present not only in the soluble fraction but was also bound to the particles. Based on the benzyl viologen assay, hydrogenase activity was approximately equally distributed between the soluble and particulate fractions.

We conclude that cell-free extracts of *Chromatium* can reduce NAD^+ and, to a lesser degree, $NADP^+$ with H_2 as the reductant. For maximal activity the reaction depends on ferredoxin and on both the soluble and particulate fractions of the cell extract. Since both hydrogenase and NAD^+ reductase are in the soluble fraction, the contribution of the particles to NAD^+ reduction is not clear. It is possible that the particles contain an unknown bound component which links hydrogenase to ferredoxin or, alternatively, that only a bound hydrogenase, and not a soluble hydrogenase, can couple efficiently to ferredoxin in the utilization of H_2.

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Light-absorption and light-scattering changes during shrinking and swelling of chloroplasts

It is now established that isolated chloroplasts undergo either shrinkage[1,2] or swelling[3,4] upon illumination. The direction of the volume change depended on the nature of the suspending medium; chloroplasts suspended in a medium containing

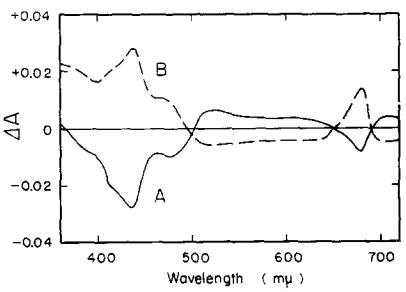
Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; $TMQH_2$, trimethyl-1,4-benzoquinol; DCIP, 2,6-dichlorophenolindophenol.

phosphate[1,2] or salts of weak organic acids[5,6] such as sodium acetate shrink on illumination, while those suspended in a medium containing methylamine[3] or sodium chloride[4] swell on illumination. The shrinkage of chloroplasts by light was accompanied by an increase in the turbidity[1] of the suspension or the 90° light scattering[2], and the swelling was accompanied by a decrease in the turbidity[3,4]. These measurements of light-scattering properties were conducted at the wavelengths where chloroplast pigments show no perceptible light absorption. DILLEY AND VERNON[5] measured the wavelength dependency of the light-induced turbidity increase, and found a maximal change around 510 mμ. Light-induced changes observed previously by these optical methods have been interpreted as due to a change of light scattering by chloroplasts in suspension, and not due to a change of light absorption by chloroplast pigments. In the present study, absorption and scattering (turbidity) by chloroplasts in suspension were measured separately and correctly to see if there is any change in the light absorption when chloroplasts shrink or swell in the light and to correlate the absorption change, if it exists, with the scattering change so far observed.

Chloroplasts were isolated from spinach (*Spinacia oleracea*) leaves as described previously[1]. To see the effect of actinic illumination on the absorbance and turbidity of chloroplast suspensions, the difference spectrum between two samples, one illuminated and the other in the dark, was measured with a Shimadzu multipurpose recording spectrophotometer model MPS-50, using 1.0-cm cells. The absorbance was measured by placing the sample closely to the large photo-cathode of the end-on type of the spectrophotometer. As fully described previously[7], the absorbance thus measured is the semi-integral attenuance, and indicates correct absorption characteristics of the pigments contained in translucent materials such as chloroplasts. The turbidity (more strictly, the rectilinear attenuance[7]) of chloroplast suspensions was measured by placing the sample with narrow slits at a remote position, 12 cm, from the photocathode. The suspension was illuminated in the sample compartment of the spectrophotometer by red light above 680 mμ (170 lux) or by far-red light above 700 mμ (70 lux) at right angles to the measuring beam, and spectral changes were observed during actinic illumination.

The absorbance of a chloroplast suspension decreased in the light during the shrinkage of chloroplasts as shown by Curve A in Fig. 1 which is the light-*minus*-dark difference spectrum obtained for the chloroplasts with 10 μM 2,6-dichlorophenolindophenol (DCIP) *plus* 1 mM ascorbate. The difference spectrum indicates distinct wavelength dependency identical with the absorption spectrum of chloroplasts. Difference maxima were located at 435, 480 and 680 mμ, and small humps around 385, 415, 520 and 650 mμ. The difference spectrum (Curve A) shows a slight increase of absorbance between 500 and 650 mμ during shrinking. This opposite change in the spectral region of weak light absorption may, however, be attributed to the effect of light reflection. The effect of backward scattering or reflection is included in the absorbance measurements as explained previously[7], although the effect of forward scattering is completely eliminated by use of the end-on type of photomultiplier. The change shown by Curve A in Fig. 1 corresponds to 2.5% of the total absorbance at 435 mμ; the absorbance value of this suspension at 435 mμ was 1.10 in the dark. It is noteworthy that the direction of the absorbance change is opposite to that of the scattering change. The absorbance change was completely reversible as observed at 435 mμ, and the absorbance increased to the original dark level when the light

was turned off (Curve A in Fig. 2). The light-induced absorbance decrease observed in the presence of 20 μM phenazine methosulfate *plus* 1 mM ascorbate was suppressed by more than 70% by addition of inhibitors for the light-induced shrinkage, such as 4 mM NH_4Cl (Curve B in Fig. 2), 30 μM carbonyl cyanide *m*-chlorophenylhydrazone, 0.6 mM 2,4-dinitrophenol and 0.5 mM pentachlorophenol. On the other hand, the turbidity of a chloroplast suspension increased during the shrinking of chloroplasts over the entire spectral region. The turbidity measured at 530 mμ changed on illumination in parallel with the absorbance change, although the directions of these changes of absorbance and turbidity were opposite (Curves A and C in Fig. 2). These results indicate the close correlation between the light-induced shrinkage of chloroplasts and the absorbance change.



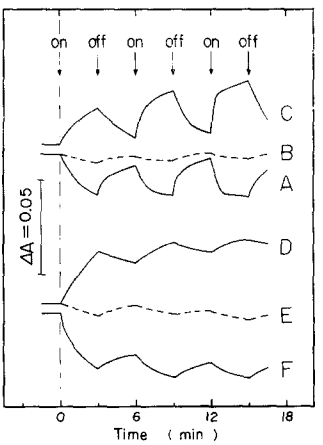


Fig. 1. The light-*minus*-dark difference spectra in terms of absorbance measured under conditions for shrinkage (Curve A) and for swelling (B). Chloroplasts (18 μg chlorophylls per ml) were suspended in 40 mM phosphate buffer containing 10 μM DCIP *plus* 1 mM ascorbate (Curve A) or 60 mM methylamine *plus* 60 μM $TMQH_2$ (B). Far-red light was turned on for 15 min (Curve A) and for 30 min (B) prior to the measurement, and the illumination was continued during the measurement.

Fig. 2. Time courses of the absorbance changes at 435 mμ (Curves A, B, D and E) and of the turbidity changes at 530 mμ (Curves C and F) as observed under conditions for shrinkage (A, B and C) and for swelling (D, E and F). Chloroplasts (22 μg chlorophylls per ml) were suspended in 40 mM phosphate buffer containing 20 μM phenazine methosulfate *plus* 1 mM ascorbate (Curves A and C) or 20 μM phenazine methosulfate *plus* 1 mM ascorbate *plus* 4 mM NH_4Cl (B) or 60 mM methylamine *plus* 60 μM $TMQH_2$ (Curves D and F) or 60 mM methylamine *plus* 60 μM $TMQH_2$ *plus* 10 μM DCMU (E). Red light was turned on or off as indicated by arrows.

If the decrease of absorption in the light is due to the shrinkage of chloroplasts, a reverse change may take place on swelling of chloroplasts. As expected, the absorbance increased in the light in the presence of 60 mM methylamine hydrochloride *plus* 60 μM trimethyl-1,4-benzoquinol ($TMQH_2$) (Curve B in Fig. 1). The shapes of the two difference spectra obtained under the conditions favorable for shrinkage and for swelling, respectively, were found to be similar, although the changes took place in opposite directions (Fig. 1). The absorbance change observed with methylamine was reversed in the dark but to a lesser extent (Curve D in Fig. 2). The change in

the presence of methylamine *plus* $TMQH_2$ was inhibited completely by 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (Curve E in Fig. 2), being compatible with the previous observation[8] that the light-induced swelling of chloroplasts is inhibited by DCMU. The turbidity change associated with the swelling in the presence of methylamine proceeded in the same manner as the absorbance change, although the directions of these changes were opposite (Curves D and F in Fig. 2).

It was thus established that the absorbance of chloroplast suspensions decreases on shrinkage of chloroplasts and increases on swelling. The light-induced absorbance changes found in the present study may be interpreted as due to the flattening effect of DUYSENS[9]. According to this effect, the absorbance of a chloroplast suspension should decrease when the projected area of the chloroplasts in suspension decreases and *vice versa*. The close similarity found between the wavelength dependency of the absorbance change on the shrinkage or swelling and the absorption spectrum of chloroplasts strongly supports this interpretation. A similar spectral change was obtained previously[10] when chloroplasts were forced to swell by use of a detergent, dodecylbenzene sulfonate. The absorbance of chloroplast suspensions may well change on varying the osmotic pressure of the medium. In fact, addition of 0.5 M NaCl or 0.5 M sucrose to chloroplasts suspended in 0.04 M phosphate caused a decrease of absorbance (approx. 4% of the total absorbance at 435 mμ) with a wavelength dependency similar to that observed during shrinking (Curve A in Fig. 1). The absorbance changes found in the present study may serve as a tool for the study of structural changes of chloroplasts. The changes in absorbance as compared to the changes in scattering can be interpreted from a more firm theoretical standpoint as changes in structure.

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