180	180	0.405	15.20
D	0	240	240	120	120	180	180	180	0.647	10.08

TABLE II

CONFORMERS OF CHLOROPHYLL *a* AFTER MINIMIZATION AND ORIENTATION AT THE INTERFACE

Torsional angles	Degree (°)			
	A	B	C	D
β_1	56	288	131	57
β_2	199	174	327	165
α_1	287	56	266	209
α_2	295	56	232	137
α_3	214	212	214	152
α_4	236	200	117	236
α_5	146	209	182	182
α_6	210	246	189	166
α_7	296	181	67	290
α_8	162	180	182	177
α_9	195	296	178	177
α_{10}	79	181	83	274
α_{11}	209	177	179	163
α_{12}	172	277	180	178
α_{13}	189	176	182	174
α_{14}	276	179	276	83
α_{15}	181	179	178	183
α_{16}	177	84	182	175
α_{17}	182	182	178	175
α_{18}	85	178	84	275

corresponding to the minimal conformational energy were retained. The structure shown in Fig. 3 corresponds to a 99% probability. In this conformation, the porphyrin ring is orientated at an angle of 45° ± 5° to the membrane interface with the phytol chain inserted into the lipid layer. This orientation of the porphyrin ring is in excellent agreement with the experimental values [1]. However, this experimental approach does not allow to exclude the possibility that the porphyrin rings lie at varying angles as compared to the membrane surface and for this reason, should be regarded as average values. Moreover, the calculated area occupied per chlorophyll molecule (108 Å^2) is in agreement with the experimental value obtained from surface pressure measurements (115 Å^2) on chlorophyll monolayers spread at the air-water interface [10]. The conformational analysis predicts an immersion of the porphyrin group into the membrane aqueous region. Another structure of the chlorophyll *a* molecule corresponding to the same orientation has, however, been proposed [11]: the hydrophilic edge of the porphyrin ring (including the cyclopentanone ring adjacent to phytol and the carbonyl group of the propionic side chain of ring IV) would remain at the aqueous membrane surface and the more hydrophobic region (ring I, II, III) would interact with the lipid acyl chain. This structure corresponds to one (B) of our minimized chlorophyll conformations, but its probability of existence after insertion into a dipalmitoylphosphatidylcholine monolayer is extremely low.

The conformational analysis allows to define a chlorophyll orientation in excellent agreement with experimental data obtained on model membranes. It would be, however, premature to extrapolate this mode of orientation to the thylakoid membrane, where it is admitted that chlorophyll are attached to proteins (Photosystem I-Photosystem II). Recent work based on ^{13}C-NMR [12] measurements of the photosynthetic spinach thylakoid membrane demonstrate, however, for a pool of chlorophyll molecules, that the motional state of phytol and lipid hydrocarbon chains are very similar, suggesting that these chlorophyll are lipid bound. In this approach, phospholipids were chosen because data concerning the orientation of chlorophyll *a* were only available in this kind of lipid matrix. Because phospholipids are only minor components of the thylakoid membranes, galactosyl diacylglycerols, which exist in high proportions, could be more valuable candidates to interact with chlorophyll molecules and to space them for efficient photoreception. The mode of association of galactosyl diacylglycerols and chlorophyll a is presently under investigation using the conformational analysis procedure. This procedure here described could be used for any molecule constituted of less than 200 atoms (carotenoids, plastoquinone).

Acknowledgements

We thank the Computing Center of the Université Libre de Bruxelles, where the calculations were performed on a C.D.C.-Cyber 170 computer, coupled to a Benson drawing table. J.D. is Aspirant of the Fonds National de la Recherche Scientifique.

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