The Secondary Structure of Proteins in the Thylakoid Membrane

WILHELM MENKE and ROLF-DIETER HIRTZ

Max-Planck-Institut für Züchtungsforschung (Erwin-Baur-Institut), Köln-Vogelsang

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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 β-structure.

Fragments of the thylakoid membrane, obtained by ultrasonication and subsequent fractioning centrifugation, gave a far ultraviolet dichroism spectrum which shows maxima at 193 to 194 (+), 208 (—) and 222 (—) nm[1]. Previously, we have tried to approximate this spectrum, by means of linear superposition of spectra of polylysine having α-helix, β- and random coil conformation, using the GREENFIELD and FASMAN[2] procedure. The result, however, was not satisfactory, despite the fact that the chloroplast spectra as well as the reference spectra were obtained under identical conditions with the same apparatus[3].

The best approximation was obtained with 42 per cent α-helix, 40 per cent random coil and 18 per cent β-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 scattering effects of the membrane preparations[4,5].

Previously, CHEN, YANG, and MARTINEZ[6] have published reference spectra for the conformation analysis of proteins, which were obtained by resolution of the circular dichroism spectra of proteins into the spectra of the three conformation parts. The authors used five proteins, the structure of which being known by X-ray diffraction. With these spectra better results were obtained when determining the secondary structure of soluble proteins than with spectra of synthetic polypeptides. If the secondary structure of the proteins of the thylakoid membrane is not considerably different to that of soluble proteins, then the reference spectra obtained by CHEN *et al.*[6] should also be suited for the conformation analysis of the proteins of the thylakoid membrane.

Requests for reprints should be sent to Prof. Dr. W. MENKE, Max-Planck-Institut für Züchtungsforschung, *D-5000 Köln 30.*

Materials and Methods

The preparation of fragments of the thylakoid membrane from chloroplasts of *Antirrhinum majus*, strain 50, was described previously[8]. The experimental spectrum is represented by 121 points which are the arithmetic average of 5-10 registrations from 5 preparations. The resolution of the experimental spectrum was carried out following $X = f_\alpha X_\alpha + f_\alpha X_\alpha + f_\alpha X_\alpha$ by trial and error[6]. The f's are the fractions of helix (α), β-structure (β) and random coil (γ) with $f_\alpha + f_\alpha + f_\alpha$ being 1. X is the mean residue ellipticity $[\Theta]$. The values of X_α, X_α and X_α have been taken from the publication of CHEN *et al.*[6]. The calibration of the spectropolarimeter (Cary 60 equipped with a model 6002 circular dichroism accessory) was carried out with D-camphor-10-sulfonic acid[7]. The points of the experimental spectrum have been corrected for the presented communication, because the experimental spectrum and the reference spectra were not affected by the same error. For the first approximation a "Du Pont 310 curve resolver" was used.

Results and Discussion

By means of the reference spectra of CHEN *et al.*[6] yielding 40 per cent helix, 18 per cent β-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

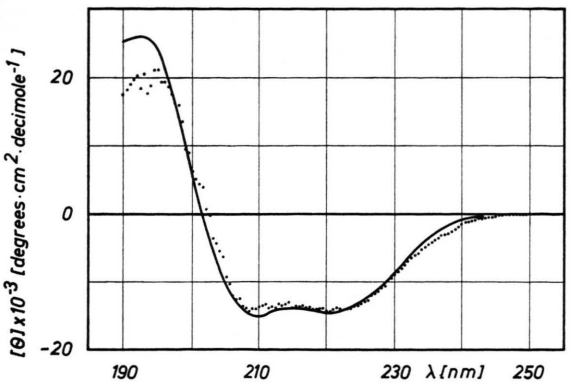

Fig. 1. Comparison of the experimental (....) with the calculated (—) circular dichroism spectrum (40 per cent helix, 42 per cent random coil, 18 per cent β-structure) using reference spectra derived from proteins by CHEN, YANG, and MARTINEZ.

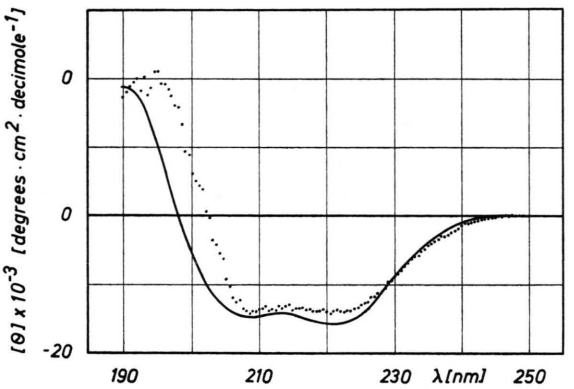

Fig. 2. Comparison of the experimental (. . . .) with the calculated (—) circular dichroism spectrum (42 per cent α-helix, 40 per cent random coil, 18 per cent β-structure) using polylysine as reference substance.

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 practized, one disregards, for the mentioned reasons, the short-wave region of the spectrum, a good approximation (Fig. 3) is reached

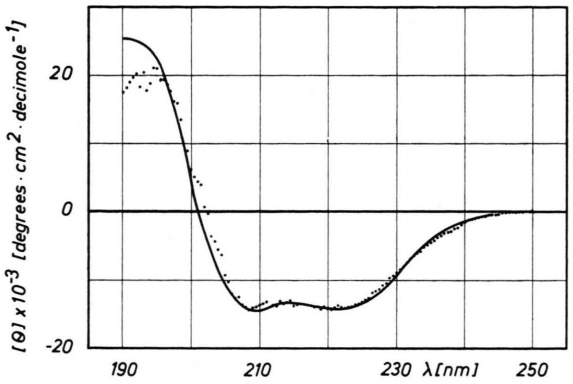

Fig. 3. With 40 per cent helix, 50 per cent random coil and 10 per cent β-structure a better fit is obtained between 200 and 250 nm than in Fig. 1.

with 40 per cent helix, 50 per cent random coil and 10 per cent β-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

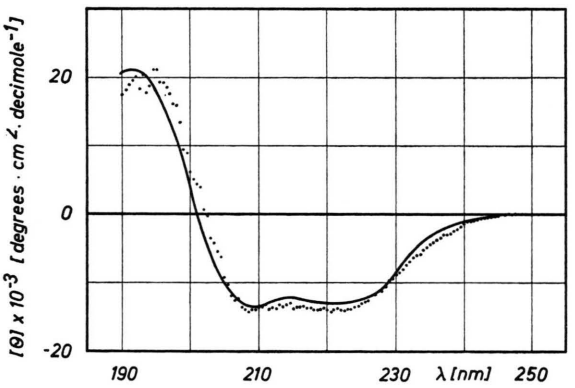

Fig. 4. A lowering of the 193 nm maximum is obtained by reduction of the helix content (34 per cent helix, 48 per cent random coil, 18 per cent β-structure).

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 β-structure.

A high helix and random coil content and a low content of β-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[9]. In addition the fragments, exhibited only photosystem I activity. Therefore, it appears doubtful wheter the results of the conformation analysis obtained with fragments are representative for the entity of proteins in the thylakoid membrane.

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