Abstract of the PhD Thesis

Fourier Transform Infrared and Electron Spin Resonance Spectroscopic Studies of Model and Biological Membranes

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Introduction

Membranes play a central role in both the structure and function of cells therefore the study of biomembranes has become a meeting ground for a number of diverse scientific disciplines ranging from biophysics to molecular biology.

Biomembranes are multicomponent dynamic systems taking part in the regulation of a number of cell processes. The major components of biological membranes are lipids and proteins. The primary role of lipids is to provide the structural framework, whereas function is mostly fulfilled by proteins. The mutual interactions of lipids and proteins are also of fundamental importance.

One of the most abundant biomembranes is thylakoid, which in the process of photosynthesis is able to transform the physical energy of light into chemical energy, and hence to organic compounds. If a leaf is grown in darkness, proplastids develop into etioplasts, which differ in structure and composition from mature chloroplasts. Upon illumination, etioplasts are transformed into chloroplasts. This transformation involves the synthesis of chlorophylls and many polypeptides, and a large reorganization of plastid structure (greening).

Besides the specific proteins and the pigments, the lipid composition of the thylakoid membranes is also uniquely specific. Monogalactosyl diacylglycerol (MGDG) and certain other lipids were shown to be involved in strong and specific lipid–protein interactions. The importance of phosphatidylglycerol (PG) – which accounts for 5–12% of the total lipid content – in the organization and functioning of thylakoid membranes has been demonstrated in several studies. For instance, correlation has been observed between the chilling sensitivity and the level of saturated and monounsaturated molecular species (known as ’high-melting-point’ molecular species) of PG for a wide variety of higher plants.

According to the importance of biological membranes, a high number of publications focused on the structure and dynamics, and on lipid–protein interactions of membranes, employing a wide variety of different physical techniques. Although much valuable information has been derived from such studies, many details are still to be explored.

Aims

In this work, mainly two complementary spectroscopic techniques, Fourier transform infrared (FTIR) and electron spin resonance (ESR) spectroscopy, were used to study membrane structure and dynamics, and lipid–protein interactions in different model and thylakoid membranes. The aims of these studies were as follows:

1. The development and validation of new methods to enhance the performance of FTIR and ESR spectroscopic techniques via
   - improved spectrum analyses studying the CH_2 stretching vibrations of lipids and ESR spectra of TEMPO spin-label,
   - the combination of complementary spectroscopic data,
2. to gain more insight into the molecular details of structural rearrangements in developing thylakoid membranes during greening, and
3. to obtain information on the structural role of PG in genetically manipulated tobacco thylakoids with different character of chilling sensitivity.

Methods

FTIR spectroscopy was used to study lipid acyl chain conformations, and protein structure and dynamics. Different model (phosphatidylcholines) and thylakoid membranes were investigated. Both the C–H stretching region and the 1500–1750 cm^{-1} region, containing the amide I–II bands and lipid carbonyl vibrations, were analysed in the FTIR spectra.

For ESR measurements, two spin-labels, 5-SASL and TEMPO, were applied and data related to membrane structure and dynamics were obtained by the analysis of their spectral parameters for barley thylakoids.

Biochemical and functional parameters of thylakoids (e.g. chlorophyll content, fatty acid composition) were determined according to literature methods.

Results

Methodological achievements

1. Development of a spectrum simulation and fitting program for the use of TEMPO spin-label was carried out. With the program, the partition of TEMPO between the aqueous

and the hydrocarbon phase of the membrane can be precisely determined from the experimental ESR spectrum. Thus it provides data on lipid packing and membrane fluidity. Furthermore, the rotational dynamics of TEMPO can also be characterized.

2. It was shown that the $\nu_{sym}CH_2$ band of the FTIR spectra can be resolved into two components, one related to the ordered, the other to the disordered segments of the hydrocarbon chains of lipids, and that the apparent temperature-induced frequency shifts of this band can be described by the competition of its two close-lying components. The method was applied on complex biological membranes as well.

Resolution of biological problems

Greening of barley thylakoids

3. An intersubtraction method was applied on the ESR spectra of 5-SASL spin-label. It provided an immobile and a mobile component spectrum, which could be used to determine the protein-associated lipid fraction in barley thylakoid membranes.
4. Our data suggest that a structural coupling between the major lipid and protein components develops during greening. The fatty acid composition and the stability of the newly synthesized light-related proteins was found to be changing in a concerted way – the appearance of more and more unsaturated lipids provides an appropriate environment for the formation of photosynthetically active protein assemblies with higher stability.
5. The formation of large protein complexes – most of them probably related to LHCII – was followed. It was shown that the mean oligomer size of the proteins increases about 7-fold during greening.
6. Three phases were proposed for the interpretation of the sequence of correlated molecular events during greening. In agreement with the Chl accumulation, greening starts with the onset phase, which is followed, by the rearrangement phase where the largest changes occur. The functional membrane is formed in the maturation phase.
7. The partial heat-denaturation of protein complexes seems to promote a large-scale lipid structural change in the thylakoid membrane – probably due to a lamellar to inverted hexagonal phase transition of the MGDG lipid species.

Genetically manipulated tobacco thylakoids

8. With the use of the two-component analysis of the $\nu_{sym}CH_2$ band, the structural role of PG molecules and their level of unsaturation have been documented on genetically manipulated tobacco plants. According to our FTIR data, PG is in intimate contact with the membrane proteins, it provides the proper dynamics of the lipid environment for the functioning of the proteins, but it is not responsible for maintaining their structural stability. It was shown that PG has a structural role, which could not have been detected by biochemical methods so far. This structural role provides an explanation for the physiological differences between the wild type and genetically manipulated tobacco plants.

Publications

Papers related to the thesis

I. Droppa, M., Kóta, Z., Páli, T., Szalontai, B., Horváth, L. I., and Horváth, G.
Structural–functional organization of thylakoids in developing chloroplasts
In *The Chloroplasts: From Molecular Biology to Biotechnology,* eds. Argyroudi-Akoyunoglou, J. H., Senger, H. (Kluwer Academic Publishers), pp. 55–60., 1999

II. Kóta, Z., Szalontai, B., Droppa, M., Horváth, G., and Páli, T.
Fourier transform infrared and electron paramagnetic resonance spectroscopic studies of thylakoid membranes
J. Mol. Struct. **480–481**, 395–400, 1999

III. Kóta, Z., Debreczeny, M., Szalontai, B.
Separable contributions of ordered and disordered lipid fatty acyl chain segments to νCH_2 bands in model and biological membranes: A Fourier transform infrared spectroscopic study
Biospectroscopy **5**, 169–178, 1999

IV. Kóta, Z., Szalontai, B., Droppa, M., Horváth, G., and Páli, T.
The formation of an inverted hexagonal phase from thylakoid membranes upon heating
Cell. Mol. Biol. Lett. **7**, 126–128, 2002

V. Kóta, Z., Horváth, L. I., Droppa, M., Horváth, G., Farkas, T., and Páli, T.
Protein assembly and heat stability in developing thylakoid membranes during greening
Proc. Natl. Acad. Sci. USA **99**, 12149–12154, 2002

VI. Szalontai, B., Kóta, Z., Nonaka, H., and Murata, N.
Structural consequences of genetically engineered saturation of the fatty acids of phosphatidylglycerol in tobacco thylakoid membranes. An FTIR study
Biochemistry **42**, 4292–4299, 2003

Other Papers

I. Kóta, Z., Páli, T., and Marsh, D.
Orientation and lipid–peptide interactions of gramicidin A in lipid membranes: polarised ATR infrared spectroscopy and spin-label electron spin resonance
Biophys. J., In press

II. Csányi, L. J., Jáky, K., Dombi, Gy., Evanics, F., Dezső, G., and Kóta, Z.
Onium-decavanadate ion-pair complexes as catalysts in the oxidation of hydrocarbons by O_2
J. Mol. Cat. A **195**, 101–111, 2003

III. Csányi, L. J., Jáky, K., Kóta, Z., and Páli, T.
Oxidation of hydrocarbons by O_2 in the presence of onium salts and onium ion-pair complexes as catalysts
J. Mol. Cat. A, In press

IV. Páli, T., Garab, G., Horváth, L. I., and Kóta, Z.
Functional significance of the lipid–protein interface in photosynthetic membranes
Cell. Mol. Life Sci. **60**, 1591–1606, 2003