CATION-INDUCED CHANGES IN THE CIRCULAR DICHROISM SPECTRUM OF CHLOROPLASTS

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1. Introduction

Circular dichroism studies of bacterial reaction center complexes [1,2] and of sub-chloroplast particles enriched in pigment system I [3] led to the general acceptance of an exciton interaction within the reaction centers. However, there has been little success in the interpretation of the circular dichroism (CD) spectra of broken chloroplast suspensions. Such spectra not only appear more intense on a chlorophyll basis than those from reaction center complexes, but they are also qualitatively different in their shape [4,5]. Philipson and Sauer [4] suggested that differential light scatter is responsible for the observed CD pattern of chloroplasts. In an opposing view, Gregory and Raps [5] suggested that the intense circular dichroism of chloroplasts reflects specific chlorophyll–chlorophyll interactions. Raps and Gregory [6] further suggested that the chloroplast CD may be a reflection of the extent of grana stacking due to the interaction of chlorophyll molecules in the juxta positioned membranes. Gregory [7] has further argued, based upon the observation of a light-induced reversible change in the presence of electron transport reagents, that the CD is an indicator of chlorophyll movement, or changes in the thylakoid–thylakoid distance, possibly related to ion movement.

In this communication chloroplasts from spinach are shown to exhibit CD spectra which are highly sensitive to the presence of low concentrations of mono- and divalent cations (when measured with the sample placed 10 or 20 cm from the photomultiplier). In spinach chloroplasts suspended in a low ionic strength medium, the addition of 3 mM NaCl diminished the intensity of the 676 nm CD peak while further addition of 3 mM $MgCl_2$ reversed this effect. Comparison of these results with those of Gross and Prasher [8] suggest that the CD spectrum, measured under the conditions stated, is a fingerprint of the degree of stacking of chloroplast membranes. Further experiments on lettuce chloroplasts indicated that the CD spectrum of these chloroplasts suspended in low ionic strength medium was sensitive only to the addition of low concentrations of divalent cations. These effects, however, are suggested to be due mainly to changes in differential selective light-scattering.

2. Materials and methods

Chloroplasts from spinach and lettuce were prepared by the method of Gross and Prasher [8] and suspended in a low ionic strength medium (100 mM sucrose buffered to pH 7.8 with Tris). The CD spectrum of the samples (containing 10 to 14 μg chlorophyll per ml of suspension) was measured in the red region (620-700 nm) of the spectrum using the JASCO Model J40A Automatic Recording Spectropolarimeter.

3. Results and discussion

Faludi-Daniel et al. [9] studied granal and agranal chloroplasts of Maize and suggested that CD was a tool capable of providing information regarding the stacking capacity of the thylakoids. More recently,

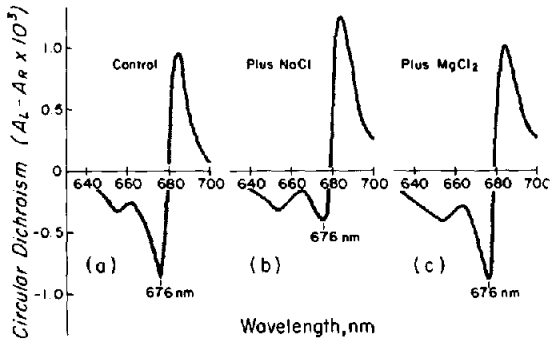

Fig.1. Effect of mono- and divalent cations on the circular dichroism (CD) spectrum of spinach chloroplasts. (a) CD spectrum of chloroplasts suspended in 100 mM sucrose buffered to pH 7.8 with Tris, (b) plus 3 mM NaCl, (c) plus 3 mM NaCl and 3 mM $MgCl_2$. Sample was placed at 10 cm from the photomultiplier.

Gross and Prasher [8] studied the effects of monovalent and divalent cations on the stacking of thylakoid membranes in spinach chloroplasts. They showed, using electron microscopy, that spinach chloroplast membranes suspended in a low ionic environment were stacked; addition of monovalent cations (3 mM) led to unstacking while further addition of divalent cations (3 mM) led to re-stacking. Our investigation (fig.1) of spinach chloroplasts under similar conditions indicates an apparent correlation between the degree of stacking of the membranes and the intensity of the 676 nm circular dichroism peak when measured with the sample at 10 or 20 cm from the photomultiplier. The CD spectrum of spinach chloroplasts suspended in a low ionic environment is shown in fig.1a; addition of 3.3 mM NaCl to these samples causes a large reduction in the magnitude of the 676 nm negative peak (fig.1b). This effect is reversed by the further addition of 3 to 5 mM of $MgCl_2$ to the sample (fig.1c). Thus, the CD spectrum can be used as an indicator of the stacking status of chloroplasts.

Lettuce chloroplasts prepared in a manner similar to that of the spinach chloroplasts yield the CD spectrum shown in fig.2a which is similar to that obtained by adding monovalent cations to the spinach chloroplast preparation. Addition of 3 mM NaCl does not change the CD spectrum of lettuce chloroplasts; however, the addition of 3 mM $MgCl_2$ gives results similar to that in spinach (fig.2b). The difference in

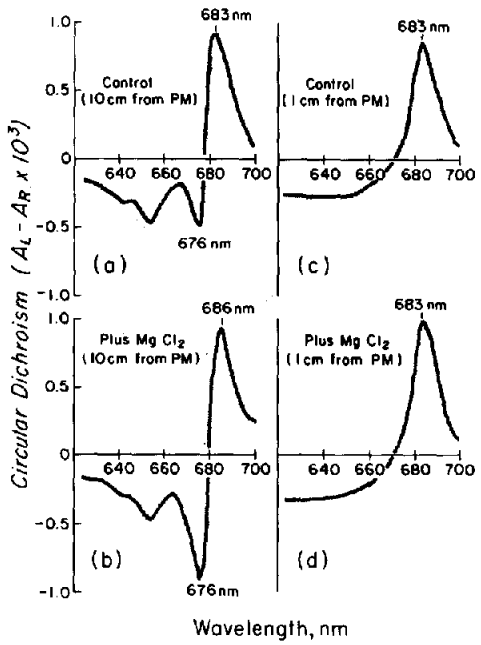

Fig.2. Effect of divalent cations and sample position on the circular dichroism spectrum of lettuce chloroplasts. (a) CD spectrum of chloroplasts suspended in 100 mM sucrose buffered to pH 7.8 with Tris and placed 10 cm from the photomultiplier, (b) plus 3 mM $MgCl_2$. (c) same as (a) with sample placed 1 cm from the photomultiplier. (d) Same as (b) but with sample placed 1 cm from the photomultiplier.

response to cation addition between spinach and lettuce chloroplasts suggests differences in thylakoid stacking status between spinach and lettuce chloroplasts. This suggestion is consistent with the observations of VanderMeulen and Govindjee [10] on the response of chlorophyll *a* fluorescence yield and 90° light scattering to the addition of monovalent and divalent cations.

The lettuce chloroplasts also exhibit a 3 nm red shift in the 683 nm positive CD peak upon the addition of divalent cations (cf. fig.2a with fig.2b). This observation is interpretable in terms of the observations of Urry and Krivacic [11] who demonstrated a red shift in CD spectra that was due to light scattering. No wavelength shift was observed for the negative CD peak upon which the cations exert their maximum effect. Furthermore, spinach chloroplasts gave no indication of any wavelength shift concommitant with cation additions.

In attempts to discover the origin of the salt effect on the CD spectrum of chloroplasts, we checked the effect of moving the sample with respect to the photomultiplier (fig.2). The spectra obtained were found to be dependent on sample position. These results suggest that the effect of 3 mM $MgCl_2$ on the CD spectrum of broken chloroplast suspensions may be a reflection of differential selective light-scattering *changes* induced by the addition of the salts. Our results do not imply that any of our spectra are free from scattering artifacts but they do underline the necessity of investigating the possibility of scattering artifacts when interpreting CD spectra of particulate material as suggested by Philipson and Sauer [4].

In conclusion, the CD spectrum of a chloroplast suspension, measured with the sample 10 or 20 cm from the photomultiplier, is suggested to be a monitor of the stacking status of the thylakoid membranes. The cation effects on the CD spectrum are suggested to be due to differential light scatter, although the possibility of an effect of cation addition on the true CD spectrum of the chloroplast samples cannot be excluded.

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