

## THE EFFECT OF MANGANESE DEFICIENCY ON THE STRUCTURE OF SPINACH CHLOROPLASTS

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Manganese is involved in the photosynthetic reactions of algae and leaves of higher plants (1-3). In tomato, manganese deficiency results in chloroplasts with a greatly reduced Hill reaction activity per unit chlorophyll (3). In spinach, manganese deficiency causes a 50 to 70 per cent reduction in the FMN-mediated photophosphorylation of isolated chloroplasts but only slightly affects the phosphorylation mediated by pyocyanine (4). Manganese is a constituent of the chloroplasts of higher plants. Photochemical activity of isolated chloroplasts is closely correlated with manganese content, and the manganese important in these reactions is not free ionic manganese but that bound within the chloroplast (5).

This paper reports the effects of manganese deficiency on the structure of spinach chloroplasts.

Manganese-deficient spinach plants (*Spinacia oleracea* L.) grown in nutrient culture solutions (3) were harvested together with healthy control plants when the leaves were showing a mild chlorosis. Small portions of the leaf were infiltrated with 0.3 M sucrose, sections were cut free hand, and the living leaf cells examined by light microscopy. Samples were fixed in  $\text{KMnO}_4$  (6) for electron microscope observations. In living tissue manganese-deficient cells contain a considerably reduced number of chloroplasts. These are characterised by dark regions which fluoresce strongly under the fluorescence microscope, and are therefore regions of chlorophyll concentration (Fig. 2), whereas chloroplasts from normal leaves contain numerous, well defined grana which fluoresce strongly under the fluorescence microscope (Fig. 1).

The basic ultrastructure of normal spinach chloroplasts (Fig. 3) is essentially similar to that reported for *Zea mays* (6), sorghum (7), and tobacco (8). The chloroplasts are seen to contain a well defined system of lamellae differentiated

into grana and intergrana regions. Stroma is present between the intergrana lamellae, but does not penetrate into the grana. The chloroplast is always enclosed by a membrane. Stroma material may form a wide band between the lamellae and the membrane, or form pseudopodia-like extensions devoid of lamellae, but enclosed by the membrane.

In chloroplasts from slightly deficient plants the intergrana lamellae are seen to be detached from the grana and lie scattered throughout the stroma, but the grana are more or less normal. With increasing severity of deficiency, fewer intergrana lamellae are present and some of these fuse end-to-end to give vesicles. "Empty" regions appear in the stroma. The grana are abnormal, tend to be elliptical in shape, and the individual lamellar discs tend to be separate. In more severe deficiency, intergrana lamellae are no longer recognizable, only vesicles, 0.01  $\mu$  in diameter, which occur randomly in the stroma. Also the grana are much disorganized and appear as clusters of loosely connected lamellar discs. These clusters probably correspond to the dark bodies which fluoresce strongly under the fluorescence microscope. In extreme deficiency, both grana and intergrana lamellae may be entirely absent, the chloroplast consisting only of a matrix, presumably stroma, with empty regions, enclosed by the membrane. As far as could be detected, manganese deficiency did not affect the structure of the mitochondria and cytoplasm.

These observations suggest that the abnormal photochemical activity associated with manganese deficiency may result in part from structural changes in the photosynthetic machinery, possibly through a reduction in the amount of lamellae. The structural disorganization described above was observed in advanced manganese deficiency. The disorganization of the lamellar system which

develops in the earlier stages of deficiency is paralleled by alterations in photochemical activity (3, 4). It is likely, however, that structural changes having profound biochemical significance are not identifiable by existing microscope techniques.

Further work is proceeding to examine the influence of other nutritional deficiencies on the relationship between the structure and photosynthetic activity of chloroplasts, and to test the specificity of the manganese observation. It is of interest that iron-deficient *Xanthium* leaves have been found to contain chloroplasts which have a granular disorganized appearance (9). We have confirmed this result for tomato and spinach, and find that the structural abnormalities produced by iron deficiency and manganese deficiency are quite different. This observation is in agreement with physiological work which has shown that these two deficiencies have different effects on the photochemical reactions of the chloroplasts of higher plants. Iron deficiency limits photosynthesis primarily *via* an effect on chlorophyll formation whereas manganese deficiency limits some other

component of the photochemical system more than it limits chlorophyll formation (3).

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#### FIGURE 1

Normal chloroplasts in living spinach cells showing numerous grana (*g*). Photographed by flash illumination under phase contrast.  $\times 1500$ .

#### FIGURE 2

Manganese-deficient chloroplasts in living cells showing dark regions (*d.r.*) and absence of distinct grana. Photographed by flash illumination under phase contrast.  $\times 1500$ .

#### FIGURE 3

Ultrastructure of normal chloroplast. The grana (*g*) consist of discs of lamellae connected by intergrana lamellae (*l*). The stroma (*s*) penetrates between the intergrana lamellae and is enclosed within the chloroplast membrane (*c.m.*). A pseudopodium-like extension (*p*) of the stroma is seen on end of the chloroplast.  $\times 20,000$ .

#### FIGURE 4

Ultrastructure of manganese-deficient chloroplast. The lamellar system is poorly developed, the grana appear as clusters of lamellae (*c.l.*) while the vesicles (*v*) correspond to the intergrana lamellae. The stroma shows empty regions (*e*). The chloroplast membrane (*c.m.*) appears normal.  $\times 20,000$ .

