Effect of Salts and Electron Transport on the Conformation of Isolated Chloroplasts. I. Light-Scattering and Volume Changes

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Summary. Whole chloroplasts isolated from the leaves of spinach (Spinacia oleracea L.) exhibit 2 types of conformational change during electron transport. Amine-uncoupled chloroplasts swell and atebrin-uncoupled chloroplasts shrink. Chloroplasts uncoupled by carbonylcyanide phenylhydrazones and by treatment with ethylenediamine tetraacetic acid do not change their volumes or light-scattering properties during electron transport. Phosphorylating chloroplasts shrink only slightly.

The rate and extent of the conformational change parallel the rate of electron transport; both the decrease in turbidity with methylamine and the increase in turbidity with atebrin are roughly proportional to the Hill reaction rate. Consequently the great volume and light-scattering changes which occur in the presence of these uncouplers can be attributed, in part, to the very high rates of uncoupled electron transport. However, for a given rate of electron transport the atebrin-induced scattering increase is very much greater than the increase observed during photophosphorylation.

When uncouplers are combined, the carbonylcyanide phenylhydrazine effect (no change) supercedes both the methylamine effect (swelling) and the atebrin effect (shrinking). The methylamine effect supercedes the atebrin (shrinking) and ethylenediamine tetraacetic acid (no change) effects. The atebrin effect supercedes the ethylenediamine tetraacetic acid effect. A similar hierarchy of effects is observed with regard to the rate of the uncoupled electron transport.

These light-scattering changes of whole chloroplasts reflect similar changes which occur in very small digitonin particles of chloroplasts. Therefore one must look among chloroplast substructures for the basic mechanism of swelling and shrinking.

Many salts (including methylamine hydrochloride) cause the chloroplasts to shrink. This phenomenon is not osmotic since comparable osmolarities of sucrose are without effect. Magnesium chloride and calcium chloride are most effective but all salts tested gave major volume decrease when less than 0.05 M. The salt-shrunken chloroplasts show greater light-scattering changes during electron transport than do low-salt chloroplasts.

Illuminated chloroplasts are known to exhibit light-scattering increases (26) accompanied by volume decreases (13). In an earlier paper (14) we reported large-scale conformational changes of chloroplasts associated with the rapid electron transport which occurs in the presence of the phosphorylation uncouplers atebrin (3) and methylamine (7). The directions of the changes induced by these different uncouplers were opposite. Atebrin induced a light-scattering increase (shrinking) while methylamine caused a light-scattering decrease (swelling). As Hind and Jagendorf (11) have recently reported, the uncouplers dicumarol and ammonia also causes chloroplasts to swell during electron transport.

Stimulation of the light-scattering changes of illuminated chloroplasts by atebrin (quinacrine) was first reported by Dilley and Vernon (4). The fact that they did not detect light-scattering changes in chloroplasts treated with ammonium chloride (19) and carbonylcyanide m-chlorophenylhydrazone (CCCP) (9) led them to suggest that these 2 compounds interfere with a series of the energy transfer reactions at an earlier stage than does atebrin. However, Hind's and our discoveries of effects of amines distinct from the effects of either CCCP or atebrin indicates that the circumstances leading to the conformational changes of chloroplasts are even more complex than Dilley and Vernon have postulated.

This paper presents further observations concerning the properties of the 2 different types of conformational changes with uncouplers. The profound effects of salts on the volume and light-scattering characteristics of chloroplasts are also described in some detail. The second paper of the series (15) will deal with an interpretation of the observed effects of salts and electron transport in terms of changes in the lamellar structure of the chloroplasts.

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Materials and Methods

Chloroplasts. Selected turgid leaves of spinach (Spinacia oleracea L.) were homogenized in a Waring blender for 10 seconds in a medium containing 0.35 M NaCl, 0.05 M sodium phosphate and 1 mM EDTA, pH 7.3. The homogenate was centrifuged at 1500 × g for 5 minutes and the pellet was resuspended in a medium containing 0.15 M sucrose and 0.05 M Tricine [Tris(hydroxymethyl)methylglycine]-NaOH buffer, pH 7.3 (8). After brief centrifugation to remove cell debris, the chloroplasts were collected at 1500 × g (5 min), washed in the sucrose-Tricine medium, and finally suspended in the same medium to give chlorophyll concentrations of approximately 1.5 mg per ml stock suspension. Chlorophyll was determined by the method of McKinney (21).

Preparation of Digitonin-Particles. Chloroplasts were treated with 0.1% digitonin at 0°C for 30 minutes as described in an earlier publication (17) except that a medium containing 0.15 M sucrose and 0.05 M Tricine buffer (pH 7.3) was used. The sediment obtained between 30,000 × g (20 min) and 100,000 × g (45 min) was washed once and resuspended in the same medium. This preparation was quite active in electron transport (approx 400 μmoles ferricyanide reduced per hr per mg chlorophyll at 15°C).

Optical Measurements. Changes in the light-scattering properties of chloroplasts were followed by recording the OD (small angle light-scattering) of suspensions at 580 mp with a Bausch and Lomb Spectronic 505 spectrophotometer modified to allow irradiation of a cuvette with a beam of actinic light (>600 mp) at right angles to the detecting beam. The rate of ferricyanide reduction was measured by following the absorbance decrease of potassium ferricyanide at 420 mp. (The light-scattering changes of chloroplasts at this wavelength were shown to be insignificant compared to the very high rate of ferricyanide reduction in the presence of uncouplers.) The temperature was 15°C. Details of the optical system have been described elsewhere (14,16).

Results

Light-Scattering Changes as Observed by Absorbance Changes. In the experiments presented here, light-scattering changes were measured as changes in the OD of chloroplast suspension at 580 mp. In our optical system roughly 80 to 90% of the apparent OD at this wavelength was estimated to be due to light-scattering. Figure 1 illustrates OD changes in chloroplast suspensions in the red region of the spectrum. These large optical differences over a wide range of wavelengths with a minimum change near the absorption peak are the changes to be expected from a modification of the light-scattering properties of pigmented particles (cf. 20). Actually one can easily observe by eye the very large changes in turbidity of the chloroplast suspensions corresponding to the recorded OD changes. In view of these facts, the term light-scattering or turbidity will be used throughout the text and the terms OD and absorbance will be avoided in order to emphasize the nature of the quantity with which we are dealing.

As clearly seen in figure 1, the addition of methylamine hydrochloride in the dark causes a considerable increase in the amount of light scattered by the chloroplasts. This scattering increase involves a shrinking of the chloroplasts (see table III). In the light in the presence of electron acceptors there is a large decrease in light-scattering which is associated with chloroplast swelling. Atebrin added in the dark also causes an increase in light-scattering but, surprisingly, this scattering increase is associated with swelling rather than shrinking (14). Illumination with atebrin in the presence of electron acceptors produces a further very large increase in the turbidity accompanied by shrinking of chloroplasts (14,15).

Light-Scattering Changes and the Rate of Electron Transport. We have already shown that these large-scale turbidity changes which occur during illumination are electron transport dependent (14). The relationship between scattering changes and rates of electron transport is shown in figure 2. The rate of electron transport was varied both by varying light intensity and by varying the concentrations of

![Figure 1](https://www.plantphysiol.org)
Fig. 2. Light-scattering changes as a function of the electron transport rate. Electron transport was varied by varying either the light intensity or the concentration of uncoupler added. Extent refers to the change in OD after 3 minutes (at least 70% of maximum) and initial rate refers to the initial slope expressed as ΔOD/2 minutes. Since salts modify the light-scattering properties of chloroplasts, the experiment involving different methylamine-HCl concentrations was carried out at constant chloride (0.06 M) through additions of appropriate amounts of NaCl. Chlorophyll 30 μg in 2 ml. Other ingredients as in figure 1. Actinic light saturating, >600 μm.
the phosphorylation uncouplers added. Kinetics of chloroplast responses were observed at each rate of electron transport. The responses were evaluated in 2 ways: A) by measuring the extent of the turbidity change after 3 minutes (by which time the chloroplasts had attained about 70% of their new steady-state light-scattering properties) and, in the case of methylamine-induced swelling, B) by measuring the initial rates of turbidity change. Both the extent of change and the rate of change showed the same correlation with the rate of electron transport. It was not possible to obtain meaningful values of initial rates of turbidity change with atebrin since the shrinking process in the presence of this uncoupler follows a markedly polyphasic course (cf. fig 3, 6). While the precise quantitative significance of our scattering measurements is obscure because of the complexity of the light-scattering phenomena and the arbitrary values of quantities measured with our apparatus, the observed parallelism between the scattering changes and the rates of electron transport is nevertheless remarkable.

Since amines reverse the direction of the turbidity changes usually observed when chloroplasts are illuminated, it is not improbable that this unique amine effect is closely associated with some unique aspect of the amine uncoupling. However, atebrin increases in extent the turbidity change which occurs in the absence of atebrin; the structural changes observed in the presence and absence of atebrin are qualitatively indistinguishable (15). Therefore, one must consider the possibility that the action of atebrin is to exaggerate the very small scattering increase, which often accompanies the slow electron transport in the absence of uncouplers, by providing a higher rate of electron transport. In other words, the effect of atebrin on the turbidity changes might be indirect and the increased turbidity change might be simply an expression of increased electron transport rate. Table I shows that this is not so. At pH 8.0, the rates of phosphorylating electron transport and atebrin-uncoupled electron transport are the same yet the turbidity change is vastly greater in the uncoupled system. Furthermore the amount of turbidity change parallels the uncoupling efficiency (as measured by the suppression of phosphorylation), and increasing the concentration of atebrin beyond the concentration required for complete uncoupling does not further increase the extent of the turbidity change.

*Interactions among Uncouplers.* Interactions among 3 different kinds of uncouplers, atebrin, methylamine and carbonylcyanide p-trifluoro-methylphenylhydrazone (FCCP) are illustrated in figure 3. The concentrations of uncouplers employed were just high enough to give maximum rates of uncoupled electron transport. It has already been reported that carbonylcyanide phenylhydrazone suppress light-scattering changes accompanying electron transport in the absence of other uncouplers (4, 11, 14, 27). As clearly seen in figure 3, FCCP also abolishes the

large-scale scattering changes induced by atebrin and methylamine. This is true when it is added initially in combination with the other uncouplers and when it is added in the light during the progress of the ate-

![Table 1. Effects of Atebrin-Uncoupled and Phosphorylation-Coupled Electron Transport on the Turbidity of Chloroplast Suspension](image)

<table>
<thead>
<tr>
<th>Atebrin</th>
<th>Electron transport rate*</th>
<th>P/2e</th>
<th>Turbidity change in light**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>390</td>
<td>1.07</td>
<td>+ 1</td>
</tr>
<tr>
<td>1 × 10⁻⁵ M</td>
<td>370</td>
<td>0.22</td>
<td>+ 18</td>
</tr>
<tr>
<td>3 × 10⁻⁵ M</td>
<td>440</td>
<td>0</td>
<td>+ 25</td>
</tr>
<tr>
<td>10 × 10⁻⁵ M</td>
<td>390</td>
<td>0</td>
<td>+ 25</td>
</tr>
</tbody>
</table>

*μmoles ferricyanide reduced per hour per mg chlorophyll.

**Percent change in OD at 580 mμ after 2-minutes illumination.

![Fig. 3. Interactions between uncouplers as shown by the effects of various combinations on the electron transport-dependent light-scattering changes. Chlorophyll 30 μg in 2.0 ml. FCCP (carbonylcyanide p-trifluoromethylphenylhydrazone) 10 μmoles. Other ingredients as in figure 1. Actinic light saturating, > 600 mμ. Methylamine effect supercedes the atebrin effect while FCCP effect (inhibition of change) supercedes both the methylamine and atebrin effects.](image)
brin-shrinking or methylamine-swelling process. The methylamine effect which is annihilated by FCCP in turn overrides completely the atebrin effect. EDTA (ethylenediamine tetraacetic acid) is known to cause uncoupling when it is added to chloroplasts in low-salt media (18) probably by releasing a coupling factor (2). As Hind and Jagendorf (11) have shown, EDTA-uncoupled chloroplasts do not exhibit changes in light-scattering properties when they are illuminated. We have confirmed this observation. In spite of the very rapid electron transport in EDTA-uncoupled chloroplasts there is no evidence of conformational change. However, we have found that these EDTA-uncoupled chloroplasts, unlike the conformationally stable phenylhydrazone-uncoupled chloroplasts, retain the greater part of their capacity for conformational change in the presence of atebrin or methylamine (see table II).

Each of the uncouplers discussed above, FCCP, methylamine, atebrin and EDTA, stimulates electron transport and the maximum rate of electron transport achieved in the presence of each uncoupler is characteristic of that uncoupler. It may be significant that the uncouplers interact with respect to electron transport rates in the same order that they interact in modifying the light-scattering changes (table II). The FCCP rate supersedes the methylamine, atebrin and EDTA rates. The methylamine rate supersedes the atebrin and EDTA rates. And the atebrin rate supersedes the EDTA rate. Few of these interactions of uncouplers on the electron transport rates can be attributed to inhibitory effects of the uncouplers since the uncouplers showed no tendency to inhibit even at higher concentrations (with the exception of FCCP). Moreover the fastest rate of all (methylamine rate) takes precedence over all of the other rates except the rate with FCCP, a situation which would be impossible if inhibition were in any way involved in the interactions. However the position of FCCP in the sequence is suspect because of its significant inhibition of electron transport at uncoupling concentrations.

One simple (and possibly naive) explanation of this hierarchical arrangement of uncoupler effects postulates that the mechanisms of uncoupling by these compounds are entirely different and that each uncoupler acts at a different stage in a sequence of energy transfers: FCCP-sensitive process (no scattering change) → amine-sensitive process (scattering decrease) → atebrin-sensitive process (scattering increase) → EDTA-sensitive process (no scattering change). However, in view of the extreme complexity of the reaction systems with which we are dealing emphasis should be placed only on the experimental observations; the existence of this hierarchy of uncouplers with respect both to electron transport rates and chloroplast conformational changes.

**Effects of Salts on the Volumes and Light-Scattering Properties of Chloroplasts in the Dark.** As described above, the addition of 0.05 M methylamine hydrochloride produces, in the dark, a rapid and considerable increase in the turbidity of chloroplast suspensions (half-time of the change about 20 sec).

**Table II. Hierarchy of Phosphorylation Uncouplers in Determining Turbidity Changes and Electron Transport Rates**

Concentrations of uncouplers employed were: FCCP $5 \times 10^{-6}$ M, methylamine-HCl 0.05 M, atebrin-HCl 1 $\times 10^{-4}$ M. These concentrations gave maximum electron transport rates. Experimental conditions were as in figure 3.

<table>
<thead>
<tr>
<th>Uncoupler</th>
<th>Turbidity change in light*</th>
<th>Electron transport rate**</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCCP***</td>
<td>0</td>
<td>370</td>
</tr>
<tr>
<td>FCCP + methylamine</td>
<td>-2</td>
<td>390</td>
</tr>
<tr>
<td>FCCP + atebrin</td>
<td>+3</td>
<td>350</td>
</tr>
<tr>
<td>FCCP + EDTA†</td>
<td>0</td>
<td>380</td>
</tr>
<tr>
<td>Methylamine</td>
<td>-39</td>
<td>960</td>
</tr>
<tr>
<td>Methylamine + atebrin</td>
<td>-37</td>
<td>850</td>
</tr>
<tr>
<td>Methylamine + EDTA†</td>
<td>-27†</td>
<td>920</td>
</tr>
<tr>
<td>Atebrin</td>
<td>+59</td>
<td>480</td>
</tr>
<tr>
<td>Atebrin + EDTA†</td>
<td>+38†</td>
<td>490</td>
</tr>
<tr>
<td>EDTA†</td>
<td>0</td>
<td>760</td>
</tr>
<tr>
<td>(None)</td>
<td>(+1)</td>
<td>(50)</td>
</tr>
</tbody>
</table>

* Percent change in OD at 580 mµ after 3-minutes illumination.

** µmoles ferricyanide reduced per hour per mg chlorophyll.

*** Carbonylcyanide p-trifluoromethylphenyldrazone.

† Chloroplasts were pretreated with 1 mM EDTA for 2 hours in a medium containing 0.15 M sucrose and 0.01 M Tricine buffer (pH 7.3).

‡ 5 mM MgCl₂ was added in the reaction mixture to supplement salt level (see section on salt effect).

![Figure 4](https://www.plantphysiol.org)
This is accompanied by a marked shrinking of the chloroplasts (14). There is no reason to believe that the phenomenon is in any way related to the uncoupling action of methylamine since all other salts tested behave in a similar manner at concentrations which do not uncouple or in any other way affect electron transport rates.

Figure 4 shows the influence of various salts and buffers on the light-scattering properties of chloroplast suspensions and Table III shows the influence of some of them on chloroplast volumes. As expected, there is an inverse correlation between the amount of light scattered and the chloroplast volume. The salt effect is clearly not related to the osmotic properties of the medium since equal or higher osmolarities of sucrose have little effect on chloroplast volumes and almost no effect on the chloroplast light-scattering tendencies. Salts of monovalent cations and monovalent anions seem to be rather similar and relatively ineffective while salts of divalent cations are extremely effective. Salts of di- and poly-valent anions are more complex in that some, such as sulfate, are relatively effective and others, such as phosphate, are relatively ineffective. The inefficacy of Tricine buffer, both in shrinking the chloroplasts and in increasing their ability to scatter light, is almost certainly due to its predominantly zwitterionic character at the pH employed. At this pH (7.3) about 80% of the buffer is zwitterionic and only 20% is anionic. Sodium Tricine (the anionic portion) is about as active as the other sodium salts of monovalent anions.

The above observations on salt effects are in good agreement with the observations of Nishida and Koshii (24). These authors also showed that prolonged incubations with salts invariably result in a gradual re-swelling of the chloroplasts. A swelling of chloroplasts even in 0.35 M NaCl has been reported (12). In our short-term experiments (5-10 min) this reverse process was negligible.

Effect of Salts on the Light-Scattering Changes Observed During Electron Transport. The light-scattering changes which occur when chloroplasts are illuminated in the absence of uncouplers are generally rather insensitive to salts. However, salts which are in themselves capable of inducing high rates of electron transport by uncoupling elicit considerably larger turbidity changes in the light. For example, citrate and phosphate are relatively effective uncouplers at high concentrations and permit quite high rates of electron transport (8). Phosphate and citrate salts in concentrations between 0.1 and 0.3 M stimulated both electron transport and light-scattering increases but both stimulations were much smaller than those produced by atebirin. Tris(hydroxymethyl)aminomethane, which is also a moderately strong uncoupler at high concentrations (8), induces an appreciable turbidity decrease in the light when the concentration is 0.2 M. Even at 0.05 M the effect is discernible. The swelling effect of Tris was irreversible as was the swelling effect of methylamine. This behavior of Tris is not surprising since it is a primary aliphatic amine and aliphatic amines are usually potent uncouplers of photophosphorylation in chloroplasts (7).

Packer et al. (29) recently reported that the swelling of chloroplasts in a medium containing NaCl and Tris-HCl was markedly accelerated by light. It seems likely to us that this phenomenon is an expression of amine-uncoupling by Tris. In our Tricine-buffered systems the swelling of chloroplasts in 0.35 M NaCl is not stimulated by light.

Salts which do not uncouple phosphorylation or otherwise stimulate electron transport rates and do not by themselves modify electron transport-induced turbidity changes (NaCl, MgCl₂, etc.) do nevertheless modify the light-scattering changes which occur in the presence of amines and atebirin. In fact the turbidity decreases characteristic of amine-uncoupled electron transport are only expressed in the presence of a certain level of salts. Ammonium chloride at its opti-

### Table III. Packed Volumes of Chloroplasts in Buffers and Other Salts

<table>
<thead>
<tr>
<th>Salt</th>
<th>Conc M</th>
<th>Chloroplast Volume (relative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>...</td>
<td>100</td>
</tr>
<tr>
<td>Tricine-NaOH</td>
<td>0.05</td>
<td>83</td>
</tr>
<tr>
<td>Tris-HCl</td>
<td>0.05</td>
<td>46</td>
</tr>
<tr>
<td>Na phosphate</td>
<td>0.05</td>
<td>43</td>
</tr>
<tr>
<td>KCI</td>
<td>0.05</td>
<td>47</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.05</td>
<td>45</td>
</tr>
<tr>
<td>Methylamine-HCl</td>
<td>0.05</td>
<td>48</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>0.01</td>
<td>39</td>
</tr>
<tr>
<td>(Sucrose)</td>
<td>(0.2)</td>
<td>(80)</td>
</tr>
</tbody>
</table>

Fig. 5. Effects of salts on amine-induced light-scattering decreases of chloroplast suspensions during electron transport. Reaction volume 2.0 ml. Chlorophyll 30 µg. Tricine-NaOH buffer (pH 7.3) 100 µmoles, potassium ferri-cyanide 2 µmoles. Actinic light saturating, > 600 mm. The inefficacy of NH₄Cl relative to methylamine-HCl is largely an expression of the lower concentration (4 mM vs. 60 mM).

Fig. 6. Effects of salts on the atebirin-induced light-scattering increases of chloroplast suspensions during electron transport. Atebrin-HCl 0.2 µmole in 2 ml. Other conditions as in figure 5.
imum uncoupling concentration (4 mM) stimulates electron transport to a level comparable to the level obtained with methylamine hydrochloride. However, this concentration of NH$_4$Cl induces only a slight increase in the turbidity of chloroplast suspension in the dark and the subsequent decrease in turbidity in the light is also very small (14). This apparent inefficiency of the ammonium ion as a swelling agent is explicable in terms of the low salt concentration of the medium: the methylamine hydrochloride (salt) concentration employed is 60 mM whereas NH$_4$Cl concentration is only 4 mM. When the NH$_4$Cl is supplemented with 100 mM NaCl, the light-scattering decrease with ammonia is comparable to that observed with methylamine. Magnesium chloride (or CaCl$_2$) is even more effective in eliciting the light-scattering decrease. Figure 5 illustrates the effect of NaCl and MgCl$_2$ on the light-scattering characteristic of ammonia- and methylamine-uncoupled chloroplasts. Atebrin-induced turbidity changes are enhanced by, but are less critically dependent on, the presence of salts (fig 6).

It is uncertain whether or not the effects of salts on the light-scattering changes reflect an ion-dependency of the underlying processes. As the next paper in this series (15) will demonstrate, the presence or absence of salts has profound effects on the organization of the lamellar structures in chloroplasts. These salt-mediated structural changes must produce major changes in the optical properties of the chloroplasts (see fig 4, 5). Therefore, it is probable that some of the salt-induced susceptibility to light-scattering changes is a manifestation of the presence of regular, highly organized arrays whose light-scattering properties are very sensitive to even a minimum of disarray. (As will be seen in the following paper, disarray is a conservative word to apply to the condition found in the amine-swollen chloroplasts.) However, there is evidence to suggest that the shrinking and swelling phenomena may themselves be critically dependent on ion fluxes (5, 23, 25).

**Light-Scattering Changes in Digitonin-Particles.**

Packer and Marchant (28) have observed small scattering changes with a fraction of chloroplast fragments sedimented at 20,000 × g, while Dilley and Vernon (4) mentioned that the "quantasome" fraction retained 36% of the capacity for absorbance changes found in crude sonicates. These reports suggest that changes in chloroplast sub-units, rather than changes in chloroplast volume per se, are the basic cause of the observed increases and decreases in the amount of light scattered by chloroplast suspensions.

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**Fig. 7.** Methylamine-induced light-scattering changes in suspensions of digitonin particles of chloroplasts during electron transport. Reaction mixture 2.0 ml. Chlorophyll 80 µg, Tricine-NaOH buffer (pH 7.3) 100 µmoles, MgCl$_2$ 10 µmoles, methylamine-HCl 100 µmoles, potassium ferricyanide 1.6 µmole. Actinic light saturating, >600 µw. Freshly prepared suspensions had so little turbidity that changes therein could not be measured. Aging or the addition of MgCl$_2$ increased the turbidity, presumably by aggregating the particles. The clumps of digitonin particles exhibited the light-scattering changes characteristic of whole chloroplasts.

**Fig. 8.** Atebrin-induced light-scattering changes in suspensions of digitonin particles of chloroplasts during electron transport. Conditions as in figure 6 except that atebbrin-HCl, 0.2 µmole, replaced methylamine-HCl.
We have confirmed this supposition by the following experiments. The fraction of digitonin-fragmented chloroplasts sedimenting between 30,000 × g and 100,000 × g (see Methods) was dispersed to give a suspension which was almost completely clear to the eye. A rough estimation from the sedimentation time indicated that the average size of the particles was the order of 0.1 μ in diameter, assuming spherical particles of density 1.2. As shown by the bottom curve of figure 7, the fresh digitonin-particles exhibit no detectable absorbance change during electron transport without uncoupler. (This is certainly to be expected since there is so little light-scattering to change). The addition of methylamine hydrochloride to this suspension in the dark produces a measurable light-scattering level (on a chlorophyll basis, about 20% of the OD observed in chloroplast suspensions) indicating that the methylamine salt causes some aggregation of the particles (cf. 22). The newly aggregated particles show a very slight absorbance decrease in the light presumably because they swell. Magnesium chloride with methylamine causes even more clumping and therefore more light-scattering. During electron transport in the light these larger aggregates again swell, and because they now scatter much more light there is a more marked decrease in the absorbance of the suspension. Aging of digitonin particles for 24 hours at −15°C induces even more aggregation (turbidity about 30% of the chloroplast level) and magnifies still more the electron transport-dependent turbidity change (fig 7).

Figure 8 illustrates a parallel series of experiments with atehrin. Clearly the small digitonin particles still retain most of the machinery involved in the light-scattering changes of whole chloroplasts.

**Discussion**

The rapidly growing literature on light-induced conformational changes in chloroplasts is replete with detailed observations but no coherent picture of the underlying process has emerged. This is doubtless a reflection of the complexity of the phenomena measured and the many, sometimes unrecognized parameters involved. The situation is not helped by the fact that one can scarcely resist the temptation to draw analogies, valid or invalid, with the equally complex and little understood swelling and shrinking of mitochondria.

The conformational changes described in this paper were observed over a wide pH range, although most of the data presented represent experiments at pH 7.3. Qualitatively and often quantitatively similar phenomena were encountered between pH 6.0 and pH 8.0. For instance the ammonia-induced, electron transport-dependent swelling of chloroplasts is similar in magnitude at all pH’s between pH 6.0 and 7.5 if the amount of NH₄Cl is adjusted so that the concentration of the unionized form remains optimal, but the swelling declines rapidly with declining electron transport rates above pH 7.5. Furthermore at all pH’s the concentrations of ammonia which are optimal for electron transport also seem optimal for swelling.

It is probable that the swelling and shrinking phenomena observed in illuminated chloroplasts are totally dependent on electron transport. Fairly large volume changes have been observed in the absence of exogenous electron acceptors (13) but many chloroplast preparations have endogenous cyclic electron transport systems (6). Our preparations seem low in cyclic electron transport (as judged by the small amount of hydrogen peroxide formed and by the low level of phosphorylation without added electron acceptors) and, in our preparations, conformational changes in the absence of electron acceptors are exceedingly small (14). Moreover, we have been able to demonstrate a relationship between electron transport rates and the extent of the consequent light-scattering changes. It follows that in evaluating the effects of various substances one must always keep in mind the possibility that the addendum is modifying the rate of electron transport rather than directly affecting the process leading to conformational changes. This possibility poses a particularly difficult problem in systems involving cyclic electron transport since in these systems the rate of electron transport cannot be measured easily.

This is not to say that either shrinking or swelling is an inevitable consequence of electron transport or that the extent of either phenomenon depends solely on the rate of electron transport. Not all kinds of electron transport affect chloroplast conformation. Electron transport coupled to phosphorylation at pH 8.0, electron transport uncoupled by FCCP and electron transport uncoupled by EDTA treatment of the chloroplasts, can all occur with little or no change in the conformation of the chloroplasts. Electron transport uncoupled by atehrin, by the detergent Triton X-100 (11) or by the sulfhydryl-activated adenosine triphosphatase (28, cf. 30) results in shrunken chloroplasts. Electron transport uncoupled by animes is accompanied by an immense and often irreversible swelling of the chloroplasts (see following paper), while electron transport uncoupled by dicumarol (11) produces a smaller, reversible swelling.

Basal electron transport occurs to some extent in all preparations and probably represents inadvertent uncoupling by an unknown number of unknown factors. This basal electron transport is very slow near pH 7.0 but in more alkaline and often in more acidic media it becomes appreciable. Above pH 8.0 the nonphosphorylating electron transport rate is 30% to 50% of the phosphorylating electron transport rate but little conformational change occurs. At pH 6.0 the nonphosphorylating electron transport depends critically on the buffer employed and is associated with a measurable chloroplast shrinking (4, 10): at this low pH the amount of shrinking is still correlated with the rate of electron transport and conse-

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sequently this conformational change seen is rarely more than 10% of the changes observed with the more efficient uncouplers atriemin and methylamine.

One is tempted to suggest that the conformational changes are induced by the uncoupling act itself; that the reaction of the uncoupler which dissipates a high energy intermediate in the phosphorylation system is intimately associated with membrane modifications (13) or ion (5, 23, 25) or water movements. For example, the effect of certain kinds of uncouplers might be to disrupt the control of membrane configuration so that the energy intended for ATP synthesis is all expended on the uncontrolled operations of contractile or osmotic systems normally employed in the maintenance of membrane integrity. This hypothesis has the advantages that it can be expanded to accommodate the qualitatively different effects of different kinds of uncoupling. Moreover it predicts that phosphorylating electron transport should not be accompanied by large conformational changes.

An alternative hypothesis is that electron transport itself imposes disruptive forces on the membranes of the chloroplasts and that these forces are normally countered by energy-requiring regulatory systems. In the presence of some kind of uncouplers the energy sources (in the form of phosphorylation intermediates) are probably broken down as soon as they are formed (11) or are never formed at all. Hence, regulation having been lost, the membranes are distorted by the reaction involved in electron transport. This hypothesis has the advantages that it helps to explain the partial inhibition of light-induced conformational changes by exogenous ATP (14) and that it relates the light-induced conformational changes of chloroplasts to the ATP-dependent conformational changes of chloroplasts in the dark (13). It has the disadvantage that it does not readily accommodate the existence of the 2 different kinds of conformational change observed; swelling and shrinking. Therefore, its acceptability may hinge on whether or not the swelling and shrinking phenomena are fundamentally different (see following paper).

Salts profoundly modify the lamellar organization of chloroplasts and in so doing cause the chloroplasts to shrink (15). Consequently, salts modify the volume and light-scattering changes by providing different base-lines and by causing major differences in the optical properties of the chloroplasts. However, salts may also influence the mechanism of swelling and shrinking itself since electron flow is known to involve an ion flux (5, 23, 25).

**Literature Cited**

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