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Effects of Cation Levels of the Nutrient Medium on the Biochemistry of *Chlorella*

II. FACTORIAL EXPERIMENT[1]

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ABSTRACT

A factorial experiment was designed to study the effects of Mg^{2+}, K^+, and Na^+ on the growth and biochemistry of *Chlorella sorokiniana*. Raising Mg^{2+} or K^+ concentration in the nutrient medium increased growth rates as well as total N levels and Mg^{2+} and K^+ accumulation by the cells. The total N effect was Mg^{2+}-dependent—if Mg^{2+} was below a certain level in the medium—increasing the K^+ concentration did not raise the total N level of cells. Low nutrient levels of K^+ decreased the levels of unsaturated fatty acids (especially 18:1 and 18:3), while increasing the levels of palmitic acid (16:0), total fatty acids, and total lipid. Increasing nutrient K^+ concentrations were accompanied by increases in levels of some unsaturated fatty acids, with a concomitant reduction in 16:0, total fatty acids and total lipid. Low Mg^{2+} levels in the nutrient medium reduced the cellular levels of palmitic acid, total fatty acids, total lipid, and certain unsaturated fatty acids (though this last effect also depended on the nutrient level of K^+). These relationships indicate that Mg^{2+} may be important in the initial steps of fatty acid synthesis, whereas K^+ may be necessary for the formation of certain unsaturated fatty acids. Variations in Na^+ concentration did not have any significant effect on the growth and biochemistry of *C. sorokiniana*.

A concentration-series experiment (11) indicated that Mg^{2+} and K^+ play major roles in lipid synthesis and total N accumulation in *Chlorella*. Growth rates and Mg^{2+} and K^+ assimilation by the cells were also affected by nutrient levels of these cations. The concentration-series experiment was designed in part to ascertain appropriate concentrations of cations for the factorial experiment which was initiated to determine the specific effects of Mg^{2+}, K^+, and Na^+ on the growth and biochemistry of *C. sorokiniana*. The factorial design was appropriate, since it revealed several interactions between Mg^{2+} and K^+ and permitted further assessment of the role of each element in *Chlorella* metabolism.

MATERIALS AND METHODS

Experimental Design. From the concentration-series experiment (9, 11) "low," "medium," and "high" levels were selected for Mg^{2+}, K^+, and Na^+. For Mg^{2+} the values were 0.008, 0.03, and 0.1 meq/l, respectively; for K^+ 0.01, 0.04, and 1.0 meq/l, respectively; and for Na^+ 0.01, 0.1, 1.0 meq/l, respectively. Ca^{2+} was allowed to vary as required from 4.6 to 6.67 meq/l, and the other nutrients were held constant at levels previously described (11). The "high" levels for Mg^{2+} and K^+ were well above the sufficiency values for these elements as indicated by growth rate data (11). "Low" and "medium" values were those levels which permitted low and medium growth rates, respectively. A requirement for Na^+ in *Chlorella* has not been demonstrated.

Culture Conditions and Analytical Determinations. The culture conditions, analytical procedures, and growth rate formula used were described previously (11). The green alga, *Chlorella sorokiniana* Shihira et Krauss was inoculated to give an initial absorbance of 0.0015. Three replications of each treatment were analyzed. Statistical analyses of these data have been presented.

RESULTS AND DISCUSION

Many of the trends in lipid synthesis, total nitrogen, and cation accumulations observed in the concentration series (11) were also seen in the factorial experiment. In this instance, however, specific cation effects and interactions could be distinguished.

Growth Rates. When Mg^{2+} and K^+ in the nutrient medium were both "high," the growth rates were significantly higher than in any other treatment (9.0 doublings per day—a normal value for healthy *C. sorokiniana* cells) (Table I). "Medium" levels of both Mg^{2+} and K^+ reduced the growth rate to 8.1 doublings per day. Growth rates were further reduced at "low" levels of either Mg^{2+} or K^+ regardless of the level of the other cation (K^+ or Mg^{2+}, respectively). These growth rates ranged from 6.4 to 6.8 doublings per day and were significantly lower than those achieved when Mg^{2+} and K^+ were both at medium or high levels. Different Na^+ levels did not significantly affect growth or levels of any of the inorganic nutrients or other cell constituents studied (10). For simplification, we present the average data for the three Na^+ treatments, which for all practical purposes can be considered replicates.

Elemental Accumulation. Raising the nutrient Mg^{2+} concentration from low to medium increased the cellular concentration of Mg^{2+}. Increasing the K^+ concentration from low to high also stimulated the accumulation of Mg^{2+} by cells (Table I). Increasing Mg^{2+} in the nutrient medium as well as increasing K^+ in the nutrient medium stimulated K^+ accumulation by *Chlorella*. However, when Mg^{2+} was low in the nutrient medium, the percentage of K^+ in the cells was extremely low and never reached the higher levels found when Mg^{2+} and K^+ were medium or high (at comparable nutrient K^+ values).

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Table I. *Effects of Cation Levels on Growth and Elemental Composition of Chlorella sorokiniana*

Cation Level[1]		Growth Rate	Cations		
Mg^{2+}	K^+		Mg^{2+}	K^+	Ca^{2+}
		doublings/day		*% dry wt*	
H	H	9.0	0.22	1.10	0.34
H	M	8.3	0.14	0.58	0.54
H	L	6.8	0.14	0.28	0.44
M	H	7.8	0.17	1.02	0.31
M	M	8.1	0.15	0.53	0.53
M	L	6.4	0.15	0.31	0.65
L	H	6.8	0.10	0.39	0.35
L	M	6.8	0.10	0.42	0.40
L	L	6.5	0.08	0.15	0.28

[1] Magnesium: low, medium, and high = 0.008, 0.03, and 0.10 meq/l, respectively. Potassium: low, medium, and high = 0.01, 0.04, and 1.0 meq/l, respectively.

The interaction between these two cations in *Chlorella* was quite different from that observed in the tomato, *e.g.*, high substrate levels of K^+ reduced Mg^{2+} accumulation (7). The cellular sufficiency values of Mg^{2+} and K^+ were 0.22 and 1.1% of the dry weight, respectively, and were significantly below those for most higher plants (5). The accumulation of Ca^{2+} was normal in that cellular levels reflected the Ca^{2+} nutrient medium levels (ref. 11 and Table I).

Total Nitrogen. At medium K^+ level, increasing Mg^{2+} from medium to high resulted in a decrease in total cellular N. In all other cases, at all K^+ levels, increasing Mg^{2+} always resulted in increased cellular N (Table II). When Mg^{2+} was either medium or high in the nutrient medium, increasing the K^+ concentration raised the cellular total N levels. However, when Mg^{2+} was low, increasing K^+ did not increase the total N concentration. Mg^{2+} concentration must be greater than 0.008 meq/l before the effect of K^+ on per cent total N is seen.

The protein level of *Chlorella* is also reduced when either Mg^{2+} or K^+ is lacking in the nutrient medium (6). In the case of Mg^{2+}, this is not surprising, since it is implicated in the aggregation of ribosomal subunits to form a functional particle, although this has not yet been demonstrated for algal ribosomes (2).

Total Lipid, Total Fatty Acids, and Individual Fatty Acids. When Mg^{2+} was low in the nutrient medium (regardless of K^+ level), there was a reduction in per cent total cellular fatty acids. At medium level of Mg^{2+}, the per cent total lipid and per cent total fatty acids increased sharply, whereas at high Mg^{2+} level each of these fractions decreased slightly (Table II).

The effect of K^+ on per cent total lipid and per cent total fatty acids in cells was opposite to that of Mg^{2+}. When K^+ was raised from low to medium total lipid and total fatty acids decreased at all Mg^{2+} levels. A further increase in K^+ to high resulted in an increase in per cent total fatty acids but the total lipid response depended upon the Mg^{2+} level. The level of a saturated fatty acid, 16:0, increased very sharply in cells grown in low K^+ culture medium (Table III). This may have been partially responsible for the increase in per cent total lipid and per cent total fatty acids in cells grown under these conditions. Levels of an unsaturated fatty acid 18:3, were low in cells grown in low K^+ nutrient medium. Furthermore, increasing K^+ concentrations in the nutrient medium were accompanied by a reduction in the level of 16:0, and a general increase in the level of the unsaturated fatty acids—this being most pronounced in the cases of 18:1 and 18:3 (Table III).

The ratio of unsaturated fatty acid-saturated fatty acid in the cells clarifies these effects. At all levels of Mg^{2+}, increasing K^+ concentrations in the nutrient medium were always accompanied by increases in the ratio of unsaturated fatty acids to saturated fatty acids. Increasing Mg^{2+} concentrations in the medium did not significantly elevate the ratio of unsaturated fatty acid-saturated fatty acid unless the K^+ level in the medium was high. Minimal values of this ratio (0.8) occurred when K^+ was low in the nutrient medium. Therefore K^+, must be present at a concentration above 0.04 meq/liter before any effect of Mg^{2+} on the ratio of unsaturated fatty acid-saturated fatty acid occurs (Table II). This effect of Mg^{2+} on the ratio of unsaturated fatty acid-saturated fatty acid was also seen in the Mg^{2+} concentration series, since the K^+ level here was above the sufficiency value of 0.10 meq/l (11).

These lipid data indicate that a certain level of Mg^{2+} is required before optimal levels of 16:0, total fatty acids, and total lipid are formed in the cells, and that K^+ stimulates the synthesis of unsaturated fatty acids. However, low K^+ levels result in the accumulation of unusually high levels of total lipid and total fatty acids. When K^+ remained low and Mg^{2+} was increased to medium or to high, the increase in 16:9, total fatty

Table II. *Effects of Cation Levels on Biochemical Composition of Chlorella sorokiniana*

Cation Level[1]		Cell Components					Unsaturated Fatty Acid-Saturated Fatty Acid
Mg^{2+}	K^+	Total nitrogen	Total lipid	Total fatty acids	Saturated fatty acids	Unsaturated fatty acids	
		% dry wt					*ratio*
H	H	9.4	21.2	6.4	1.9	4.4	2.3
H	M	7.7	21.7	4.6	1.9	2.7	1.4
H	L	7.5	27.8	7.8	4.3	3.5	0.8
M	H	9.1	25.0	7.3	2.4	5.0	2.1
M	M	8.4	20.8	5.5	2.5	3.1	1.2
M	L	7.1	38.6	8.8	5.0	3.9	0.8
L	H	5.6	14.6	4.8	1.9	2.9	1.5
L	M	6.2	18.2	4.1	1.8	2.3	1.3
L	L	6.1	22.7	4.7	2.6	2.1	0.8

[1] Magnesium: low, medium, and high = 0.008, 0.03, and 0.10 meq/l, respectively. Potassium: low, medium, and high = 0.01, 0.04, and 1.0 meq/l, respectively.

Table III. *Effects of Cation Levels on Fatty Acid Composition of Chlorella sorokiniana*

Cation Level[1]		Fatty Acids								
Mg^{2+}	K^+	14:0	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
		% cell dry wt								
H	H	0.24	1.70	0.44	0.60	0.17	0.02	0.93	1.08	1.20
H	M	0.27	1.60	0.36	0.39	0.15	0.05	0.58	0.76	0.48
H	L	0.56	3.60	0.81	0.44	0.24	0.16	0.78	0.81	0.37
M	H	0.44	1.90	0.65	0.76	0.11	0.06	1.03	1.30	1.12
M	M	0.27	2.10	0.46	0.39	0.15	0.12	0.68	0.87	0.59
M	L	0.66	4.30	0.23	0.80	0.31	0.08	0.88	1.23	0.47
L	H	0.25	1.60	0.29	0.23	0.16	0.03	0.78	0.51	0.90
L	M	0.26	1.50	0.32	0.18	0.20	0.05	0.60	0.43	0.58
L	L	0.31	2.10	0.54	0.25	0.15	0.22	0.49	0.44	0.24

[1] Magnesium: low, medium, and high = 0.008, 0.03, and 0.10 meq/l, respectively. Potassium: low, medium, and high = 0.01, 0.04, and 1.0 meq/l, respectively.

acids, and total lipid indicated that Mg^{2+} was involved at an initial stage in lipid synthesis (Tables II and III). The very high concentration of 16:0 under the conditions of low K^+ also indicated that certain levels of K^+ were required for the conversion of 16:0 or one of its precursors into unsaturated fatty acids. This effect is highlighted by the increases seen in the levels of unsaturated fatty acids (especially 18:3) and in the ratio of unsaturated fatty acid-saturated fatty acid, when increasing the K^+ concentration at each level of Mg^{2+}.

The exact site of Mg^{2+} and K^+ activity in lipid synthesis has yet to be determined. Mg^{2+} is an essential cofactor in several biochemical conversions, one such step being the production of acetyl CoA from pyruvate. Mg^{2+} deficiency could inhibit the conversion of pyruvate to acetyl CoA. This, in turn would reduce the level of acetyl CoA available for conversion into fatty acids and lead to a general reduction in the levels of the individual fatty acids, total fatty acids, and total lipid. Whereas the major effect of Mg^{2+} on fatty acid synthesis occurs before chain elongation begins, K^+ is necessary for the production of 18-carbon unsaturated fatty acids, especially 18:3. Formation of these fatty acids is linked to that of 16:0, since they accumulate in the cell at its expense. The exact role of K^+ is not clear, other than it may be involved in some unknown mechanism entailing either desaturation of an 18- or a 16-carbon intermediate, or else elongation of a short chain unsaturated precursor.

The changes in lipid synthesis recorded here must be connected with, and influenced by, metabolic events at other sites in the cell. Others have observed that a deficit of K^+ leads to an increase in lipid and a reduction in protein of *Chlorella* (6). This is not surprising, since fat accumulation of N-limited algal cultures is a well known phenomenon (3, 8, 12, 13), although this effect may be confined to batch-cultured cells (14). The data from the work described in this paper indicate that, since *Chlorella* cells grown under low K^+ conditions have low total N levels, then the metabolic products normally destined for protein synthesis could be rerouted into lipid synthesis to give the observed increases in levels of 16:0, total fatty acids, and total lipid. These increases were more pronounced if Mg^{2+} was medium or high in nutrient medium, since Mg^{2+} is essential at the initial stages of lipid synthesis (Tables II and III). Regardless of the K^+ level, if Mg^{2+} was low in the nutrient medium, the initial cellular concentrations of fatty acids and total N were so low that rerouting of other metabolic products (if it occurs at all in these treatments) did not cause a net gain in lipid synthesis (Table II). At the other Mg^{2+} levels, as the nutrient medium K^+ concentration was increased to medium to high, protein synthesis accelerated and the synthesis of lipid took place via the usual pathways.

A control mechanism similar to that proposed by Atkinson (1) for N-starved tissue may operate in cells low in K^+ because these have impaired N accumulation. He suggested that in N-starved tissues the level of ATP increases since protein synthesis is limited. The level of AMP decreases and reduces the activity of isocitric dehydrogenase. Citric acid accumulates, activity of acetyl CoA carboxylase is increased, and thus the conversion of acetyl CoA to fatty acids is catalyzed. A control mechanism like this, operating in cells grown under low K^+ conditions, could account for the increases in 16:0, total fatty acids, and total lipid in these cells. This control mechanism could not increase lipid synthesis under Mg^{2+} deficiency conditions, since Mg^{2+} is necessary to catalyze conversion of pyruvate to acetyl CoA. Therefore, when Mg^{2+} is deficient for growth, impaired fatty acid synthesis is likely, owing to low levels of acetyl CoA, even though Mg^{2+} is also essential for N accumulation in *Chlorella* (Table II). Inasmuch as fatty acid levels of *Chlorella* are low under Mg^{2+} deficiency conditions (Tables II and III), the metabolic products normally incorporated into protein may be shunted into carbohydrate synthesis. Such an increase in carbohydrate level of *Chlorella* has been observed by Galling (4) when protein synthesis was inhibited by a deficiency of Mg^{2+}.

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