Manganese as Substitute for Magnesium During Magnesium-limited Growth of the Cyanobacterium *Anacystis nidulans*

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(Received 3 December 1982; revised 20 January 1983)

An apparent inhibition of cell division in the cyanobacterium *Anacystis nidulans*, caused by low Mg\(^{2+}\) concentrations, was abolished by increasing the medium Mn\(^{2+}\) concentration. Thus the mean cell volume of this organism growing in a Mg\(^{2+}\)-limited chemostat culture decreased from an average of 1.3 to 0.4 \(\mu\text{m}^3\) following an increase in the reservoir Mn\(^{2+}\) concentration from 9.5 to 15 \(\mu\text{M}\). This increase in Mn\(^{2+}\) had no effect on the steady-state biomass concentration, while a further elevation of the Mn\(^{2+}\) concentration lowered the biomass concentration, seemingly by making Mg\(^{2+}\) less available to the organism. The cellular Mn\(^{2+}\) concentration increased, while cellular Mg\(^{2+}\) was unaltered, following an increase in the medium Mn\(^{2+}\) concentration.

**INTRODUCTION**

Although Mn\(^{2+}\) cannot replace Mg\(^{2+}\) for growth (Tempest, 1969; Utkilen, 1982), there is evidence indicating that Mg\(^{2+}\) can be replaced by Mn\(^{2+}\) in some cellular processes (Kennell & Kotoulas, 1967; Webb, 1968). Cell division could be one of the events where Mn\(^{2+}\) might substitute for Mg\(^{2+}\). Deprivation of either Mn\(^{2+}\) (Alberts-Dietert, 1941) or Mg\(^{2+}\) (Finkle & Appleman, 1953) resulted in cell enlargement of *Chlorella*, and cell enlargement was also found when a diatom was deprived of Mn\(^{2+}\) (Von Stosch, 1942).

In the cyanobacterium *Anacystis nidulans*, cell division is influenced by Mg\(^{2+}\) (Utkilen, 1982) and cell division was dissociated from biomass production when the organism was grown in media containing 5 \(\mu\text{M-Mg}^{2+}\). In order to examine whether Mn\(^{2+}\) could replace Mg\(^{2+}\) in cell division of *A. nidulans*, the concentration of Mn\(^{2+}\) in the growth medium of a chemostat limited by Mg\(^{2+}\) was progressively increased.

**METHODS**

**Organism.** *Anacystis nidulans* strain UTEX 625 of the Culture Collection of Algae, Department of Botany, University of Texas, was used.

**Growth conditions.** The organism was grown in a Mg\(^{2+}\)-limited chemostat as described earlier (Utkilen, 1982). The feed medium was designed to give a final concentration of 5 \(\mu\text{M-MgCl}_2\), but when the Mg\(^{2+}\) concentration in feed medium was measured it turned out to be about 6 \(\mu\text{M}\) (Table 1). The difference between added and measured Mg\(^{2+}\) could be due to impurities from other chemicals, but the assay errors were large (25 and 15\%), which also might account for some of the difference. The desired Mn\(^{2+}\) concentration was obtained by adding MnCl\(_2\), which was autoclaved separately unless otherwise stated.

**Cell number and volume.** These were determined by a Coulter electronic particle counter (Model ZB, industrial, Coulter Electronics Ltd, U.K.), as described earlier (Utkilen, 1982).

**Analytical methods.** Macromolecule and dry weight estimations were performed as previously described (Utkilen, 1982). Mn\(^{2+}\) and Mg\(^{2+}\) were determined by atomic absorption spectrophotometry (Perkin Elmer 306, Connecticut, U.S.A.), using air/acetylene.

**RESULTS AND DISCUSSION**

Marler & Van Baalen (1965) showed that about 60 \(\mu\text{g H}_2\text{O}_2\ 1^{-1}\) was formed in medium C (Kratz & Meyers, 1955) during autoclaving. This was due to a reaction between citrate and...
Mn\textsuperscript{2+}. The same authors demonstrated that the growth of \textit{A. nidulans} was extremely sensitive to H\textsubscript{2}O\textsubscript{2}. In order to examine whether increasing the Mn\textsuperscript{2+} concentration would have any inhibitory effect as a consequence of such a reaction, the concentration of this cation was increased to 20 pM (9.5 µM in medium C) in the reservoir before or after autoclaving. The results revealed that the steady-state biomass was about 80 µg ml\textsuperscript{-1} and the chlorophyll content 0.8\% of dry weight in both cases. The different ways of handling Mn\textsuperscript{2+} therefore had no effect on the growth of \textit{A. nidulans}. As a result of these preliminary experiments the additional amount of Mn\textsuperscript{2+} was autoclaved separately, since there was a heavy precipitation during autoclaving media that contained 20 µM-Mn\textsuperscript{2+}.

The steady-state dry weight for the Mg\textsuperscript{2+}-limited (6 µM) chemostat at \( D = 0.1 \) h\textsuperscript{-1} was 106 µg ml\textsuperscript{-1} when the reservoir contained 9.5 or 15 µM Mn\textsuperscript{2+}. Increasing the Mn\textsuperscript{2+} concentration to 20 or 100 µM reduced the steady-state dry weight to 85 µg ml\textsuperscript{-1}. The reduction in steady-state biomass was caused by an inhibition of Mg\textsuperscript{2+} uptake, since Mg\textsuperscript{2+} was detected in the culture medium at 20 µM-Mn\textsuperscript{2+} (Table 1). It was also found that the Mn\textsuperscript{2+} concentration in the feed medium was about 5 or 13 µM, when 9.5 or 20 µM-Mn\textsuperscript{2+}, respectively, was added to the reservoir (Table 1). This difference, which was not found for Mg\textsuperscript{2+}, could be due to precipitation in the reservoir. These results indicate that Mn\textsuperscript{2+} had a constant inhibitory effect on biomass production of \textit{A. nidulans} over a wide range of concentrations above 13 µM in a chemostat limited by 6 µM-Mg\textsuperscript{2+}.

The most pronounced effect of increasing the Mn\textsuperscript{2+} concentration was on mean cell volume, since increasing the reservoir Mn\textsuperscript{2+} concentration from 9.5 to 15 µM resulted in a decrease of cell volume from about 1.4 to 0.4 µm\textsuperscript{3}. These minute cells were also obtained with 20 or 100 µM-Mn\textsuperscript{2+} in the reservoir. These cell sizes and the results in Table 1 were used to calculate intracellular concentrations of Mn\textsuperscript{2+} and Mg\textsuperscript{2+}. The cellular Mg\textsuperscript{2+} concentration was found to be about 100 mM at both 5 and 13 µM-Mn\textsuperscript{2+}, while the cellular Mn\textsuperscript{2+} concentration increased from about 9 to 35 mM over the same range of extracellular Mn\textsuperscript{2+} concentrations. Thus, although the cellular Mn\textsuperscript{2+} concentration increased almost fourfold, the organism was able to maintain its Mg\textsuperscript{2+} concentration. But \textit{A. nidulans} could no longer deplete the medium of Mg\textsuperscript{2+} at 13 µM-Mn\textsuperscript{2+} or higher. A competitive inhibition of Mg\textsuperscript{2+} uptake by Mn\textsuperscript{2+} was unlikely, since the lowering of biomass concentration was the same with either 20 or 100 µM-Mn\textsuperscript{2+} in the reservoir.

The cell size of \textit{A. nidulans} growing in a Mg\textsuperscript{2+}-limited chemostat culture decreased with increasing growth rate, while it increased with growth rate when SO\textsubscript{4}\textsuperscript{2-} was the limiting nutrient (Utkilen, 1982). The mean cell volume, as a function of growth rate in a Mg\textsuperscript{2+}-limited chemostat culture with additional Mn\textsuperscript{2+}, followed the same pattern as for a non-Mg\textsuperscript{2+}-limited culture (Fig. 1). A Mg\textsuperscript{2+} shift-up from 5 µM to 1 mM, during balanced growth, resulted in a synchronized cell division of \textit{A. nidulans} after 90 min and was accompanied by a marked decrease in cell volume (Utkilen, 1982). In order to investigate whether Mn\textsuperscript{2+} would have the same effect on cell division, the organism was grown in batch cultures as described earlier (Utkilen, 1982) and Mn\textsuperscript{2+} shift-ups to 15, 20, 50 and 100 µM were made by adding MnCl\textsubscript{2}. These shifts revealed that the cell volume began to decline about 60 min after the Mn\textsuperscript{2+} shift-up, but the decrease in cell volume was not as marked as with a Mg\textsuperscript{2+} shift-up (Utkilen, 1982) and there was no synchronized cell division accompanying the decrease in cell volume.

### Table 1. Mn\textsuperscript{2+} and Mg\textsuperscript{2+} concentrations in feed medium and culture medium, when 9.5 or 20 µM-Mn\textsuperscript{2+} was added to the reservoir of a chemostat limited by 6 µM-Mg\textsuperscript{2+} (\( D = 0.01 \) h\textsuperscript{-1})

The steady-state cell number at the two Mn\textsuperscript{2+} concentrations is also shown. The concentrations of the cations are given ± S.D. (six determinations), while cell numbers are average values obtained from two samples.

<table>
<thead>
<tr>
<th>Concentration (µM) in feed medium</th>
<th>Concentration (µM) in culture medium</th>
<th>10(^{-1}) x No. of cells ml\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg\textsuperscript{2+}</td>
<td>Mn\textsuperscript{2+}</td>
<td></td>
</tr>
<tr>
<td>6.0 ± 1.5</td>
<td>4.7 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>6.0 ± 0.9</td>
<td>13.2 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

The steady-state Mn\textsuperscript{2+} concentration in the feed medium was about 5 or 13 µM, when 9.5 or 20 µM-Mn\textsuperscript{2+}, respectively, was added to the reservoir (Table 1).
Mn$^{2+}$ effect on Mg$^{2+}$-limited A. nidulans

The results presented here indicate that Mn$^{2+}$ could functionally replace Mg$^{2+}$ in the cell division process during Mg$^{2+}$-limited growth. In doing so, Mn$^{2+}$ was apparently more efficient than Mg$^{2+}$, since very small cells were obtained although most of the added Mn$^{2+}$ was not taken up by the organism (Table 1). Increasing the concentration of Mg$^{2+}$, which was depleted from the medium, did not result in the same decrease of cell volume (Utkilen, 1982) though it resulted in a corresponding increase in biomass concentration (Utkilen, 1982). Therefore only a fraction of the additional Mg$^{2+}$ would be available for cell division, in contrast to Mn$^{2+}$ where no increase in biomass was observed. Mn$^{2+}$ might in fact be less effective than Mg$^{2+}$ in cell division, since no synchronized cell division was observed during a Mn$^{2+}$ shift-up in contrast to that of a Mg$^{2+}$ shift-up (Utkilen, 1982).

REFERENCES


