The Secondary Structure of Proteins in the Thylakoid Membrane

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With reference spectra derived from proteins of known structure (CHEN, YANG, and MARTINEZ, Biochemistry 11, 4120 [1972]) a better approximation of the circular dichroism spectrum of fragments of the thylakoid membrane is achieved, than by the use of polylysine as reference substance. Most probably the protein in the thylakoid membrane consists of 40 per cent helix, 42 per cent random coil and 18 per cent $\beta$-structure.

The best approximation was obtained with 42 per cent $\alpha$-helix, 40 per cent random coil and 18 per cent $\beta$-structure. The greatest difference between the experimental and calculated spectra consisted in a shift of the positive extremum from 191 to 193-194 nm. As the investigated fragments of the thylakoid membrane exhibited a mean diameter of 108 Å, it appeared impossible to attribute this red shift only to lysis of proteins, which were obtained by resolution of proteins of the thylakoid membrane.

By means of the reference spectra of CHEN et al.\(^6\) yielding 40 per cent helix, 18 per cent $\beta$-structure and 42 per cent random coil, a better approximation to experimental spectrum (Fig. 1) is achieved, than with polylysine as reference substance (Fig. 2). The maximum of ellipticity lies in the calculated spectrum at 193 nm and coincides almost fully...
Fig. 1. Comparison of the experimental (-----) with the calculated (---) circular dichroism spectrum (40 per cent helix, 42 per cent random coil, 18 per cent \( \beta \)-structure) using reference spectra derived from proteins by Chen, Yang, and Martinez.

Fig. 2. Comparison of the experimental (-----) with the calculated (---) circular dichroism spectrum (42 per cent \( \alpha \)-helix, 40 per cent random coil, 18 per cent \( \beta \)-structure) using polylysine as reference substance.

with the maximum of the experimental curve, which is situated between 193 and 194 nm. However, both spectra differ in their amount of ellipticity in the maximum, the difference in the height of the maximum of the experimental and calculated curve being approximately 6,000. For the calculated curve a standard deviation of 7,000 is obtained. Besides this the maximum of the experimental curve should be lowered by light scattering, which is greater with particles of an average diameter of 108 Å than with the soluble proteins, used for the determination of the reference spectra. Since, furthermore, the high absorption of the chloroplast preparation influences unfavourably the signal to noise ratio, it is not clear, whether the depression of the maximum of ellipticity is real. If, as is frequently practised, one disregards, for the mentioned reasons, the short-wave region of the spectrum, a good approximation (Fig. 3) is reached

Fig. 3. With 40 per cent helix, 50 per cent random coil and 10 per cent \( \beta \)-structure a better fit is obtained between 200 and 250 nm than in Fig. 1.

with 40 per cent helix, 50 per cent random coil and 10 per cent \( \beta \)-structure. However, the maximum of ellipticity is in this case, shifted to shorter wavelengths. A lowering of the 193 nm maximum of ellipticity can, as shown in Fig. 4, be achieved by a lowering of

Fig. 4. A lowering of the 193 nm maximum is obtained by reduction of the helix content (34 per cent helix, 48 per cent random coil, 18 per cent \( \beta \)-structure).

the helix content. By this means, however, a shift of the maximum and an inferior fit in the region of negative ellipticity cannot be avoided. Since all attempts to obtain a better fit, were unsuccessful, we like to retain as the most probable result up to now, that in the fragments of the thylakoid membrane the polypeptides have approximately 40 per cent helix, 42 per cent random coil and 18 per cent \( \beta \)-structure.
A high helix and random coil content and a low content of \( \beta \)-structure is also in agreement with the infrared spectra\(^1\). Furthermore it can be seen that the far ultraviolet dichroism spectrum of the fragments of the thylakoid membrane is in reasonable agreement with the spectrum of soluble proteins of corresponding conformation. For the interpretation of the remaining differences, especially those between 200 and 205 nm, new measurements with an improved signal to noise ratio are necessary. In addition the weak circular dichroism of the lipids should be considered.

It is not clear yet whether the ultraviolet circular dichroism spectrum of the fragments agrees with the circular dichroism of the entire thylakoid membrane, in other words, whether the spectrum of the fragments may be considered representative for the lamellar system. Since the fragments contain approximately the same amounts of chlorophyll, colorless lipids and proteins as the whole lamellar system\(^8\) we felt that the question could be answered positively. A more thorough investigation, however, revealed that the fragments have a different peptide composition than the lamellar system\(^8\). In addition the fragments, exhibited only photosystem I activity. Therefore, it appears doubtful whether the results of the conformation analysis obtained with fragments are representative for the entity of proteins in the thylakoid membrane.

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5. D. J. GORDON, Biochemistry 11, 413 [1972].