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Effect of trivalent lanthanide cations on chlorophyll fluorescence and thylakoid membrane stacking

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We have used trivalent lanthanide metal cations in the buffering media of pea chloroplasts to probe the stacking arrangement of thylakoid membranes and the spatial distribution of chlorophyll-protein complexes of Photosystems I and II. Measurements of steady-state chlorophyll fluorescence emission spectra of pea chloroplasts at room temperature demonstrate that, within this tripositive valency group, the extent of membrane appression is a function of hydrated metal ionic radius. These results are in agreement with a recent investigation using monovalent and divalent metal cations (Karukstis, K.K. and Sauer, K. (1985) Biochim. Biophys. Acta 806, 374–389). In addition, the lanthanide cation concentration effective in producing the maximum chlorophyll fluorescence intensity upon grana formation is dependent on hydrated ionic size. The current investigation supports the proposed hypothesis that cation screening ability defines the extent of intermembrane separation as well as the extent of lateral distribution of chlorophyll-protein complexes.

Introduction

Thylakoid membrane appression via electrostatic screening can be experimentally induced by varying the cationic composition of the chloroplast suspension medium [1,2]. High ionic strength media preserve the in vivo structural differentiation of thylakoid membranes into stacked (grana lamellae) and exposed (stroma lamellae) regions [3–5]. In addition, a lateral distribution of three supramolecular chlorophyll-protein complexes between grana and stroma regions is also maintained. In the grana partitions, an enrichment occurs of light-harvesting chlorophyll complexes (LHC II) and chlorophyll core complexes associated only with Photosystem II (PS II). In contrast, stroma-exposed thylakoids are enriched in Photosystem I (PS I) chlorophyll antenna complexes [6]. Thus, the structural differentiation of higher plant chloroplasts into grana stacks and stroma-exposed thylakoids is paralleled by a functional differentiation. Chloroplast incubation in low ionic-strength media fully unstacks thylakoid membranes and randomly distributes PS I, PS II, and LHC I and II complexes along the membrane plane.

Theoretical analyses [2,7] suggest that electrostatic screening of fixed negative charges on the thylakoid membrane surface by added cations is a possible mechanism for the reversible stacking and unstacking of chloroplast membranes and the associated fluorescence variations [1]. Such electrostatic screening to reduce coulombic repulsion between membrane surfaces also encourages aggregation of complexes within the membrane plane

Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_{max}, maximum fluorescence level of chlorophyll fluorescence with Photosystem II electron acceptor Q reduced; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LHC, light-harvesting chlorophyll *a*/*b*-protein complex; PS I, II; Photosystem I, II.

itself. Supporting experimental evidence for an electrical mechanism of membrane appression is based on the observation of a differential effect in restacking of monovalent and divalent cations, with divalent cations (3–5 mM) more effective than monovalent cations (50–100 mM) in this reconstitution [8,9]. With regard to extent of screening, a general lack of specificity between species of a particular charge group has generally been considered as characterisitc of effects mediated via the membrane surface potential [7]. However, recent studies by this investigator [1] have observed a specific dependence of the extent of stacking on the cation identity as reflected in cation-induced chlorophyll fluorescence intensities. Structural variations are proposed to be a consequence of differences in the screening ability of metal ions within the same valency group as influenced by the hydrated metal ionic radius. In general, ions with smaller hydrated ionic radii exhibit more effective electrostatic screening.

To characterize further the cation effect on chloroplast photoprocesses, the current investigation tests the hypothesis correlating the extent of cation-induced variation of chlorophyll fluorescence with cation screening ability by extending the study to a series of trivalent metal ions of the lanthanide elements. For lanthanides, the radii of the hydrated ions vary in the same manner as the crystal ionic radii [12], decreasing with increasing atomic number. Correspondingly, the observed chlorophyll fluorescence level upon lanthanide incubation under electrostatic screening conditions is expected to be at a maximum for La^{3+} and decrease with increasing atomic number. This investigation focuses on the following questions. (1) Can differences among lanthanide ion effects on the room-temperature PS II chlorophyll fluorescence of plant chloroplasts be distinguished? (2) Do these differences in cation effects correlate with the previously observed results [1] in which larger hydrated metal ions induce more pronounced fluorescence variations upon cation addition? (3) Furthermore, can distinct effects of lanthanide ions on chlorophyll fluorescence be discerned at different levels of added cation? The results of this investigation are used to validate the current model of the origin of cation-induced chlorophyll fluorescence variations.

Materials and Methods

Chloroplasts were isolated from freshly harvested growth-chamber peas in a medium containing 0.4 M sucrose/50 mM Hepes-NaOH (pH 7.5)/10 mM NaCl. After centrifugation at $6000 \times g$ for 10 min and washing with the same medium, the chloroplasts were resuspended in a medium of 0.1 M sucrose/10 mM Hepes-NaOH (pH 7.5)/10 mM NaCl. Following centrifugation at $6000 \times g$ for 10 min, the pellet was resuspended in the same medium, kept in the dark for 15 min at approx. 0°C, and then a similar centrifugation step was performed. Portions of this final pellet were then resuspended in a medium of 0.1 M sucrose/50 mM Hepes-NaOH (pH 7.5) at the desired concentration of metal cation to give approximately 1 mg Chl per ml. The lanthanides considered include the chloride salts of La^{3+}, Pr^{3+}, Eu^{3+}, Tb^{3+} and Dy^{3+} in the concentration range of 0–100 μM. The chloroplasts were allowed to equilibrate in these buffers for 30 min to ensure complete changes in stacking and reorganization of chlorophyll-protein complexes [13]. For fluorescence measurements the chloroplast suspension was diluted with this final buffer to a concentration of 10 μg Chl per ml.

Room temperature fluorescence emission spectra were measured with a Perkin-Elmer LS-5 fluorescence spectrophotometer. Chlorophyll fluorescence was induced by excitation at 620 nm and detected over the range of 650 to 750 nm. Maximum fluorescence emission occurred at 684 nm. Previous studies [1] demonstrated that variations in cation-induced PS II chlorophyll fluorescence intensities are most pronounced at the F_{max} level when the PS II electron acceptor Q is reduced [1]. To ensure that all measurements were made at the F_{max} level of fluorescence, 10 μM DCMU was added to each sample [14]. For experiments examining the concentration dependence of lanthanide effects, 25 μM 9-aminoacridine was added to chloroplasts. 9-Aminoacridine fluorescence was excited at 398 nm and detected over the range of 420 to 620 nm, with a maximum emission at 455 nm.

Results

Fig. 1 presents the chlorophyll fluorescence emission spectra of pea chloroplasts incubated in the presence of 20 μM La^{3+}, Eu^{3+} and Tb^{3+} and of pea chloroplasts under low (5 mM NaCl) and high (5 mM $MgCl_2$) ionic strength conditions. Of the spectra presented for lanthanide-enriched chloroplasts, La^{3+} induces the largest increase in chlorophyll fluorescence intensity at all wavelengths and Tb^{3+}, the smallest. All lanthanide-induced chlorophyll fluorescence levels in the presence of 20 μM cation are lower than the chlorophyll fluorescence level of Mg^{2+}-enriched (5 mM) chloroplasts.

The F_{max} 684-nm peak chlorophyll fluorescence intensity as a function of added lanthanide concentration is plotted in Fig. 2 for La^{3+}-, Pr^{3+}-, and Dy^{3+}-incubated chloroplasts. The data reveal an additional phenomenon – the lanthanide cation concentration effective in producing the maximum chlorophyll fluorescence level increases as hydrated ionic size decreases. For the lanthanides used in this investigation, the maximum intensity of cation-induced chlorophyll fluorescence occurs at approx. 20 μM La^{3+}, 50 μM Pr^{3+}, 60 μM Eu^{3+}, and at least 100 μM Tb^{3+} and Dy^{3+}.

The maximum relative chlorophyll fluorescence intensities induced by the lanthanide-enriched pea chloroplasts are summarized in Table I. The maximum chlorophyll fluorescence level achieved upon lanthanide incubation decreases as the lanthanide atomic number increases, that is, as the hydrated lanthanide ionic radius decreases. In the given concentration range, the largest hydrated lanthanide ion, La^{3+}, induces a maximum chlorophyll fluorescence increase of 1.63-fold at 684 nm, while the smallest hydrated lanthanide cation studied, Dy^{3+}, induces a maximum chlorophyll increase of only 1.18-fold at this same wavelength.

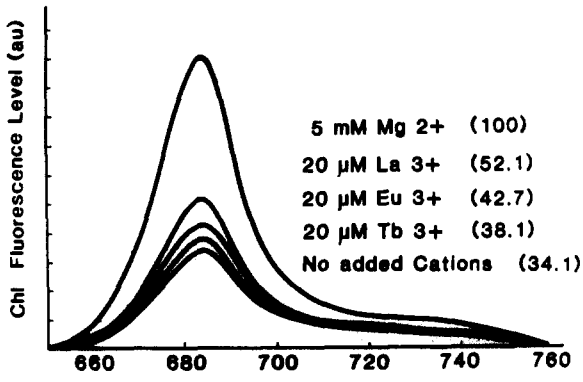

Fig. 1. Chlorophyll fluorescence emission spectra of pea chloroplasts at F_{max} as a function of ionic composition of the chloroplast suspension medium.

TABLE I

MAXIMUM RELATIVE CHLOROPHYLL FLUORESCENCE INTENSITIES OF PEA CHLOROPLASTS INCUBATED IN TRIVALENT LANTHANIDE-ENRICHED MEDIA

These data show the effects of added trivalent lanthanide cation on the 684-nm F_{max} chlorophyll fluorescence intensity of pea chloroplasts. The fluorescence intensity levels are normalized such that the maximum chlorophyll fluorescence intensity at 684 nm in the presence of 20 μM La^{3+} equals 100. The lanthanide cation concentration effective in producing the maximum chlorophyll fluorescence level is also tabulated. The relative chlorophyll fluorescence intensities in the absence of added lanthanide ion under low ionic strength conditions (5 mM NaCl only) is 61.4.

Ln^{3+} cation	Maximum intensity of chlorophyll fluorescence	Approx. concentration for fluorescence maximum (μM)
La^{3+}	100	20
Pr^{3+}	87.1	50
Eu^{3+}	82.2	60
Tb^{3+}	76.7	$\geqslant$100
Dy^{3+}	72.4	$\geqslant$100

The data of Table I reveal a variation in the lanthanide concentrations which induce the maximum chlorophyll fluorescence intensities upon grana formation. To examine this phenomenon further, we used the diffusible fluorescent dye 9-aminoacridine as a probe of the electrical properties of the thylakoid membrane surfaces. Searle et al. [15] have demonstrated that 9-aminoacridine fluorescence is quenched in unstacked chloroplasts when the protonated monovalent cation of 9-aminoacridine is in the vicinity of negatively charged thylakoid membrane surfaces. Addition of cations to the chloroplast suspending medium displaces 9-aminoacridine from the membrane surface and restores the 9-aminoacridine fluorescence level. Table II summarizes the present study of 9-aminoacridine fluorescence intensities in the

TABLE II

MAXIMUM RELATIVE 9-AMINOACRIDINE FLUORESCENCE INTENSITIES IN CATION-ENRICHED PEA CHLOROPLASTS

These data show the effect of cations on the 455-nm 9-aminoacridine fluorescence intensity of 9-aminoacridine-incubated pea chloroplasts. The fluorescence intensity levels are normalized such that the maximum 9-aminoacridine fluorescence intensity at 455 nm in the presence of 5 mM Mg^{2+} equals 100.

Cation	Concentration (μM)	9-Aminoacridine fluorescence intensity maximum
Mg^{2+}	0	45
	5000	100
La^{3+}	0	45
	20	64
	100	59
Tb^{3+}	0	45
	50	49
	100	56

presence of La^{3+} and Tb^{3+} cations at selected concentrations. For reference, we present data for Mg^{2+}-enriched chloroplasts which are in excellent agreement with those of the earlier studies [15].

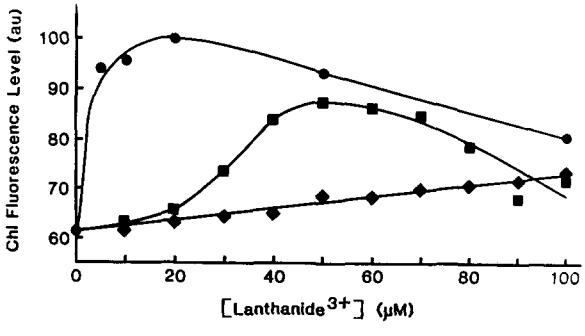

Fig. 2. Chlorophyll fluorescence intensity at 684 nm of pea chloroplasts at F_{max} as a function of added trivalent lanthanide concentration. The symbols are defined as follows: La^{3+}-incubated chloroplasts (●); Pr^{3+}-incubated chloroplasts (■); Dy^{3+}-incubated chloroplasts (◆).

Discussion

Recent studies involving La^{3+} [1,10] suggest that lanthanide cations induce grana formation in chloroplasts via both electrostatic screening and charge neutralization mechanisms. At low concentrations (up to 20 μM), La^{3+} cations mimic the action of screening cations (e.g., Na^{+} and Mg^{2+}) in promoting thylakoid stacking and in increasing chlorophyll fluorescence. At higher concentrations, La^{3+} ions presumably bring about surface charge neutralization by binding to the thylakoid membrane. The accompanying membrane stacking is associated with a less pronounced increase in chlorophyll fluorescence [1,2,10,11].

The variations observed in lanthanide-induced chlorophyll fluorescence under electrostatic screening conditions (i.e., low lanthanide concentrations) follow the proposed model: as the hydrated ionic radius decreases, the induced PS II chlorophyll fluorescence decreases. These results confirm that the effectiveness of an ion to reduce membrane surface potential is directly related to the distance of closest approach. The smaller the hydrated ionic radius, the closer the approach and the more effective the electrostatic screening.

The data of Fig. 2 are also consistent with the hypothesis of concentration-dependent mechanisms of grana formation by added lanthanide cations [1,10]. The present study demonstrates that the threshold cation concentration of electrostatic screening varies with the lanthanide ion hydrated radius. The 9-aminoacridine studies of La^{3+}- and Tb^{3+}-enriched chloroplasts in Table II also support a change in membrane surface electrical properties with lanthanide concentration. La^{3+} cations, which induce a maximum chlorophyll fluorescence intensity at 20 μM and increasingly lower intensities at higher concentrations, also induce a lowering of 9-aminoacridine fluorescence as La^{3+} concentration is increased from 20 to 100 μM. This decrease in the extrinsic 9-aminoacridine fluorescence at higher La^{3+} concentration is consistent with increased access of the 9-aminoacridine cations to the chloroplast membrane surface as La^{3+} concentration is increased [15]. Tb^{3+}-enriched chloroplasts, which do not exhibit a saturation in chlorophyll fluorescence intensity over the concentration range studied, show a correspond-

ing increase in 9-aminoacridine fluorescence intensity at higher Tb^{3+} concentrations. This effect is consistent with increased displacement of 9-aminoacridine from the vicinity of the membrane surface as more pronounced electrostatically-controlled grana formation occurs at higher Tb^{3+} concentrations.

The significance of these findings is in the revelation of the shortcomings of the current theory used to describe electrical phenomena at the surface of thylakoid membranes [7]. In this theory counterions at the charged membrane surface are considered to be point charges which therefore can approach the membrane at any distance. Thus, any phenomena governed in a straightforward manner by the surface potential resulting from negative charges on the membrane should, for a particular valency group, be independent of the chemical nature of the cation. The extent of membrane stacking and the observed chlorophyll fluorescence intensity would then be expected to be related only to cation valency and to be essentially independent of the cation identity. Consequently, the size-dependent behavior which is observed in this study and in a previous investigation [1] indicates that the above theory represents an oversimplification. In practice, the closest plane of approach of an ion is limited by its finite size and its extent of hydration. Although cation valency effects dominate, ignoring consideration of hydrated ionic size can lead to only semiquantitative approximations of cation effects on thylakoid membranes.

In conclusion, we have investigated the cation-induced variations in chlorophyll fluorescence of pea chloroplasts resulting from grana formation via electrostatic screening by added trivalent lanthanide ions. We have observed that these tripositive metal ions differ in electrostatic screening ability as influenced by the hydrated metal ionic radii. Specificially, we have noted that the smaller the hydrated lanthanide ionic radius, the lower the cation-induced chlorophyll fluorescence intensity. These results reveal the importance of hydrated metal ionic size as a factor in determining the spatial organization of thylakoid membranes. The clearer delineation of the role of metal cations in photosynthetic membrane organization is a significant step toward further understanding of the physicochemical aspects of photosynthesis.

Acknowledgments

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