

NOVEL PROTON NMR CHARACTERIZED STRUCTURES FOR CHLOROPHYLL A AND B AGGREGATES
IN SOLUTION.

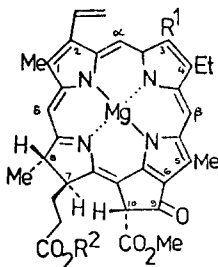
Raymond J. Abraham and Kevin M. Smith

The Robert Robinson Laboratories, University of Liverpool, P.O. Box 147,
Liverpool, L69 3BX, England, and
Department of Chemistry, University of California, Davis, California 95616, U.S.A.

Summary: Novel aggregate structures for both chlorophyll a and b are presented, derived from a quantitative analysis of the observed proton NMR aggregation shifts.

Despite intense interest over the past two decades, (1) the structures of Chl aggregates formed in solution, which could provide a model for the organization of Chl antenna and reaction-center pigments in vivo, (2) have not been satisfactorily established. Large NMR aggregation shifts have been documented, carefully analyzed, (1,3) and reinterpreted (4). Infrared (5) and circular dichroism (6) studies have also been described and have confirmed the overall validity of the NMR work. On the basis of the NMR and infrared work, (1,3,5) Katz et al., suggested that the C-9 keto function was the primary donor group to magnesium, while Fong and Koester's optical studies (4) favoured a head-to-tail dimer in which the magnesium atom was co-ordinated to the C-10 carbomethoxy group. Other aggregate structures involving interstitial water molecules have been proposed by Ballschmitter and Katz, (7) and a number of Chl "special pair" dimers, all involving water molecules, have been put forward (8). Significantly, the crystal structure of ethyl chlorophyllide a dihydrate shows (9) a bridging link in which two water molecules are placed between the C-9 carbonyl of one molecule and the magnesium of a neighbour. No definite proposed structure exists for the Chl b aggregate, but Closs et al., have suggested (3) that the NMR aggregation shifts could be consistent with the presence of two dimers of comparable stability, or of aggregates in which the magnesium is co-ordinated with both the C-3 and C-9 carbonyl groups.

On the basis of a refined accurate model of the Chl ring current which we recently described (10) we herein propose novel structures for the Chl a and b aggregates which we feel adequately account for the observed NMR shifts.



Chlorophyll a: $R^1 = \text{Me}, R^2 = \text{Phytyl}$
 Chlorophyll b: $R^1 = \text{CHO}, R^2 = \text{Phytyl}$
 Methyl Chlorophyllide a: $R^1 = R^2 = \text{Me}$
 Methyl Chlorophyllide b: $R^1 = \text{CHO}, R^2 = \text{Me}$

RESULTS AND DISCUSSION

Two possible types of Chl a structures were investigated. The first was the C_2 symmetrical dimer of Fong and Koester (4), Figure 1A. Trial calculations showed that a structure of this type could reproduce the observed shifts, so the molecular separation and displacement were varied until the best agreement with observed shifts was obtained, using only those protons of fixed geometry relative to the Chl nucleus. Having found the displacement co-ordinates, the orientations of the C-2 vinyl, C-7 propionate, and C-10 carbomethoxy groups were searched for the best agreement with these proton shifts to give an r.m.s. error (obs-calc shifts) of 0.20 p.p.m. Another possible structure is a non-symmetric one in which the two molecules in the dimer have different environments. An acceptable structure of this type was found in which the Chl a molecules are rotated 180° with respect to each other (Figure 1B). As before, the co-ordinates were optimized with protons of fixed geometry, and then the side chain orientations were optimized to give an r.m.s. error of 0.11 p.p.m.

Trial calculations showed that there is no symmetric structure capable of explaining the observed aggregation shifts for Chl b, but a possible non-symmetric structure does exist. The displacement co-ordinates of the dimer geometry were then scanned for the best agreement of the observed and normalized shifts, in view of the polymeric nature of the aggregate in this case,¹¹ to give an r.m.s. error of 0.18 p.p.m. and the geometry of Figure 1C.

The calculated shifts for the symmetric dimer of Chl a show moderate agreement with the observed data, whilst those for the non-symmetric structures for both Chl a and b constitute, we suggest, the first quantitative explanation of the observed aggregation shifts for these molecules. The Chl a dimer (Figure 1B) allows both one C-10 carbomethoxy and one or both of the C-9 keto groups to participate in binding to the magnesium. In the structure, the C-9 keto groups are positioned over the magnesium atom. However, the separation between the parallel planes of the dimer (ca. 6 Å) makes it unlikely that there could be a direct attachment, and raises the question of whether there is an interstitial molecule of water in the bridging interaction. Our proposed structure is similar to that of Ballschmitter and Katz (7) for the Chl-water-Chl interaction, except that in our case each Chl molecule is opposite to each adjoining molecule, rather than aligned. The proposed structure for Chl b

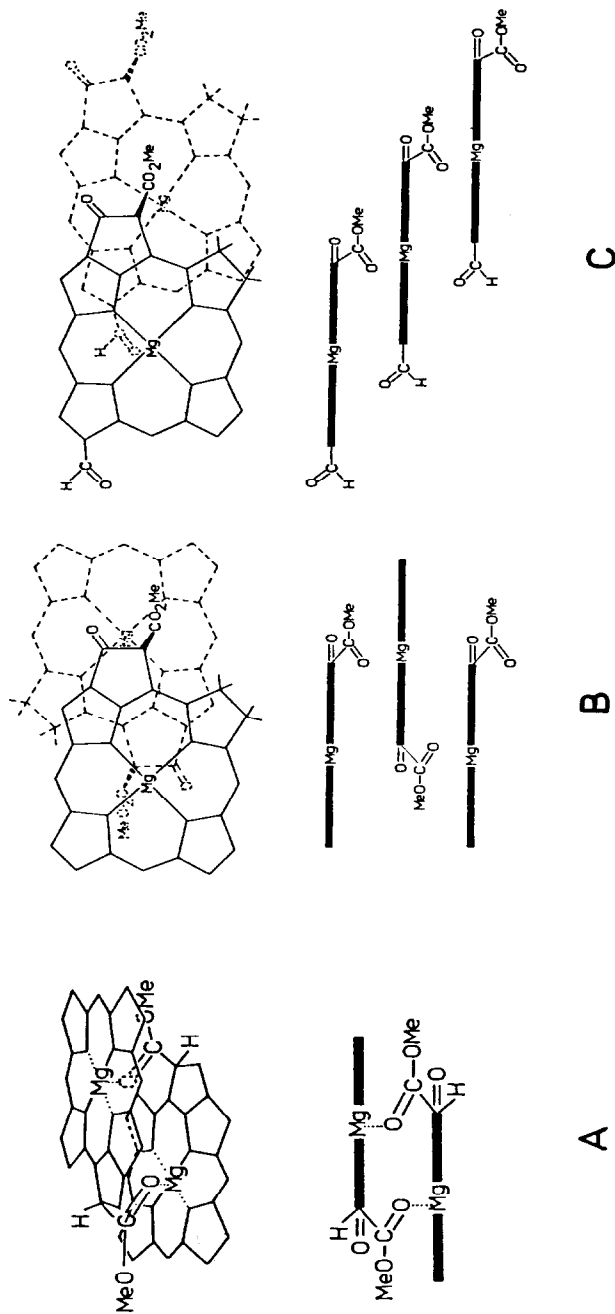


FIGURE 1. Proposed dimer structures and end-on projections A, the C_2 symmetrical Chl a - Chl a dimer; B the unsymmetrical Chl a adduct; C the Chl b adduct. Peripheral substituents have been omitted for clarity.

(Figure 1C) allows interaction between the magnesium atoms and both the C-3 formyl group and the carbonyl system at C-9, 10, and this is consistent with the original suggestion of Closs *et al.*, (3). As in Chl a, the estimated interplane distance (ca. 6 Å) appears to be too large for a direct magnesium to carbonyl bond as even the ester carbonyl is still 3.7 Å above the plane of the neighbour.

The proposed structures (Figures 1B and C) have one important conceptual advantage over the previous dimer structure (Figure 1A), in that they allow the ready formation of (experimentally observed) higher aggregates. The third molecule can be attached in exactly the same way as in the dimer bonding, and the difference between the Chl a and b aggregates is that in the former case the alternation of the molecules gives rise to a stacking arrangement in which the next but one neighbours are vertically above each other; in Chl b the aggregate chains are laterally displaced. A stepped aggregate of the type shown in Figure 1C has been suggested (12) for the antenna pigment array in Chlorobiaceae, and it would be of considerable interest to know whether these stacking arrangements are of any biological relevance in other organisms.

ACKNOWLEDGEMENTS

This research was supported by grants from the National Science Foundation (CHE-81-20891) and the Scientific Affairs Division of N.A.T.O. (RG 256.80).

REFERENCES

1. Katz, J.J., Shipman, L.L., Cotton, T.M., and Janson, T.R., (1978). The Porphyrins, Dolphin, D., ed., Vol. V, Academic Press, New York, pp. 402 - 458.
2. Chlorophyll Organization and Energy Transfer in Photosynthesis (1979). Ciba Foundation Symposium No. 61, Excerpta Medica, Amsterdam, p.374.
3. Closs, G.L., Katz, J.J., Pennington, F.C., Thomas, M.R., and Strain, H.H., (1963). *J.Am.Chem.Soc.*, 85, 3809 - 3821.
4. Fong, F.K., and Koester, V.J., (1975). *J.Am.Chem.Soc.*, 97, 6888 - 6890.
5. Katz, J.J., Closs, G.L., Pennington, F.C., Thomas, M.R., and Strain, H.H., (1963). *J.Am.Chem.Soc.*, 85, 3801 - 3809.
6. Houssier, C., and Sauer, K., (1970). *J.Am.Chem.Soc.*, 92, 779 - 791.
7. Ballschmitter, K., and Katz, J.J., (1969). *J.Am.Chem.Soc.*, 91, 2661 - 2677.
8. Pellin, M.J., Wasielewski, and Kaufmann, K.J., (1980). *J.Am.Chem.Soc.*, 102, 1868-1873.
9. Chow, H.C., Serlin, R., and Strouse, C.E., (1975). *J.Am.Chem.Soc.*, 97, 7230 - 7237.
10. Abraham, R.J., Smith, K.M., Goff, D.A., and Lai, J.-J., (1982). *J.Am.Chem.Soc.*, 104, 4332 - 4337.
11. Ballschmitter, K., Truesdell, K., and Katz, J.J., (1969). *Biochim.Biophys.Acta*, 184, 604 - 613.
12. Smith, K.M., Kehres, L.A., and Fajer, J., (1983). Submitted for publication.

(Received in UK 3 February 1983)