# X-RAY DIFFRACTION FROM CHLOROPLAST MEMBRANES ORIENTED IN A MAGENTIC FIELD

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## 1. Introduction

There is as yet no clear consensus in the literature as to the general features of the structure of photosynthetic membranes. Much of the interpretation of the physiology of chloroplasts does not require that the thylakoid membranes differ fundamentally as concerns these general features from other membranes. Current thinking would favour as a scheme for most membrane structures the mosaic model, with a lipid matrix (which may be 'bilayer-like') which is penetrated to various extents by proteins. Of the work specifically concerned with the structure of photosynthetic membranes, there is some support for the application of this model [1,2]. A number of other suggestions are on record however: among these are the existence of separate lipid protein and water layers [3] and the membranes being assemblies of lipoproteins [4]. This work is aimed at providing more evidence upon which to decide this issue.

## 2. Experimental

Spinach leaves which were grown under controlled conditions were homogenized using a Warring Blender, filtered through Miracloth; the suspension was centrifuged at 4000 *g*; the pellet was then resuspended, and the suspension centrifuged again. The medium was 0.1 M sucrose, 30 mM Tricine-NaOH adjusted to pH 7.3, and 5 mM $MgCl_2$. In some cases the chloroplasts were resuspended in a similar medium but not containing $MgCl_2$. To a centrifuge pellet of chloroplasts a sufficient quantity of washing medium was added in order to achieve fluidity: this corresponded to about 5 mg chlorophyll/ml. This concentrated suspension was put in a glass capillary, and mounted between the poles of an electromagnetic on a low angle X-ray camera. In several cases this was a Franks camera employing film recording, requiring 12 h exposure. Otherwise the beam was linear, focussed with curved glass. The recording was then with a position sensitive proportional counter [6]. Counting times were typically one hour. Signals from unoriented specimens were corrected or distortion [7].

## 3. Results

Fig.1 shows a photographic recording of the oriented signal. The arcs are in the direction of the magnetic field, and are attributable to the enhanced

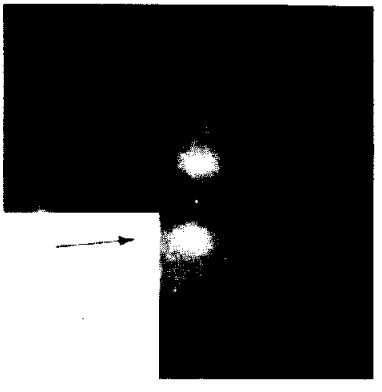

Fig.1. Photographic recording of low angle scattering from a chloroplast suspension in a magnetic field of approximately 10 kG. Sucrose 0.4 M, Tricene–NaOH 50 mM, $MgCl_2$ 5 mM. The arrow indicates a Bragg spacing of 40 Å.

scatter when the diffraction vector $s(s = 2 \sin \theta/\lambda)$ is parallel to the lamellar normals. This confirms that the lamellar normals are indeed aligned along the magnetic field [5]; in addition we can estimate a degree of orientation of approximately $\pm 15^\circ$ arc. The lack of Bragg peaks in fig.1 shows in addition that there is not a stacking of membrane sacs into a one-dimensionally ordered morphology, as was the case for the technique of controlled evaporation [2]. There is however a quite detailed structure to the scatter which is meridional in fig.1.

Fig.2 and 3 show counter results for two ionic conditions. Diffraction from a sample was recorded with and without the magnetic field. The same qualitative features are noted as using the photographic technique; in addition it is observed that for $s < 0.01$ Å$^{-1}$ scatter in the absence of the field is about 60% of the signal with field. From this we can infer a significant non-meridional intensity, as discussed below.

## 4. Interpretation

To analyze the results further the following two effects must be considered. There will be a component of the scattered intensity which is off the meridian; its dependence on the azimuthal angle will depend on

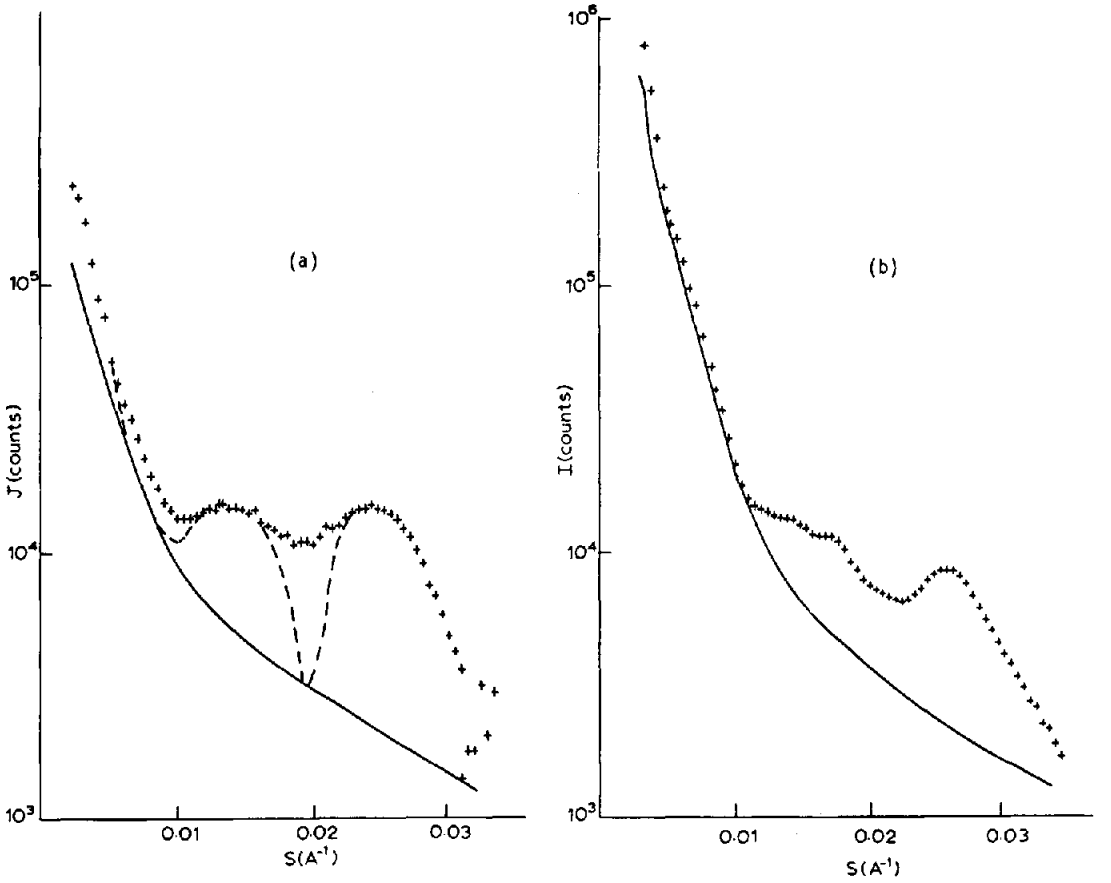


Fig.2. Low angle scattering of chloroplast suspension, with subtraction of scatter from the buffer solution, 0.1 M sucrose, 50 mM Tricene–NaOH, 5 mM $MgCl_2$ (a) with magnetic field (b) no field after correction [7]. The solid lines refer to an approximate separation of meridional scatter (see text). The broken line refers to the meridional scatter which would be expected from a model based on previous interpretations [2].

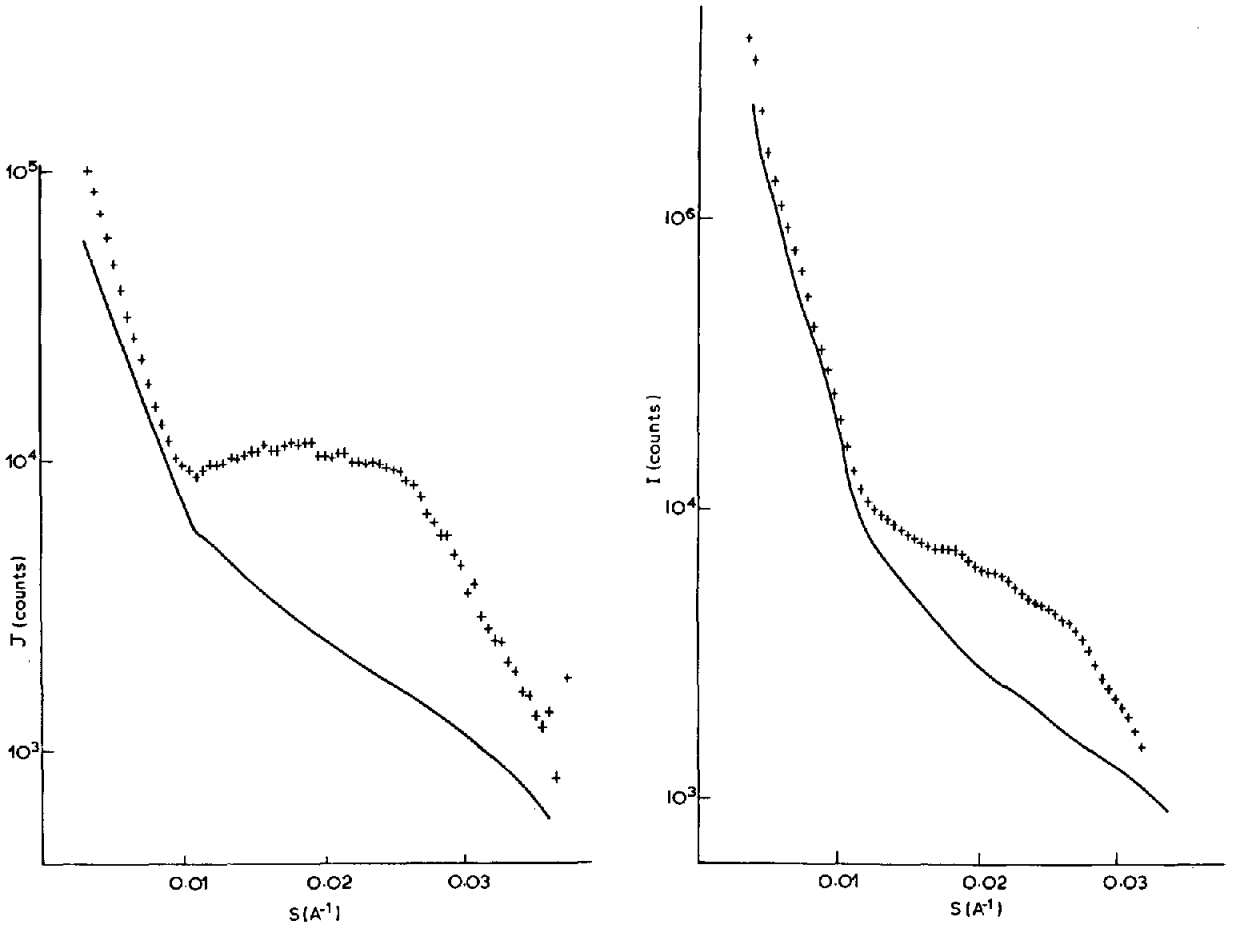


Fig.3. Results analogous to fig.2, but with no $MgCl_2$ in the medium.

variations in electron density corresponding to structure within the plane of the membrane, for example from proteins in the membrane. Secondly there may be scattering from structures not integral to the membranes e.g. proteins attached to the outer surfaces of the membranes or trapped in the 'stroma' regions between membranes. Although insufficient data is available to analyze these effects in detail, an assessment of their importance can be made in the following way.

The membranes, like all lamellar systems, will have a structure in reciprocal space consisting in the first place of a 'spike' normal to the membrane (the 'meridional' direction). In the case of non-uniform lamellae, this pike will correspond to the Fourier transform of the electron density of the membrane as projected on a line normal to the lamellae. Lack of uniformity in the lamellae will result in the structure in reciprocal space not being confined to this spike. If the contribution to the intensity of scattering corresponding to the spike was large compared with other contributions, there will be an approximate relation between the signal from the oriented sample (J) and the unoriented signal (I) after correction for collimation distortion by:

$$Js \propto Is^2$$

where s and $s^2$ are the Lorentz factors for lamellar systems, oriented and unoriented respectively. The measurements show that this proportionality does not hold; in particular the unoriented signal at

$s < 0.01$ Å$^{-1}$ is much too large. It should be emphasized that this cannot be resolved in terms of poor orientation; indeed fig.1 demonstrates the rather good orientation. A measure of the departure from proportionality is provided by the nominal 'background' $J_0$ and $I_0$ which are shown in the figures as (solid) lines. These were derived by trial and error so that,

$$J_1 s \propto I_1 s^2$$

where $J_1$ and $I_1$ are the intensities after subtraction of $J_0$ and $I_0$. This procedure corresponds to a nominal separation of the signal into $J_1$ (corresponding to uniform lamellae) and a signal $J_0$ which has no preferred azimuthal orientation with respect to the membranes. If the structures which give rise to the departure from uniform lamellae (e.g. proteins in the sample) do not have well defined positions with respect to the mid lines of the membranes, this separation procedure would be a good approximation. In that case, the lamellar diffraction $J_1$ would be interpreted in terms of the broken line in fig.2. There are several protein species associated with the membranes, (cf. retinal membranes [8] and purple membranes [9]) so that variability in protein positions may be correspondingly higher than in these other cases. In addition there are proteins such as cytochrome *f* which are not likely to be incorporated entirely within the membrane sheets, and there is no reason to suppose that all the stromal proteins have been removed from the preparations. Hence it is no surprise that there is a signal $J_0$ which is not of negligible intensity compared with $J_1$.

The orientable signal shows an additional structure (fig.2(a)) when magnesium is present in the suspending medium compared with when magnesium is absent (fig.3(a)). The presence of magnesium is known to preserve the partition structure which links together many of the membrane sacs in an ordered way [10]. Thus we can identify the presence of additional maxima with the presence of membranes which are predominantly paired. It may be anticipated that the double membrane (with partitions) may, to a first approximation, be considered as the simple addition of single membranes with no fundamental change in membrane structure. This would seem to be borne out by the oriented scatter in fig.2 and 3, whereby the former can be related to the latter by a pair correlation term of the form $\cos^2(\pi\, sd)$ where d is the membrane separation within the pair (across the partition). On this basis by matching a pair correlation term with the minimum in scatter at $s = 0.73$ Å$^{-1}$, d is 77 Å. This agrees very well with electron density distribution derived previously for chloroplasts from *Euglena* [2].

## 4. Conclusions

It has been shown that X-ray scattering signals can be readily obtained from chloroplast under conditions where measurements of their physiology are normally made.

The magnetic orientation used for optical measurements has been confirmed, and a degree of orientation estimated.

The information gained from the orientation has shown that there is a non-meridional component to the scatter which is comparable in intensity to the meridional component. This is interpreted on the basis of proteins associated with the membranes.

Differences in ionic conditions have demonstrated the effect of membrane pairing in the partition region. A distance between the centres of the membranes across the partition is estimated as 77 Å, in good agreement with previous interpretations [2]. Hence the results bring additional support for a mosaic model structure for the chloroplast membrane.

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