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Changed lateral migration of phospho-LHCII in the thylakoid membrane upon acclimation of spinach to low temperatures

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Movement of proteins along the plant thylakoid membrane is of importance for several physiological events, such as state transitions and turnover and repair of the photosystem II complex. Such lateral migrations are impaired at low temperatures, which could contribute to the increased sensitivity of plants to photoinhibitory damages at low temperatures. The migration behaviour of phospho-LHCII in thylakoid membranes isolated from cold-acclimated spinach was studied and compared to that in control membranes. The rate of migration of phospho-LHCII at low temperatures is increased 2- to 3-fold and the apparent activation energy of the migration is decreased after the cold acclimation.

Cold acclimation; Lateral migration; Membrane fluidity; Phospho-LHCII; Thylakoid membrane

1. INTRODUCTION

Protein phosphorylation of the major light-harvesting chlorophyll *a/b* protein complex of photosystem II (LHCII) and the accompanying reduction in the photosystem II (PSII) antenna are regarded as mechanisms involved in the short-term [1,2] and possibly long-term [3] regulation of light harvesting, as well as in the protection of PSII against photodamage [4]. Phosphorylation of LHCII is catalyzed by a membrane-bound kinase that is controlled by the redox state of the plastoquinone pool [5,6] and the cytochrome *b/f* complex [7–10]. The rearrangements in the light-harvesting antenna following its phosphorylation include the dissociation of a specific subpopulation of phospho-LHCII from PSII and the lateral migration of this population from the grana stacks and their margins [11] to the PSI-rich stroma-exposed thylakoid membrane regions [12,13].

In a recent communication [14] protein phosphorylation in spinach thylakoids at low temperature was studied in vitro. It was found that the kinase is still quite active at 0°C but that the rate of the subsequent lateral migration of phospho-LHCII is severely impaired at temperatures below 10°C, as judged by subfractionation experiments. Moreover, there is no decrease in the functional antenna size of PSII below that temperature. However, when the temperature is raised above 10°C after protein phosphorylation in the cold, the migration of phospho-LHCII and the accompanying reduction of the PSII antenna readily occur. This subfractional approach has been used to determine the rate and temperature dependence of the migration of phospho-LHCII [14], as well as to analyze its diffusion characteristics by Monte-Carlo simulation [15].

In cold climates plants regularly experience temperatures where the effective viscosity of the thylakoid membrane will be reduced, and consequently the lateral migration of phospho-LHCII severely restricted. Such limitations are likely to be of significance for the increased sensitivity to photoinhibition at low temperatures [16,17], due to impairment of protective rearrangements in the antenna, as well as events involved in biosynthesis and assembly required for repair of PSII. It is consequently of interest to investigate whether any acclimation with respect to such migratory processes takes place when plants are grown at low temperatures.

2. MATERIALS AND METHODS

Cold acclimation of spinach was performed using 5-week-old plants essentially as described in [18]. The temperature of growth was successively decreased down to 2–3°C night/5–6°C day, over a period of 10 days. The light regime was 250 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$. The spinach was kept in the cold for an additional 4–5 days before use. Control plants were grown at 25°C under the same light regime.

Thylakoids were prepared as described earlier [19] and suspended in 50 mM Tricine, pH 7.8, 0.1 M sorbitol, 20 mM NaCl and 5 mM $MgCl_2$. Protein phosphorylation at −2°C and digitonin fractionation of thylakoid membranes were performed as described in [14]. The thylakoid membranes were phosphorylated at 300 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$ in the resuspension medium, at a chlorophyll concentration of 0.4 mg/ml in the presence of 0.4 mM ATP containing [γ-^{32}P]ATP (0.02 mCi/mg chlorophyll) and 10 mM NaF. Thylakoid subfractionation was performed at a digitonin concentration of 0.5–1%/mg chlorophyll and the stroma-exposed thylakoid membranes were collected by differential centrifugation [14]. SDS-PAGE was carried out according to [20] using a 12–22% polyacrylamide gradient in the separation gel. The gels were autoradiographed and the films were analyzed by laser densitometry.

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Chlorophyll *a/b* was determined according to Arnon [21], the amount of protein according to Markwell et al. [22], and total lipid content as described in [23].

3. RESULTS

For the purpose of studying acclimation of phospho-LHCII migration to low temperatures, spinach was grown in the cold [18]. An analysis of some of the general properties of the thylakoid membranes isolated from cold-acclimated and control spinach revealed no major differences with respect to either PSII or whole-chain electron transport. Thylakoids isolated from cold-grown plants had a somewhat higher chlorophyll *a/b* value (3.1 ± 0.1) than control-spinach (3.0 ± 0.1). No difference in the yield and the chlorophyll *a/b* values of the stroma lamellae membranes were found. The protein:chlorophyll ratio of the thylakoid membrane showed a decrease from 6.6 to 5.3 upon cold acclimation, whereas the protein:lipid ratio remained constant. The rates and degree of protein phosphorylation, as well as the pattern of phosphoproteins were virtually identical for thylakoids isolated from both control and cold-acclimated spinach.

The lateral migration of phosphorylated LHCII along the stacked thylakoid membrane in acclimated spinach thylakoids was analyzed and compared to that in plants grown at normal temperature (Fig. 1). To this end, protein phosphorylation was performed at −2°C. As indicated above, at this low temperature a substantial degree of phosphorylation still takes place while the rate of lateral migration is very slow (Fig. 1). Subsequent to the protein phosphorylation at −2°C, the temperature of the thylakoid suspension was quickly raised to the desired values. At specified time points samples were withdrawn, subjected to digitonin-mediated fragmentation and the stroma thylakoid membranes were isolated by differential centrifugation, analyzed for their relative content of phospho-LHCII, which was finally plotted against temperature (Fig. 1).

In control thylakoid membranes only very low levels of phospho-LHCII appear at temperatures below 10°C, in accordance with our previous study [14]. Notably, however, the rate of appearance of phospho-LHCII in the stroma-exposed membranes in thylakoids from the cold-acclimated spinach is 2- to 3-times higher at the lower temperatures. The most obvious difference is seen at 10°C, where the rate in the acclimated spinach thylakoids is almost maximal, whereas it is still very slow in the normal thylakoid membranes. As the temperature is raised the difference becomes less pronounced, and at 20°C the rates are more-or-less equal in the two samples. There are even indications that the migration is more restricted in the acclimated than in the control membranes at room temperature.

Fig. 2 depicts the complete set of data used in Fig. 1 transformed into an Arrhenius plot. In this kind of plot, the slope is related to the apparent activation energy of the overall process, which in turn is mainly dependent on the activation energy of the rate-limiting step of the process. For control spinach thylakoids, the good linearity ($R = 0.97$) of the experimental data, indicates that no phase transitions that could influence the LHCII migration takes place within the temperature interval

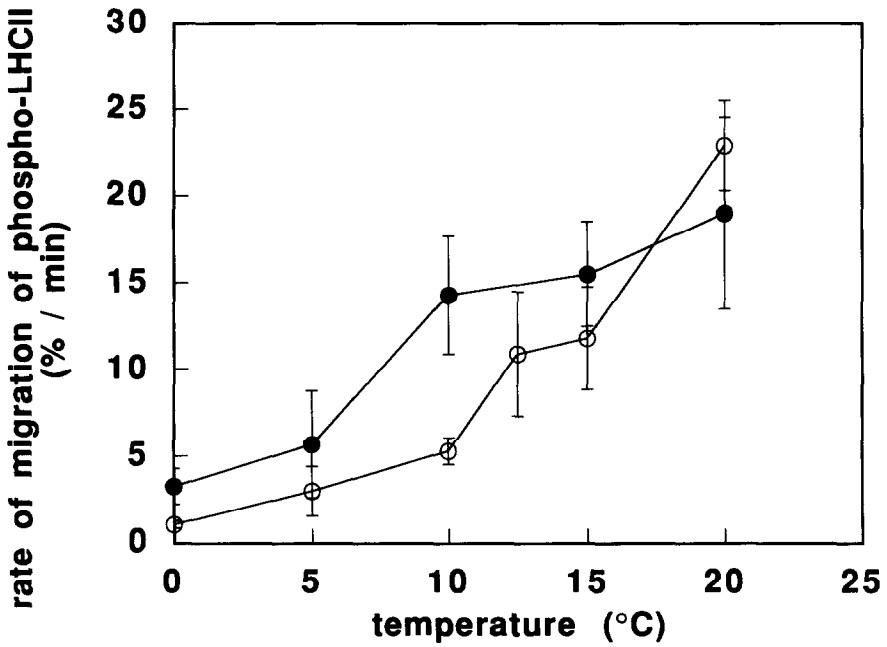

Fig. 1. Initial rate of migration of phospho-LHCII as a function of temperature. ○, control thylakoid membranes; ●, thylakoid membranes isolated from cold-acclimated spinach. The rate is expressed as percent per minute of the relative amount of phospho-LHCII that has appeared in the stroma thylakoid membranes after 15 min at 20°C (100%). Each point represents the mean of 2–5 experiments. The error bars show the standard deviation.

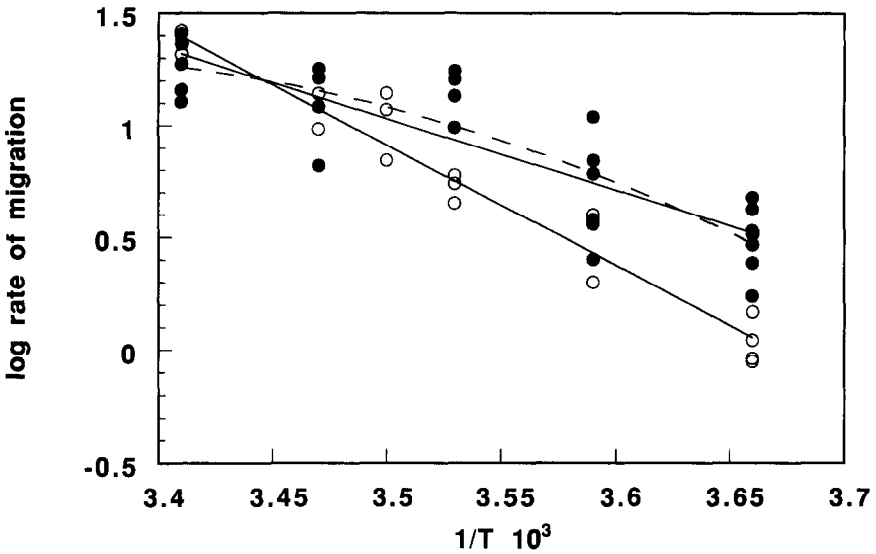

Fig. 2. Arrhenius plot of the rate of migration as a function of temperature. ○, control thylakoid membranes; ●, thylakoid membranes isolated from cold-acclimated spinach. Solid lines, linear fit to experimental data from control and acclimated membranes, respectively. Dashed line, non-linear fit to the data from acclimated membranes.

studied, since such changes would give rise to discontinuities in the slope. This is in agreement with studies by Murata and co-workers showing that no gel-phase formation occurred between 5°C and 40°C in any of the lipid classes that were isolated from spinach thylakoids [24]. The lateral migration, furthermore, appears to be controlled by one single rate-limiting step under these conditions.

In the case of cold-acclimated membranes, the migration behaviour appears to be more complicated (Fig. 2). A limited regression analysis shows only a slightly better fit for a simple polynomial curve ($R = 0.86$) than for a straight line ($R = 0.85$). If we assume a linear dependence in both cases, the apparent activation energies can be calculated from the slopes of the appropriate lines in Fig. 2. According to these calculations, cold acclimation decreases the apparent activation energy for the migration of phospho-LHCII into the stroma-exposed thylakoid regions from 24.5 to 14.6 kcal · mol^{-1}. On the other hand, if we assume a non-linear temperature dependence of the migration in cold-acclimated membranes, this would indicate that the migration process is no longer controlled by a single rate-limiting step. Such a situation could, for example, occur if the dissociation from PSII and the subsequent diffusion of phospho-LHCII both contribute significantly to the apparent migration rate. Alternatively, there could be different populations of phospho-LHCII migrating with different temperature dependencies. Irrespective of the reason for a possible non-linear temperature dependence, the activation energy is significantly decreased in the acclimated thylakoids, especially at temperatures above 10°C.

4. DISCUSSION

According to our earlier observations [14], a decrease in the functional antenna size of PSII due to light-induced phosphorylation, and the subsequent lateral movement of LHCII, will virtually not occur at temperatures below 10°C in normal spinach thylakoid membranes. Apart from reducing the plant's capacity to optimize the capture of excitation energy, this impairment could also lead to a higher risk for over-excitation of PSII and be of significance for the increased sensitivity to photoinhibition of plants in the cold [16,17]. Furthermore, the slow migration of phospho-LHCII at low temperatures also reflects a general decrease in the effective viscosity of the thylakoid membrane, and is consequently of relevance for a number of other migration events, such as those associated with turnover and biosynthesis of PSII: in particular, transport of newly synthesized proteins from the stroma exposed to the appressed thylakoid regions, as has been shown to occur for the D1 protein [26] and subunits of LHCII [27]. In the latter case accumulation of newly synthesized LHCII has been shown to occur in the cold.

In the present investigation we show that when spinach is grown in the cold, an acclimation of the lateral migration of phospho-LHCII takes place (Figs. 1 and 2). The result of this acclimation is a 2- to 3-fold increase in the rate of appearance of phospho-LHCII in the non-appressed thylakoid membrane regions at low temperatures (Fig. 1). However, in spite of this acclimation, the migration rate at the temperature of growth for the two spinach populations is still lower in the acclimated membranes than in the control membranes, indicating

that the physiological compensation for the lowered temperature is only partial. On the other hand, at 10°C the rate of migration appears to already have approached the maximal rate in the acclimated thylakoid membranes, while it is still very low in control thylakoids.

Apart from a general increase in the migration rate, it is also found that the movement of phospho-LHCII in the acclimated membranes is less temperature dependent and consequently has a lower apparent activation energy than that in the control membranes (Fig. 2).

There are several reports in the literature on the correlation between cold tolerance of plants and fatty acid composition of their membrane lipids (e.g. [24]). However, in contrast to what has been found for cyanobacteria [28], growth at low temperatures seems to have a relatively limited effect on the unsaturation level of plant thylakoid lipids [29,30]. This has been confirmed also for the spinach used in the present experiments (Trémolières, A., Carlberg, I. and Andersson, B., unpublished). In spite of this, plants have been shown to increase their membrane fluidity in response to cold treatment, as judged by fluorescence polarization measurements of the hydrophobic probe 1,6-diphenyl-1,3,5-hexatriene [25]. This is consistent with the fact that the effective membrane viscosity is not only a consequence of the unsaturation level of the lipids but also depends markedly on other factors, such as lipid–protein interactions [31]. In addition the migration of a relatively large protein molecule in a biomembrane is most likely strongly influenced by protein–protein interactions. Recent estimates of the diffusion rate for phospho-LHCII in spinach thylakoid membranes indicate that it is a relatively slow process ($K_d = 2\text{–}4 \times 10^{-12} \cdot cm^2 \cdot s^{-1}$) [15]. This, as well as the relatively high activation energies (24.5 and 14.6 kcal $\cdot$ mol^{-1}) for the migration found in the present work, indicates strong interactions between protein complexes and proteins and lipids in the membrane as discussed in [15]. In a recent study on the lateral diffusion of ubiquinone in the mitochondrial inner membrane, Chazotte and Hackenbrock have shown that a decreased protein content leads to a decrease in the temperature dependence and the activation energy of the diffusion [32]. Although cold acclimation in some plants, as well as in cyanobacteria, has been reported to be accompanied by an increased lipid-to-protein ratio [28,29], our present observations do not reveal any major changes in the amount of lipid per protein in the case of spinach during the acclimation process. This does not, however, rule out specific or localized changes in the relative content of some of the minor lipids. Li et al. observed a preference for phosphatidylglycerol in lipid–protein interactions in cold-tolerant plants [33], and Trémolière et al. have suggested that phosphatidylglycerol (PG) with *trans*-16:1 is functionally associated with LHCII [34]. In the case of cereals, Huner et al. have shown that cold acclimation leads to a decrease in the content of the *trans*-16:1 fatty acid with a concomitant in vitro reorganization in LHCII [30]. However, this decrease in *trans*-16:1 of PG appears to be species specific and has not been observed in spinach [30].

At present, the specific molecular changes in the thylakoid membrane underlying the increased rate at low temperatures and decreased apparent activation energy of lateral migration of phospho-LHCII in cold-acclimated spinach thylakoids remain unknown and thus further experimentation is needed. Despite this, our study points to a physiological response that allows for an acclimation of the lateral migration of proteins in the thylakoid membrane at low temperatures, which should be of significance for both short- and long-term regulation of light harvesting, as well as biosynthetic events such as repair of photodamaged PSII reaction centres.

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