THE FORMATION OF AN INVERTED HEXAGONAL PHASE FROM THYLAKOID MEMBRANES UPON HEATING

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Abstract: Barley thylakoid membranes were studied with FTIR and EPR spectroscopy. Thylakoids were exposed to elevated temperatures in order to induce structural changes. As temperatures increased through physiological to even higher levels, no features changed, but upon heating to above 45ºC, the fraction of lipid acyl chain segments with *gauche*-type vibration increased, accompanied by a sharp drop in the membranous spin probe component. These apparently conflicting observations in fact concur with the formation of an inverted hexagonal (H_{II}) phase, supporting its putative role in protecting the photosynthetic machinery in thylakoid membranes against thermally-induced disassembly.

Key Words: Photosynthesis, Non-Bilayer Lipid, Thylakoid, FTIR, EPR (ESR)

The light absorbing and energy converting machinery of photosynthesis is hosted in thylakoid membranes in which MGDG accounts for about 50 % of the total lipid content [1]. MGDG cannot form lamellar phases above its chain melting transition temperature when dispersed in water, due to its conical shape [2]. It is believed that MGDG is excluded from the thylakoid membrane at elevated temperatures, resulting in the formation of an inverted hexagonal (H_{II}) phase and morphological alterations of the chloroplast [3]. We investigated both lipid acyl chain (CH_2) vibrations, with FTIR spectroscopy, and the partitioning of TEMPO between the thylakoid membrane and the aqueous phase, with EPR spectroscopy, as functions of temperature. Barley thylakoid membranes were isolated from leaves as described in [4]. Samples for FTIR and EPR experiments and spectral analyses were done as in [5]. The symmetric stretching mode of the CH_2 groups (~2850 cm^{-1}), dominated by lipid acyl chain vibrations in a biomembrane [6], was analyzed to separate the contributions of *gauche* and *trans* vibration character [7]. TEMPO partitions between the membranous and

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Abbreviations: TEMPO, 2,2,6,6,-tetramethylpiperidine-1-oxyl; EPR, electron paramagnetic resonance; FTIR, Fourier transform infrared; MGDG monogalactosyl diacylglycerol.

aqueous phases which leads to separated spectral components in the EPR spectrum (*M* and *W*). The partitioning depends on the state of the bilayer [8] and can be described, using signal amplitudes, as *M*/(*M*+*W*). These parameters are shown in Fig. 1.

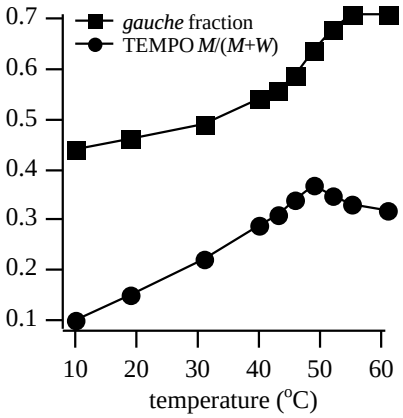

Fig. 1. Temperature dependenc of the acyl *gauche* fraction of membrane lipids (squares) and the membrane-associated TEMPO fraction (circles) in aqueous dispersions of thylakoid membranes isolated from barley leaves.

Neither TEMPO nor the *gauche* fraction indicates transition-like changes below 46ºC. A high wavenumber for the symmetric CH_2 vibration band, and a correspondingly high *gauche* content [7], was reported for the H_{II} phase in model systems [6]. Therefore, the changes in the parameters above 49ºC in the opposite sense can indeed be assigned to the formation of the H_{II} phase, assuming that TEMPO does not partition into it. This assumption is very likely to hold, considering the tightly packed headgroup region in the H_{II} phase [2]. Since MGDG is excluded together with its poly-unsaturated acyl chains, and considering that the light harvesting complex has high specificity towards MGDG [9], it is likely that the exclusion of these lipids leaves the thylakoid membrane in a more rigid state to delay the complete disassembly of the antenna complexes upon heating.

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