

Light-Dependent Chloroplast Volume Changes in Chloride Media*

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Abstract

In the past, some workers have reported that isolated *Spinacea oleracea* chloroplasts show light-induced swelling in chloride media, while another group showed that chloroplasts may exhibit light-induced shrinkage in chloride media. The difference cannot be due to nuances of individual technique because the present authors, one from each of the above-mentioned groups, worked together each using his usual techniques and found that the chloroplasts used in this study did not show light-induced swelling in chloride media. A slight shrinkage occurred with these plastids in chloride media. The criteria used to determine the nature of the volume changes were 0°-90° light-scattering measurements and Coulter Counter particle volume measurements.

It is not known exactly what determines whether chloroplasts will show light-induced shrinkage or swelling in a chloride medium.

Introduction

The purpose of this report is to clarify conflicting data from two laboratories concerning the effect of chloride media on isolated spinach chloroplast light-induced volume changes. Deamer and Packer² and Crofts *et al.*³ presented evidence which suggested that their chloroplast preparations underwent a light-induced shrinkage in acetate media and a light-induced swelling in NaCl, choline-Cl or Tris-Cl media. Since swelling in chloride media was generally observed in their experiments, they proposed such swelling to be a general response of isolated chloroplasts. Contrary to this generalization, Dilley and colleagues have observed light-scattering and ultrastructural changes in chloride media (KCl and Tris-Cl) which were interpreted as light-induced shrinkage.^{4,5} On occasion the latter group has also observed apparent light-induced swelling in chloride media (unpublished results), but the phenomena was not predictable or controllable. The

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only consistent differences in experimental conditions between the two groups of workers has been the individual differences of technique in preparing and handling the chloroplasts and the source of spinach. In an attempt to resolve this problem the present authors have carried out experiments using commercial summer Ohio spinach and the standard chloroplast preparation procedures employed by the respective groups. The results show that no generalization may be made regarding whether spinach chloroplasts shrink or swell upon illumination in a chloride media. Both phenomena occur, apparently depending on the type of spinach and/or the state of the spinach chloroplasts used in the experiments.

Methods

Local market spinach (Ohio) was used for two types of chloroplast preparations. The first utilized the standard procedures of Deamer *et al.*¹ in which 100 g of leaves were deveined, homogenized in 150 ml 0.35 M NaCl using a Waring blender for 45 sec. The brei was strained through eight layers of cheese-cloth without squeezing. After a 2-min centrifugation at $200 \times g$, the supernatant was centrifuged at $1200 \times g$ for 10 min, and the pellet washed once in the homogenization media and resuspended in the same media ten-fold diluted. In the second preparation, spinach leaves were homogenized 45 sec in a Waring blender with a medium of 0.40 M sucrose, 0.01 M KCl and 0.02 M Tricine pH 7.8, washed once in the same media, and resuspended in the same media ten-fold dilute.

Chlorophyll content, and assays for light scattering and turbidity changes, and Coulter counter volume measurements were as described before^{1,9} with any changes noted in the figure legends. A dual phototube light-scattering device (Phoenix Precision Instruments) allowed measurements of 90° light scattering and 0° turbidity measurements to be made in the same instrument.

Results and Discussion

A major difficulty in correlating light-scattering data with particle counter volume changes arises from the highly complex ultrastructure of chloroplasts. Three membrane structures may be involved in apparent volume changes within chloroplast populations, including the outer limiting membrane, the external grana and stroma membranes and the partition membranes of the grana stacks. Electronic particle counters sense only the outermost membrane which is resistant to ionic current, whereas light-scattering changes probably reflect volume and refractive-index changes within the entire mass of the membranes. Although it is possible for particle counter volume changes and light-scattering changes to arise from the same total volume change, it is also possible for paradoxical results to emerge from such a comparison. For instance, a ballooning of the outermost thylakoid membrane has often been observed (see Fig. 4 and ref. 2) which may be accompanied by a more compact packing of inner thylakoid lamellae. This would result in an increase of volume as measured by a particle counter, but an apparent decrease in volume as indicated by increased light scattering.

Thus, any study of light-induced volume changes in chloroplasts must consider at least three parameters.

(1) Total chloroplast volume as measured by electronic particle counters is represented by the outermost membrane resistant to ionic flux. In Class II chloroplasts, this would be the external grana and the stroma membranes. It is not clear what membranes are sensed in Class I chloroplasts, since the outer limiting membrane may be quite permeable to ions.

(2) Volume changes within the inner grana stack membranes probably exert the greatest influences on light-scattering changes, since the major portion of chloroplast

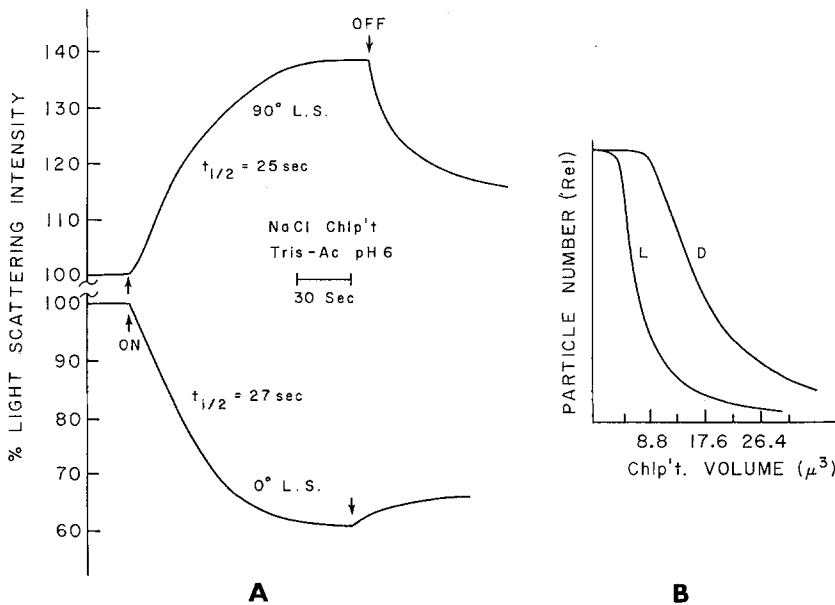


Figure 1. A: Light-scattering changes of chloroplasts prepared in NaCl and suspended in a Tris-acetate reaction medium. The reaction conditions were: 100 mM Tris-acetate pH 6.0, 0.5 mM $MgCl_2$, 1.5×10^{-5} M pyocyanine, 20 μg chlorophyll per milliliter, and a total volume of 3 ml. The top tracing gives the results for 90° light-scattering and the bottom for 0° light-scattering (turbidity). The half-time values for the light-scattering change are listed by the tracing. Red light was obtained with a Corning 2403 filter.

B: Coulter counter data for particle size distribution in light and dark. The curves drawn trace the peak values of counts in each of 12 channels recorded from the counter. The reaction-conditions were similar to Fig. 1A, except the chlorophyll concentration was 16 $\mu g/30$ ml total volume. A 50- μ aperture was used for counting the particles.

membrane mass is accumulated therein. Volume changes within the thylakoid space are not necessarily related to total chloroplast volume.

(3) A third factor which must be considered is the refractive property of single membranes. Light-scattering properties of single membranes can be strongly influenced by binding of protons and divalent ions.⁸

The interplay between these various parameters is not at all obvious, and chloroplasts from different sources and under different conditions will certainly vary in their responses. We will attempt to discuss the present results with the above considerations in mind.

Figure 1A shows that the NaCl-prepared chloroplasts in Tris-acetate media gave the typical large amplitude shrinkage as measured by an increase in 90° light-scattering

intensity which corresponds to a turbidity increase. The general equation for light-scattering intensity predicts that organelle shrinkage will give an increased light-scattering because the refractive index in the interior of the particles increases as water is lost. (See ref. 6 for detailed discussions of light scattering by organelles.)

That the light-scattering changes in Fig. 1A do result from shrinkage is clearly seen in Fig. 1B, which shows a Coulter counter volume distribution curve. The shrinkage measured here in 0.10 M Tris-acetate pH 6, is of the same order of magnitude as that reported by Deamer *et al.*¹ using sodium acetate and Tris-acetate.

The NaCl-prepared chloroplasts showed relatively little light-induced shrinkage when suspended in 0.1 M KCl at pH 6 (Fig. 2A and B). These are conditions which have been reported to give a light-induced swelling¹ [also occasionally observed in the other author's (R.A.D.) laboratory in the past]. It is apparent, however, that no swelling

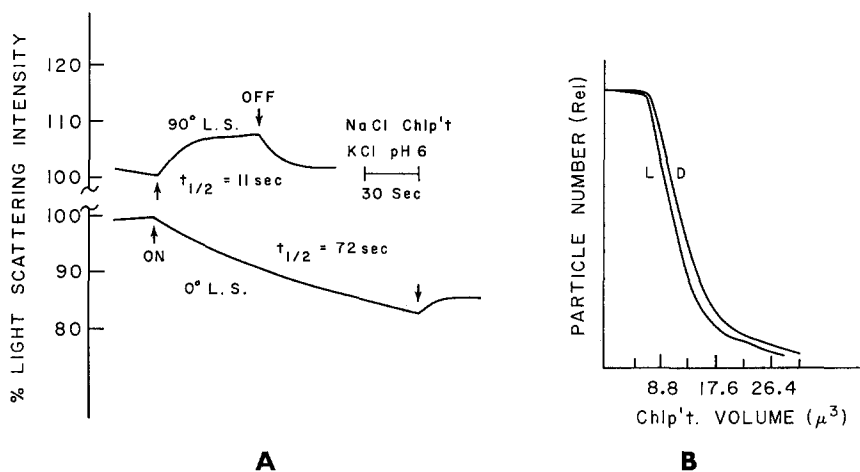


Figure 2. A: Light-scattering changes of chloroplasts prepared in NaCl and suspended in a KCl reaction medium. Reaction conditions similar to Fig. 1A, except that 100 mM KCl replaced the Tris-acetate. The pH was 6.3.

B: Coulter counter data for particle size distribution in light and dark. Reaction conditions as for Fig. 1A, except the chlorophyll concentration was 16 $\mu\text{g}/30 \text{ ml}$.

occurred with the spinach chloroplasts used in these experiments by either light-scattering or Coulter counter criteria. Similar experiments were carried out on three occasions from July through September of 1968 with essentially the same results. All the above results were obtained with chloroplasts prepared in NaCl in the standard way used by Deamer *et al.*¹ in their studies. Chloroplasts prepared in sucrose as used by Dilley and colleagues^{4,5} also showed large amplitude shrinkage in Tris-acetate medium (Fig. 3A and B) and a slight shrinkage in the KCl medium (Fig. 4A and B).

As pointed out by Deamer *et al.*¹ 0° light-scattering or turbidity measurements reveal a different phenomenon in part from that which gives rise to 90° light-scattering changes. Figures 1A, 2A and 4A show this, in that the kinetics of the 0° and 90° light-scattering changes are strikingly different; i.e., the $t_{1/2}$ of the onset phase differs by a factor of over 6 in Fig. 2A; and in Fig. 4A there is a 10-sec lag in the onset of the change at 0° but no such lag in the onset of the 90° measured changes. Deamer *et al.*¹ showed that the 0° light-scattering changes closely matched volume changes as assayed by the

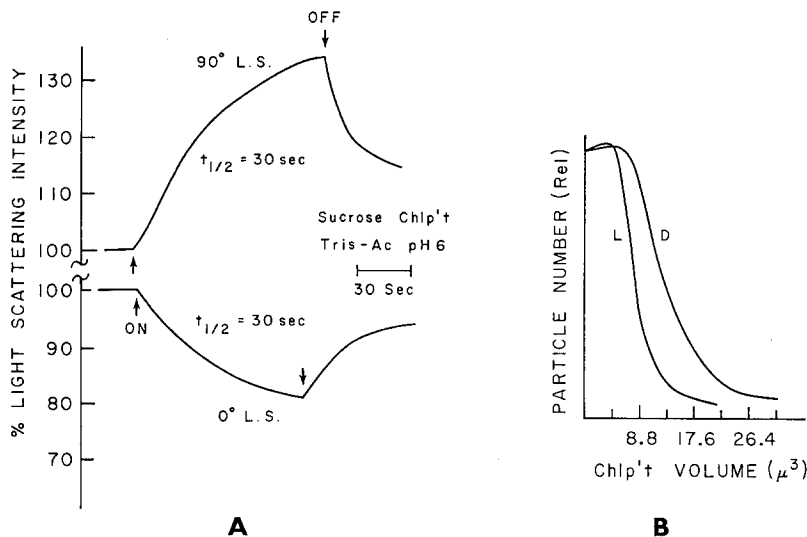


Figure 3. A: Light-scattering changes in chloroplasts prepared in the sucrose medium and suspended in the Tris-acetate reaction medium. Reaction conditions as in Fig. 1A.

B: Coulter counter data for particle distribution. Reaction conditions as in Fig. 1B.

Coulter counter, whereas the 90° light-scattering changes do not always match those at 0° light scattering. They attributed some of the 90° light-scattering changes as due to alterations in the physical state of the chloroplast membrane, resulting from the light-induced proton uptake into the grana membrane which is known to occur.⁷ The angular dependence of light-scattering intensity could well be different for the two phenomena. Data of Dilley and Rothstein⁸ also showed the light-induced light-scattering changes of chloroplasts involve more than a simple volume change in response to osmotic pressure changes following ion transport. Their data clearly implicated some change

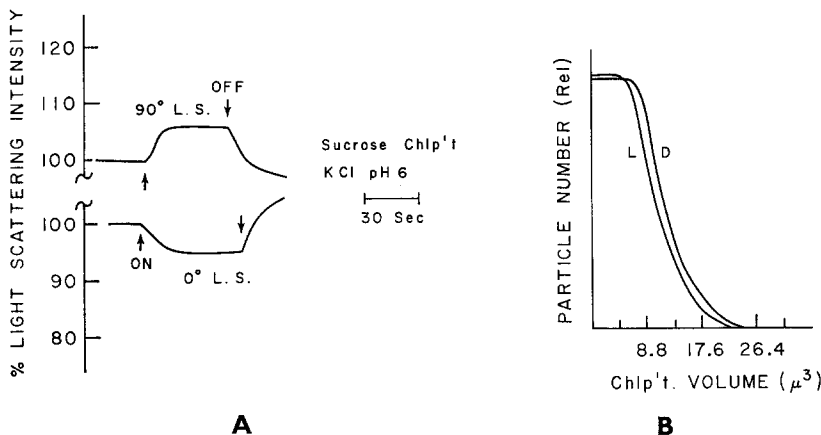


Figure 4. A: Light-scattering changes in chloroplasts prepared in the sucrose medium and suspended in the KCl reaction medium. Reaction conditions as in Fig. 2A.

B: Coulter counter data for particle size distribution.

in the physical state of the membrane driven by increased H^+ ion concentration, since the light-scattering increase evoked by acidification was similar to that induced by electron transport. The osmotically driven light-scattering increase was not large enough to match the light-induced or pH-induced change in the scattered light intensity. However, it is very likely that part of the volume and light-scattering changes driven by light or pH changes reflect changes in osmotic pressure resulting from shifts in the ionic content of the grana. The clearest evidence for this is that both electron flow⁹ and acidification in the dark⁸ lead to H^+ -cation (K^+ , etc.) exchange and loss of water from the grana. These data have been interpreted as due to the binding of protons to fixed negative charge groups in the grana membrane; groups which in the dark resting state (or high pH state) have K^+ , Mg^{2+} , etc. as soluble counter-ions.⁹ The loss of the K^+ or Mg^{2+} ion concomitant with the formation of a protonated acid group could lead to decreased osmotic potential within the membrane and a loss of water to the medium.

The above explanation seems to account for observations on some chloroplast preparations in which a relatively small light-induced shrinkage occurs in the absence of weak acid anions. In the presence of weak acid anions, a shrinkage of much greater magnitude is seen which can best be explained by loss of both cations and weak acid anions from the chloroplasts.^{3, 10} However, it does not account for the observations on other chloroplast preparations in chloride media in which a marked swelling is observed by particle counter measurements and electron microscopic examination.^{1, 2} The latter phenomenon is best explained in terms of chloride uptake to balance the inward movement of protons.¹⁰

It is not known precisely what membrane differences exist between chloroplast preparations which exhibit the Cl-swelling effect compared to those not showing the effect. One obvious direction to look is toward differences in ion permeability. The more permeable the grana membranes, the more likely the prospect of accumulating chloride anions to balance the H^+ ion uptake. In less permeable membranes, H^+ ion uptake may be balanced by the cation efflux previously documented.⁹ Further studies are required for a more precise elucidation of the relation between total chloroplast volume, thylakoid volume and ion transport.

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