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BIOCHEMICAL STUDIES OF MICROELEMENTS IN GREEN PLANTS

IV. STATUS OF CHLOROPLASTS AND RATE OF PHOTOSYNTHESIS IN MICROELEMENT DEFICIENT BARLEY LEAVES

By

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A shortage of microelements usually makes plant leaves chlorotic, more or less. Chlorosis means the decrease of the chlorophyll content in the leaf-tissue. Chlorophyll has been recognized to exist solely in chloroplasts, which multiply, enlarge and become chlorophyllous from colorless proplastids within the cell during its development. Among the chlorosis caused by microelement deficiencies, the best known is the iron chlorosis. On the iron chlorosis of sunflower leaves, Jacobson (1) has studied the relation between iron and chlorophyll contents and suggested that iron is involved in the chloroplast formation *via* protein synthesis, directly or indirectly. Bogorad *et al.* (2) have recently found by the electronmicroscope that the chloroplasts of the leaf-tissue of iron deficient *xanthium* are about the same as those of the green tissue in size but considerably differ from the latters having poor lamellar structure.

It is of interest with relation to the microelement metabolisms to study the status of chloroplasts in microelement deficient leaves involving iron deficient ones. Almost no work, however, has been done on this subject except for that of Bogorad *et al.* (2). Thus, in the present work, chloroplast fractions were separated by differential centrifugation from the macerates of barley leaves which had revealed the characteristic deficiency symptoms of the respective microelements and investigated on their status. At the same time, the rate of photosynthesis in these leaves was measured and discussed in connection with the chloroplast status.

Materials and Methods

Deficient culture of barley: The two week old barley seedlings were grown on the cultural solutions from which iron, manganese, zinc, copper, and mo-

lybdenum was eliminated individually as described before (3). After the culture of 50 days, when the microelement deficiency symptoms were observable, the plants were taken for the following experiments.

Preparation of chloroplasts: About 5 g of leaf blades were taken from these plants and macerated in a mortar chilled on an ice bath with 5 *ml* of 0.35 *M* NaCl solution. The macerate was filtered through three layers of gauze and to the filtrate was added an appropriate volume of 0.35 *M* NaCl solution then centrifuged at 200 G 2 min by the refrigerated centrifuge to precipitate the unbroken cells, cell debris or nuclei. The supernatant obtained was then centrifuged at 1000 G 30 min. After the supernatant was discarded, the precipitate was again suspended in 0.35 *M* NaCl solution and the centrifugation repeated. The chloroplast fractions were thus seperated as the precipitate.

Technique of measurements: Chlorophyll in the leaf blades and chloroplast fractions was extracted with 80 per cent acetone and the optical densities at 645 and 663 mμ of the acetone exract were measured, then the concentration of chlorophyll was calculated following in Arnon's equations (4). The nitrogen contents of the plant materials were determined by Kjeldahl's method.

The rate of photosynthesis was measured by counting the radioactivity of ^{14}C incorporated as CO_2 into the leaf blade. The $^{14}CO_2$ incorporation was made in a closed glass-chamber which had been described previously (5). Each one of the respective microelement deficient plants was kept 6 hr in dark beforehand, then taken together into the chamber. 200 μc of $^{14}CO_2$ were diffused within it and photosynthetic CO_2 assimilation was brought about under the irradiation of about 20,000 lux by incandescent lamps for 20 minutes at 20°C.

Results

Status of leaf blades: Total- and protein-nitrogen contents in dry matter of leaf blades used for the present experiment are shown in Table 1. The protein levels of the microelement deficient leaf blades did not differ largely from the normal level except for the zinc deficient ones, of which the protein nitrogen was characteristically low. The total-nitrogen of all deficient leaves

Table 1. Total- and protein-nitrogen in microelement deficient barley leaf blades. (dry weight basis)

Sample	Total-N	Protein-N
Complete	4.40%	3.62%
-Fe	6.16	3.40
-Mh	5.26	3.82
-Zn	5.32	2.48
-Cu	5.34	3.96
-Mo	4.46	3.56

except molybdenum deficient ones was clearly higher than that of the normal. These results may be regarded as the effects of deficiency of the microelements on the nitrogen status of the leaf blades.

The data obtained for chlorophyll content are given in Table 2. Iron deficient leaf, on which the most severe chlorosis had been observed visually, was lowest of all in chlorophyll content. In manganese, zinc, and copper deficient leaves, the contents of chlorophyll were also considerably reduced. The molybdenum deficient leaf showed no chlorotic symptom and their chlorophyll was also as high as the normal level. The ratio of chlorophyll *a* to *b* in the microelement deficient leaves deviated to some degree from the normal value. Especially it appears that the deficiency of iron causes the augmentation of this ratio whereas that of manganese causes the diminution of it. Though the mechanism involved in such phenomena has not been comprehended yet, it seems to be noticeable that there exists a clear contrast of this ratio between apparently similar chlorosis produced by the iron and the manganese deficiency.

Table 2. Chlorophyll contents in microelement deficient barley leaf blades. (dry weight basis)

Sample	Chlorophyll *a*	Chlorophyll *b*	Total-chlorophyll	*a*/*b*
Complete	0.671%	0.190%	0.861%	3.54
-Fe	0.238	0.046	0.284	5.18
-Mn	0.509	0.187	0.696	2.72
-Zn	0.389	0.111	0.500	3.50
-Cu	0.524	0.131	0.655	4.00
-Mo	0.711	0.183	0.894	3.88

Status of chloroplasts: The results obtained by the analysis of isolated chloroplast fractions are given in Table 3. In chloroplasts from iron deficient leaf the total-chlorophyll decreased strikingly and those from manganese, zinc, and copper deficient leaves also lowered to some degree. On the other hand, the protein-nitrogen decreased in general not so largely from the normal level as the total-chlorophyll, even in iron deficient chloroplasts. The mass ratio of chlorophyll to protein in iron deficient chloroplasts, therefore, was about one third of the normal value.

The percentage of the amount of chloroplasts present in the leaf blade was obtained by comparing the concentration of total-chlorophyll in the formers to that in the latter (6). The ratio of chlorophyll *a* to *b* obtained on chloroplasts was found to differ a little from that on the leaf blade in all cases. These deviations, which might be caused by some physicochemical effects in the isolating process of chloroplasts, produce inevitable errors on the chloroplast contents according to whether chlorophyll *a*, *b* or total-chlorophyll is used as the basis

of this comparison. Taking account of this point, however, it may be mentioned from the data given in Table 3 that the chloroplast content of iron deficient leaf was never low as compared with that of the normal leaf whereas in zinc deficient leaf the chloroplast content was reduced to a great extent.

Table 3. Status of chloroplasts from microelement deficient barley leaves. (dry weight basis)

Sample	Chlorophyll *a*	Chlorophyll *b*	Total-chlorophyll	*a*/*b*	Protein-N	Chlorophyll /Protein (N×6.25)	Chloroplasts /Leaf blade
Complete	5.54%	1.63%	7.17%	3.40	8.11%	0.141	12.0%
-Fe	1.57	0.31	1.88	5.07	6.03	0.050	15.1
-Mn	3.98	1.54	5.52	2.58	7.83	0.113	13.2
-Zn	5.07	1.47	6.52	3.45	7.42	0.140	7.7
-Cu	4.72	1.19	5.91	3.96	8.83	0.107	11.1
-Mo	5.71	1.60	7.31	3.57	7.80	0.150	12.2

Rate of photosynthesis: Table 4 shows the index number of the counts of photosynthetically incorporated ^{14}C per unit dry weight of leaf and that per unit weight of chlorophyll. As the matter of convenience, the former is designated as the rate of photosynthesis and the latter as the assimilation number.

Table 4. Index number of rate of photosynthesis in microelement deficient barley leaves.

Sample	dry weight basis	chlorophyll basis
Complete	100.0	100.0
-Fe	59.6	180.5
-Mn	44.5	55.0
-Zn	25.7	44.4
-Cu	45.9	60.3
-Mo	107.0	103.0

Except for the molybdenum deficient leaf, the rate of photosynthesis became reduced in all deficient leaves, particularly to the greatest degree in zinc deficient one. The assimilation number decreased in manganese, zinc, and copper deficient leaves, though in the iron deficient leaf it increased definitely. In this connection, Loustalot *et al.* (7) have found that there is a highly significant reduction in CO_2 assimilation even with normal appearing leaves in copper and zinc deficient tung trees and Pirson (8) has stated that the decrease of photosynthesis is clearly independent of any influence on the chlorophyll level at the beginning of manganese deficiency in green algae. On the other hand, it has been described that the assimilation number of iron deficient chlorotic plants was found to be not very different from that of normal plants (sometimes

smaller, sometimes larger) (9).

Discussion

The data gained on the iron deficient leaf indicate that iron chlorosis is caused by the retardation of chloroplast formation not quantitatively but qualitatively. On other words, in iron deficient chlorotic leaf, chloroplasts themselves are chlorotic. This view is well consistent with the observation made by Bogorad *et al.* (2). Moreover, since protein content in iron deficient chloroplasts was not so low as the chlorophyll content, Jacobson's opinion (1) can hardly be accepted as such, especially if his protein means vaguely the chloroplast protein as a whole, though the possibility of the presence of such a specific chloroplst protein that directly catalize the chlorophyll formation and is sensitively affected by iron shortage should be kept in mind.

Two specific iron proteins have already been found in the chloroplast and identified as cytochrome b_6 and f (10). Hill *et al.* (11) have presumed that these cytochromes might participate to the photosynthetic oxygen evolution. If the formation of these iron proteins decreased in iron deficient chloroplasts, it should not exceed the decrease of chlorophyll level, because the assimilation number was high in iron deficient leaf and consequently the rate-determining factor in photosynthetic process is considered to be chlorophyll. At any rate, from the results for the chloroplast status and for the assimilation number, it may be pointed out that the shortage of iron affects at first on chlorophyll formation, as Jacobson (12) stated in his early report.

The chloroplasts from both manganese and copper deficient leaf are also chlorotic and have low chlorophyll/protein ratios, though not so greatly as in iron deficient chloroplasts. Assimilation number, however, is very small in both leaves contrarily with that of the iron deficient leaf. Therefore, the rate of photosynthesis in both leaves is considered to be determined in the non chlorophyll participating step. Probably there are some functional defects in chloroplast protein of them. Katoh *et al.* (13) have recently isolated a copper protein from the chloroplast of chlorella and spinach and named it plastcyanin. This copper protein has been suggested to play an important part in the photosynthetic mechanism in green plants. The shortage of copper might cause the retardation in making up this protein within chloroplasts and subsequently reduce the rate of photosynthesis.

In zinc deficient leaf, the assimilation number decreases to the greatest degree. Therefore, its chloroplasts must be functionally incomplete though their chlorophyll/protein ratio does not differ from that of normal chloroplasts.

Neither chloroplast status nor rate of photosynthesis seems to be affected at the beginning of molybdenum deficiency, for there was no definite difference between the molybdenum deficient leaf and normal one. This status may be a

cauce to produce a high content of carbohydrate in molybdenum deficient plants (14).

Summary

Chloroplasts were separated from microelement deficient barley leaves by differential centrifugation and investigated on some status. In addition, the rate of photosynthesis in these leaves was measured and discussed.

In iron deficient leaf, the chloroplast content did not reduce but chlorophyll content in chloroplasts themselves was very low. Mass ratio of chlorophyll/protein in iron deficient chloroplasts was about one third of the normal level and the rate of photosynthesis per chlorophyll (assimilation number) in iron deficient leaf was found to be higher than in the normal leaf.

Chloroplasts from manganese and copper deficient leaves also showed a somewhat low chlorophyll content and chlorophyll/protein ratio but the assimilation number was considerably low in both cases.

In zinc deficient leaf, the chloroplast content was greatly reduced whereas the chlorophyll/protein ratio of chloroplasts was almost the same as the normal value. Assimilation number of zinc deficient leaf was smallest of all.

In molybdenum deficient leaf, there was no definite difference from the normal leaf on chloroplast status and rate of photosynthesis.

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