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Polyamine-sensitive magnesium transport in *Saccharomyces cerevisiae*

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Abstract

In *Saccharomyces cerevisiae* we found a toxic effect of polyamines, well-known metabolites important for cell proliferation; in magnesium-limited (50 μM Mg^{2+}) synthetic medium, cell growth was severely inhibited by spermine, spermidine and putrescine in descending order. In conjunction with a decrease in the growth rate by the addition of 0.5 mM spermine, the internal Mg^{2+} content decreased and the spermine content increased. When cell growth ceased, the Mg^{2+} content had finally decreased to about 40% of the value before the addition of spermine (120–130 nmol/mg dry weight), and the spermine content concomitantly increased 30-fold (from 1 to 30 nmol/mg dry weight); $spermine^{4+}$ apparently took the internal place of Mg^{2+} with a probable stoichiometry of 1:2. However, the total amount of Mg^{2+} retained in the cells remained constant even with the addition of spermine, suggesting that spermine blocks Mg^{2+} accumulation. In high (2 mM) Mg^{2+} medium, cell growth was hardly affected by polyamines, and an exchange of spermine and Mg^{2+} was minimal. Energy-dependent Mg^{2+} uptake by whole cells was inhibited by spermine, spermidine and putrescine in a similar manner as the growth rates. On the other hand, Mg^{2+} inhibited spermine uptake. These results suggest that competition takes place between extracellular spermine and Mg^{2+} for their accumulations. It is thus clear that polyamine-sensitive Mg^{2+} transport system is indispensable for the physiology of this organism.

Key words: Polyamine toxicity; Magnesium ion transport; Spermine transport; (*S. cerevisiae*)

1. Introduction

Magnesium is the most abundant divalent cation within all living cells [1–4]. More than 90% of the internal Mg^{2+} can be assumed to be bound (largely to the intracellular polynucleotides), so that it is not osmotically free but readily exchangeable. It has been considered that internal Mg^{2+} acts as a membrane-stabilizing agent and as a cofactor with ATP in enzymatic reactions. However, recent studies showed significant changes in the intracellular free Mg^{2+} concentration after a variety of physiological stimuli, and there is increasing evidence about the hormonal regulation of Mg^{2+} fluxes in mammals [5–8]. Consequently, a variety of regulatory functions for Mg^{2+} have been postulated [9–13].

To understand the physiological roles of the intracellular Mg^{2+}, its membrane transport process must be elucidated. The magnesium content within cells is generally maintained within a narrow range despite large variations in its external concentration [1–4]; in bacterial cells the intracellular Mg^{2+} is equivalent to 20–40 mM if all Mg^{2+} is free. This implies the existence of a specialized magnesium transport system in the cell membrane. The active uptake of Mg^{2+} has been studied in several bacteria [14,15]. Genetic studies in *Escherichia coli* and *Salmonella typhimurium* showed the presence of several Mg^{2+} transport systems [16,17–20]. Three independent Mg^{2+} transport systems (CorA, MgtA and MgtB) have been reported in *S. typhimurium*. Their properties are now being characterized at the molecular level [21–23]. In mammals, recent observations have shown that, under physiologial and pathological conditions, a major redistribution of Mg^{2+} in cells and organelles occurs in liver, heart and other tissues [24–27]; mitochondria are the major intracellular Mg^{2+} reservoir [1,4]. However, the mechanism of net fluxes of Mg^{2+} across the membranes is less understood. It has been demonstrated in mammalian and non-mammalian cells that extracellular Na^+

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is important for permitting Mg^{2+} efflux [28,29]; Na^+/Mg^{2+} antiporter may be present [4]. On the other hand, studies on Mg^{2+} transport in unicellular eukaryotes have so far been limited. Mg^{2+} movement has been reported in yeast [30–32] but has not yet been fully characterized.

In this paper we report a feature of Mg^{2+} accumulation of *Saccharomyces cerevisiae*. From the investigation on the toxicity of polyamines, important metabolites in all living cells [33,34], we found that Mg^{2+} transport is specifically inhibited by polyamines, especially spermine.

2. Materials and methods

2.1. Strain and culture conditions

The haploid strain of *Saccharomyces cerevisiae*, X2180-1A, from the Yeast Genetic Stock Center, Berkeley, CA, was grown in a completely synthetic medium [35] devoid of $CaCl_2$, containing different concentrations of $MgSO_4$ at 30°C with shaking.

2.2. Measurements of internal Mg^{2+} and polyamines

The cells were collected on cellulose acetate filters (pore size, 0.45 μm; Advantec, Japan) and washed twice with 5 ml of 100 mM K^+-phosphate buffer (pH 5.6) to remove Mg^{2+} and polyamines bound to the cell exterior. The filters were suspended in 1 ml of 5% trichloroacetic acid, heated at 70°C for 10 min, and then centrifuged at 3000 × *g* for 10 min. The magnesium and polyamines extracted in the supernatant were measured with an atomic absorption spectrophotometer (Z-7000; Hitachi, Japan) and by high performance liquid chromatography (Toso, Japan) [36], respectively. The internal contents were referred to the dry weight of the cells.

2.3. Transport experiments

Mg^{2+} uptake. Cells grown on Mg^{2+}-limited (50 μM) medium were used, since the internal Mg^{2+} of cells grown to the stationary phase in this medium was only about 40% of that of exponential phase cells. Washed cells were suspended at 2.5 mg dry weight per ml in 20 mM MES-NaOH (pH 5.6) containing 1% glucose and incubated at 30°C, and the reaction was initiated by the addition of 0.25 mM $MgSO_4$. At various intervals, 1.0 ml of the suspension was overlaid on 0.5-ml of an oil mixture (corn oil/di-*n*-butylphthalate = 10:3) in a 2.0-ml microcentrifuge tube, and the cells were immediately centrifuged through the oil at 12000 × *g* for 10 s. After the supernatant and oil were removed, the cellular Mg^{2+} was extracted from the pellets as described above. The Mg^{2+} uptake was calculated from both the decrease in the supernatant Mg^{2+} and the increase in the Mg^{2+} contents of the pellets. The Mg^{2+} uptake by these cells was temperature-dependent and showed a velocity of 3.6 ± 0.4 nmol/min per mg dry weight. Polyamines and various metal ions were added 5 min before the reaction was started.

Uptake of Ni^{2+} and spermine. Washed cells, which were harvested in the mid-logarithmic phase, were

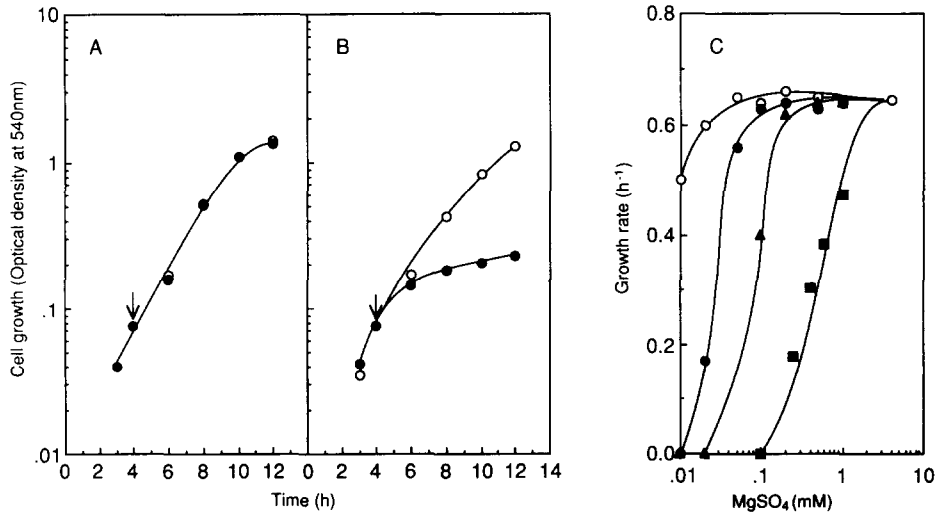

Fig. 1. Effect of spermine on the growth of *S. cerevisiae*. (A and B) Inhibition by spermine of the cell growth in low-Mg^{2+} medium. *S. cerevisiae* X2180 was cultured in a synthetic medium containing 2 mM $MgSO_4$ (A, high-Mg^{2+} medium) or 50 μM $MgSO_4$ (B, low-Mg^{2+} medium). Spermine (0.5 mM) was added at the point indicated by arrows, and cell growth was monitored. Symbols: ○, control; ●, plus spermine. (C) Effect of spermine on growth rates in media containing various concentrations of $MgSO_4$. Growth rates were calculated as described in Materials and methods. Symbols: ○, control; ●, 0.05 mM spermine; ▲, 0.2 mM spermine; ■, 1 mM spermine.

suspended at 4 mg dry weight per ml in the same buffer as used for Mg^{2+} uptake, and incubated at 30°C. The reaction was started by the addition of 800 μM $^{63}NiCl_2$ (15 MBq/mmol) or 10 μM [^{14}C]spermine (18.5 MBq/mmol), and 0.5 ml aliquots were filtered through cellulose acetate filters (pore size, 0.45 μm) at intervals. The filters were washed twice with 2 ml of the same buffer containing 20 mM $NiCl_2$ for ^{63}Ni uptake or 20 mM spermine for [^{14}C]spermine uptake, respectively, and the radioactivity trapped on the filters was counted in a liquid scintillation counter. All these uptake activities were referred to the dry weight of the cells.

2.4. Others

Cell growth was monitored by measuring the optical density at 540 nm with a spectrophotometer (model 20A, Shimadzu, Japan). The growth rates were determined between optical densities of 0.2 and 0.4. $^{63}NiCl_2$ (123 GBq/mmol) and [^{14}C]spermine (4 GBq/mmol) were purchased from New England Nuclear. Other reagents used were of analytical grade.

3. Results

3.1. Inhibition by polyamines of the growth of S. cerevisiae in a low Mg^{2+} medium

In *S. cerevisiae*, polyamines (spermine, spermidine and putrescine) are especially important for aerobic growth [37,38]; mutants which cannot synthesize these polyamines, absolutely require external spermidine or spermine for growth [39]. Fig. 1 shows the effect of spermine on the growth of *S. cerevisiae* in synthetic media containing different concentrations of $MgSO_4$. As X2180 is the wild type strain, external spermine is not required for cell growth; the growth in a medium containing 2 mM $MgSO_4$ (high Mg^{2+} medium) was hardly affected by the addition of 0.5 mM spermine (Fig. 1A). In medium containing 50 μM $MgSO_4$ (low Mg^{2+} medium), however, 0.5 mM spermine did inhibit cell growth, which ceased completely 6 h after the addition (Fig. 1B). Inhibition of cell growth by spermine depended on the Mg^{2+} concentration in the medium (Fig. 1C). In the presence of 2 mM $MgSO_4$, 10 mM spermine was required for growth inhibition (data not shown). Thus, the growth of *S. cerevisiae* is critically influenced by the concentration ratio of spermine and Mg^{2+} in the medium. Spermidine and putrescine also inhibited cell growth in a low-Mg^{2+} medium (Fig. 2). However, among these polyamines, spermine was the most toxic for this organism. In 50 μM Mg^{2+} medium, cell growth was not inhibited by the addition of basic amino acids (L-arginine, L-lysine and L-histidine) and L-ornithine at concentrations of 5 mM, respectively, but was inhibited by reagents used as polyamine analogues such as paraquat, benzyl viologen and methylglyoxal bis(guanylhydrazone) [40] (data not shown). These results suggest that the growth inhibition observed in low-Mg^{2+} medium is specific for polyamines.

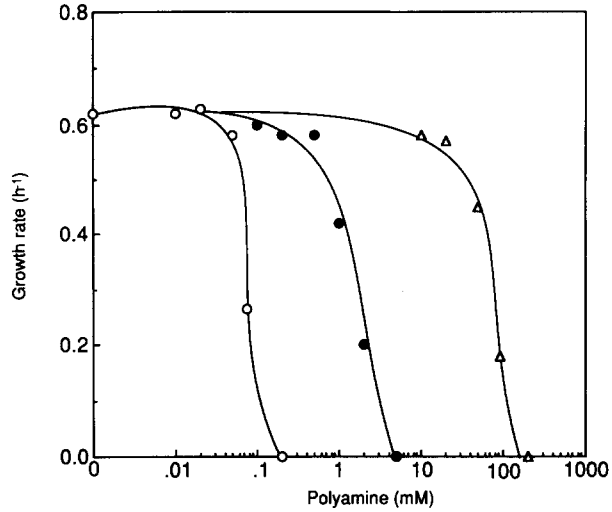

Fig. 2. Effect of various polyamines on growth rates of *S. cerevisiae*. Cell culture was started in a low-Mg^{2+} medium. Various concentrations of polyamines were added at the point as shown in Fig. 1, and growth rates were calculated. Symbols: ○, spermine; ●, spermidine; △, putrescine.

3.2. Apparent exchange of internal Mg^{2+} for external spermine

As both Mg^{2+} and polyamines are important cationic constituents of the cell, it is most conceivable that the toxicity of polyamines in a low-Mg^{2+} medium is related to changes in the cellular amounts of these constituents. The internal contents of Mg^{2+} and polyamines were followed after the addition of spermine (Fig. 3). Without its addition, the Mg^{2+} content (120–130 nmol/mg dry weight) of cells growing in both low- and high-Mg^{2+} media remained relatively constant from the early to the late log phase (Fig. 3). The cellular polyamine content, largely represented by spermidine, was also scarcely influenced by the Mg^{2+} concentration in the medium during the log phase of growth (Fig. 3 and Table 1).

In a low-Mg^{2+} medium, the Mg^{2+} and spermine contents were significantly changed by the addition of 0.5 mM spermine (Fig. 3A). As cell growth ceased, the Mg^{2+} content decreased to about 40% of that before the addition of spermine. Concomitantly, the intracellular spermine level increased about 30-fold, from 1 to 30 nmol/mg dry weight although the intracellular spermidine content showed little change (Table 1). The decreased amount of Mg^{2+} was almost twice that of

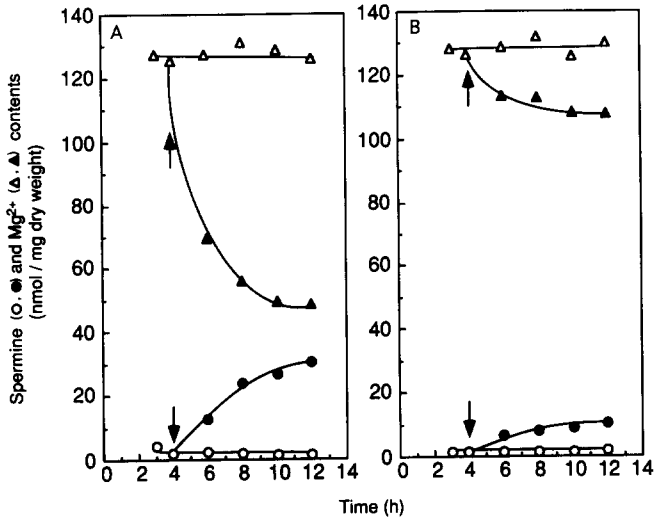

Fig. 3. Effect of external spermine on internal Mg^{2+} and spermine content. Cells were cultured in a low-Mg^{2+} medium (A) or a high-Mg^{2+} medium (B) as shown in Fig. 1., and 0.5 mM spermine was added at the point indicated by arrows. At various intervals the cells were harvested and the internal contents of Mg^{2+} (triangles) and spermine (circles) were measured as described in Materials and methods. Symbols: open, control; closed, plus spermine.

Table 1
Effect of external spermine on internal polyamine contents

$MgSO_4$ in medium (mM)	Addition	Sampling time after addition	PUT (nmol/mg dry wt.)	SPD (nmol/mg dry wt.)	SPM (nmol/mg dry wt.)
0.05	None	0	1.8	9.2	4.0
		2	2.6	10.8	2.5
		4	2.4	10.0	1.7
		8	2.4	10.3	1.4
	SPM	0	1.9	10.8	2.8
		2	1.0	12.4	12.7
		4	n.d.	12.8	23.7
		8	n.d.	10.6	30.1
2	None	0	1.9	7.5	1.6
		2	1.9	7.1	1.5
		4	2.2	7.5	1.5
		8	1.2	7.0	1.4
	SPM	0	3.5	8.1	2.5
		2	1.1	8.4	6.7
		4	0.1	5.8	8.1
		8	0.2	2.1	10.3

Cells were cultured in a synthetic medium containing 0.05 mM or 2 mM $MgSO_4$. At $A_{540} = 0.08$, 0.5 mM spermine was added, and the cells were collected at various intervals. Internal polyamines were measured as described in Materials and methods. PUT, putrescine; SPD, spermidine; SPM, spermine. Each point represents the mean of duplicate experiments. n.d., not detected.

the increase of spermine. As $spermine^{4+}$ is exclusively present at usual pH values, the charge balance between the spermine taken up and the Mg^{2+} released was nearly constant ($spermine^{4+}/Mg^{2+} = 1:2$). When spermidine was added, the ratio of the amount of decreased Mg^{2+} to that of increased spermidine was about 1.4; the charge balance ($spermidine^{3+}/ Mg^{2+} = 2:3$) was also maintained. Thus, the growth inhibition by polyamines in a low-Mg^{2+} medium probably resulted from the exchange of internal Mg^{2+} for external polyamine. However, an exchange of Mg^{2+} for spermine is not likely to be a direct transport reaction such as a $spermine^{4+}/Mg^{2+}$ antiport. The time course of the apparent exchange of Mg^{2+} for spermine paralleled that of the decrease in growth rate. After the addition of spermine, the total amount of Mg^{2+} retained in the cells did not change, suggesting that

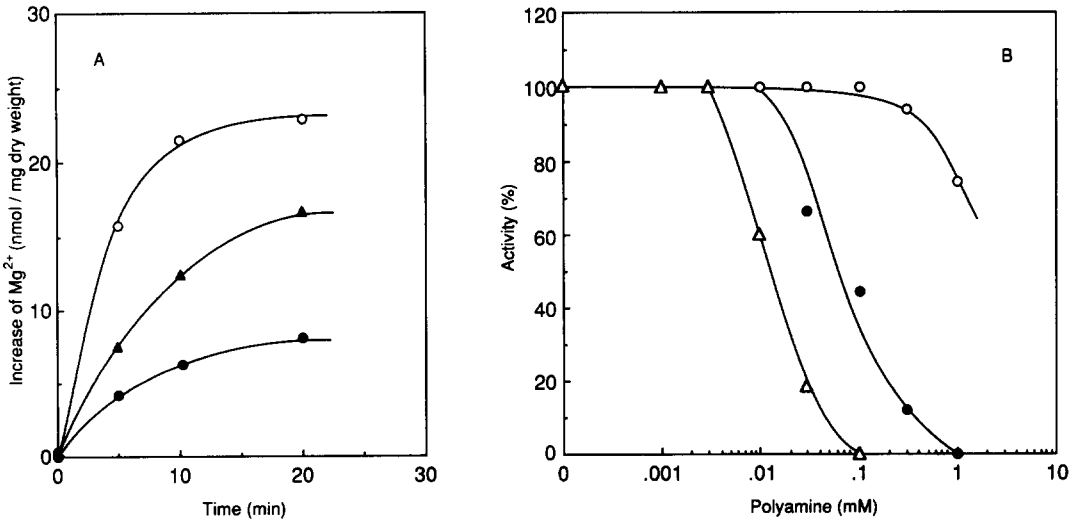

Fig. 4. Inhibition of net Mg^{2+} uptake by polyamines. (A) Time course of Mg^{2+} uptake. Net Mg^{2+} uptake was measured using the cells from which internal Mg^{2+} was partially depleted as described in Materials and methods. Spermine (0.05 mM) or $NiCl_2$ (0.5 mM) was added 3 min before initiating the reaction. Symbols: ○, control; ●, plus spermine; ▲, plus $NiCl_2$. (B) Effect of various polyamines on Mg^{2+} uptake. Various concentrations of polyamines were added 3 min before starting the reaction. The initial rate of Mg^{2+} uptake was measured as the Mg^{2+} accumulation after 5 min. Symbols: △, spermine; ●, spermidine; ○, putrescine.

internal Mg^{2+} is not released but that Mg^{2+} accumulation may be blocked by spermine. Thus, the internal Mg^{2+} content per cell decreased as cell growth proceeded.

In a high-Mg^{2+} medium, where spermine did not inhibit cell growth (Fig. 1A), cellular Mg^{2+} and polyamine contents were slightly changed; Mg^{2+} decreased by about 10% (Fig. 3B). Although the internal spermine content increased from 1.5 to 10 nmol/mg dry weight, the spermidine content decreased from 8 to 2 nmol/mg dry weight by the addition of spermine (Table 1). Ornithine decarboxylase may be inhibited by this spermine level in cells cultured in a high-Mg^{2+} medium.

3.3. Polyamine-sensitive Mg^{2+} transport by S. cerevisiae

Fig. 4A shows the time course of magnesium accumulation by *S. cerevisiae*. In this experiment the net Mg^{2+} influx was examined as described in Materials and Methods; preincubation with 1 mM sodium cyanide or 1 mM sodium azide inhibited the initial uptake of Mg^{2+} by 80% and 70%, respectively, suggesting that it is energy-dependent. All three polyamines inhibited the Mg^{2+} uptake (Figs. 4A and 4B). Their inhibitory effect (spermine > spermidine > putrescine in this order) showed a strong correlation with their effects on cell growth (Fig. 2). These results suggest that the polyamine-sensitive Mg^{2+} accumulating activity is important for the growth of *S. cerevisiae* in a low-Mg^{2+} medium. Mg^{2+} uptake was inhibited by various divalent cations such as Mn^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+}, but there was only a small inhibition by Ca^{2+}, Na^{+} and K^{+} (data not shown). It is noteworthy that inhibition by spermine was more severe than that by Ni^{2+} (Fig. 4A).

Table 2
Inhibition of uptake of Ni^{2+} and spermine by Mg^{2+}

Substrate	K_m (μM)	K_i (μM)	Inhibition type
Ni^{2+}	620 ± 50	28 ± 15 (Mg^{2+})	Competitive
		115 ± 20 (spermine)	Competitive
Spermine	6 ± 4	640 ± 95 (Mg^{2+})	Non competitive
	65 ± 18	85 ± 30 (Mg^{2+})	Competitive

Uptake of $^{63}Ni^{2+}$ and [^{14}C]spermine were assayed as described in Materials and methods. Kinetic constants were estimated from the initial velocities of uptake. The K_i values were determined by varying the sustrate concentration in the presence of a fixed inhibitor concentration. Standard deviations were from triplicate experiments.

In order to determine more precisely the properties of the net Mg^{2+} uptake by this organism during the log phase of growth, we looked for other metal ions which could be used as substrates of the transport, since radioactive Mg is prohibitively expensive and has an extremely short half-life. It has been reported that Ni^{2+} is a substrate for all three Mg^{2+} transport systems in *S. typhimurium* [21]. It has also been pointed out that Ni^{2+} competes with the Mg^{2+} movement in *S. cerevisiae* [31]. In the present study we examined the properties of $^{63}Ni^{2+}$ uptake. Accumulation of $^{63}Ni^{2+}$ was suppressed by energy inhibitors such as cyanide and azide (data not shown), and it was competitively inhibited by Mg^{2+} (Table 2). The maximal velocity of $^{63}Ni^{2+}$ uptake (4.6 ± 0.3 nmol/min per mg dry weight) was almost the same as that of Mg^{2+} uptake as measured by flame photometry, indicating that Ni^{2+} can be used as a substrate for this Mg^{2+} transport system.

Fig. 5 shows the effect of polyamines on $^{63}Ni^{2+}$ uptake. As expected, Ni^{2+} uptake was inhibited by spermine, spermidine and putrescine in this order (Fig.

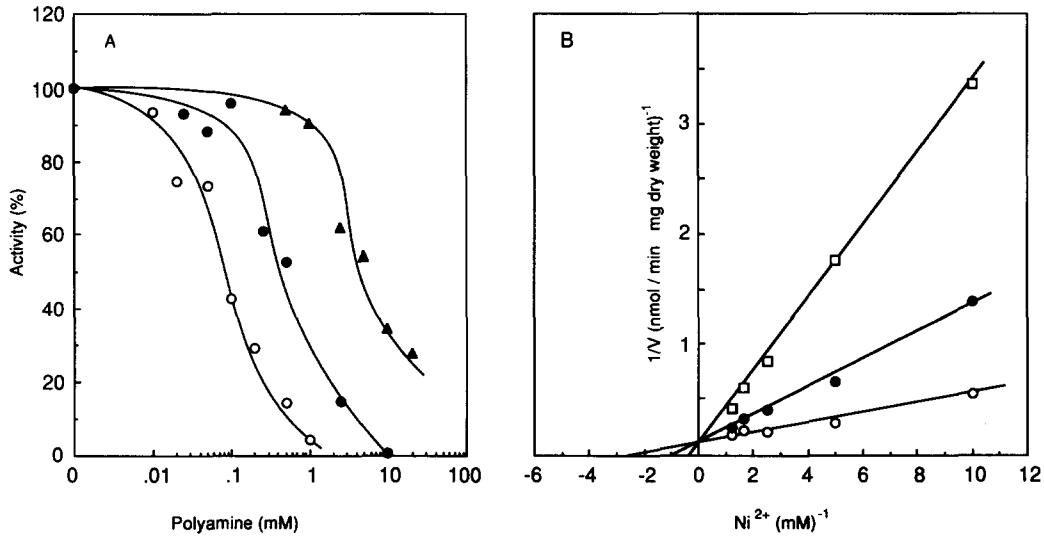

Fig. 5. Inhibition of $^{63}Ni^{2+}$ uptake by polyamines. *A*. Effect of various polyamines on $^{63}Ni^{2+}$ uptake. The $^{63}Ni^{2+}$ uptake was assayed as described in Materials and methods. Various concentrations of polyamines were added 3 min before starting the reaction, and the initial rates were calculated. Symbols: ○, spermine; ●, spermidine; ▲, putrescine. *B*. Effect of spermine on the Lineweaver-Burk plot of $^{63}Ni^{2+}$ uptake. The effect of spermine on the initial rates of $^{63}Ni^{2+}$ uptake at various Ni^{2+} concentrations was examined by its addition 3 min before starting the reaction. Symbols: ○, control; ●, 0.1 mM spermine; □, 0.2 mM spermine.

5A); it was not inhibited by the basic amino acids arginine, lysine and histidine (data not shown). The inhibition of Ni^{2+} uptake by these polyamines showed patterns similar to those of Mg^{2+} uptake (Fig. 4B) and of the cell growth in low-Mg^{2+} medium (Fig. 2). The Ni^{2+} uptake was competitively inhibited by spermine (Fig. 5B and Table 2).

3.4. Properties of spermine uptake

Even when spermine was added simultaneously with Mg^{2+} or $^{63}Ni^{2+}$, the uptake of these metal ions was inhibited (data not shown). In addition, when cells preincubated with spermine for 10 min were washed with spermine-free buffer, Mg^{2+} uptake rate fully recovered, suggesting that spermine acts externally on the Mg^{2+} transport system. Hence, spermine may compete with Mg^{2+} for transport, as indicated by its apparent exchange with Mg^{2+} and its competitive inhibition of $^{63}Ni^{2+}$ uptake.

The effect of Mg^{2+} on [^{14}C]spermine uptake by *S. cerevisiae* was examined. Spermine uptake was not observed at 0°C, and it was inhibited by energy inhibitors such as cyanide and azide, suggesting that it is energy-dependent. The spermine taken up was not in a free form because accumulated [^{14}C]spermine was not exchanged with external spermine (data not shown). A Lineweaver-Burk plot of the initial spermine uptake showed a biphasic pattern (Fig. 6), suggesting the presence of two uptake systems with different K_m values (6 ± 4 μM and 65 ± 18 μM) for spermine (Table 2). Conversely, magnesium inhibited spermine uptake, the Lineweaver-Burk plot of spermine uptake in the presence of 0.5 mM $MgSO_4$ being also biphasic. Inhibition

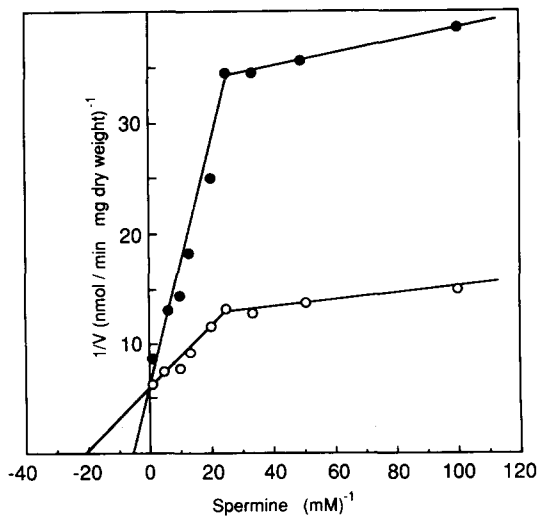

Fig. 6. Lineweaver-Burk plot of the effect of Mg^{2+} on spermine uptake. The initial rates of [^{14}C]spermine uptake at various concentrations of spermine were assayed as described in Materials and methods. $MgSO_4$ (0.5 mM) was added 3 min before starting the reaction. Symbols: ○, control; ●, plus 0.5 mM $MgSO_4$.

of the high-affinity spermine uptake system by Mg^{2+} was not competitive, while that of the low-affinity spermine transporter was competitive. Thus, it is clear that spermine and Mg^{2+} compete for their accumulations.

4. Discussion

At the outset of this work, our interest was directed toward the polyamine transport of *S. cerevisiae*. In order to understand the physiological role of polyamine transport, it is important to establish the culture conditions under which the system will function. As polyamine uptake was inhibited by Mg^{2+} (Fig. 6), we attempted to limit the concentration of magnesium ions in the medium as much as possible. Then, to our surprise, in a low-Mg^{2+} medium where the polyamine uptake systems work, we found that polyamines inhibited the growth of this organism (Fig. 1B). Polyamine toxicity in Mg^{2+}-depleted cultures was also recently observed in mouse FM3A cells [41].

Growth inhibition by spermine was accompanied by a decrease in internal Mg^{2+} and an increase in internal spermine (Fig. 3). As the growth inhibition by polyamines paralleled that of Mg^{2+} uptake (Figs. 2 and 4B), the toxicity of polyamines may be a direct result of the decrease in the internal Mg^{2+} content. Although depletion of the internal Mg^{2+} was partial, polyamines may selectively release the Mg^{2+} bound to key sites for cell proliferation or decrease the internal free Mg^{2+} concentration. On the other hand, overaccumulation of spermine may be critical for cell growth, although excess amounts of polyamines are thought to be stored in vacuoles [42–44]. In mouse FM3A cells cultured in a low-Mg^{2+} medium, mitochondria were damaged by the addition of spermine [41].

The growth of *S. cerevisiae* was influenced by the concentration ratio of Mg^{2+} and polyamines in the medium (Fig. 1). In addition, inhibition of cell growth in a low-Mg^{2+} medium containing 0.5 mM spermine was completely recovered by the addition of 2 mM $MgSO_4$ (Maruyama, T. Kakinuma, Y. and Igarashi, K., unpublished results); internal Mg^{2+} increased to the normal level and the overaccumulated spermine decreased in concert with the recovery of cell growth, supporting our notion that spermine and Mg^{2+} compete for their accumulations in cells. It has been demonstrated that several divalent cations are accumulated in *S. cerevisiae* by an energy-dependent, low-specificity uptake system with an affinity series of Mg^{2+}, Co^{2+}, $Zn^{2+} > Mn^{2+} > Ni^{2+} > Ca^{2+}$ and Sr^{2+} [30,31]. Our examination of the cation selectivity of net Mg^{2+} uptake and $^{63}Ni^{2+}$ uptake was mostly consistent with the previous report [31]; the Mg^{2+} uptake system recognizes Ni^{2+} as substrate. It is of particular interest

that $^{63}Ni^{2+}$ was competitively inhibited by spermine (Fig. 5B) and that Mg^{2+} competitively inhibited spermine uptake with a low affinity (Fig. 6). Although to draw conclusions from these kinetics data would be premature, the results are most simply explained by the assumption that Mg^{2+} and polyamines are transported by a common system in *S. cerevisiae*.

It has been reported that spermine interacts with copper ions [45]. It may be supposed that the inhibition of spermine uptake by magnesium or the Mg^{2+} uptake by spermine is caused by a loss of free spermine and Mg^{2+} with the formation of a Mg^{2+}-spermine complex. However, NMR analysis of the interaction between spermine and Mg^{2+} under the same conditions as the transport assay indicated that this possibility can probably be ruled out. Spermine uptake activity by mouse FM3A cells was not inhibited by less than 5 mM Mg^{2+} (data not shown). It has also been reported that the uptake of spermidine and putrescine by *E. coli* cells is not inhibited by magnesium ions [46]. Although the sensitivity of Mg^{2+} transport to polyamines has not been investigated in other organisms, the present results suggest that the interaction between polyamines and Mg^{2+} for transport may be unique in yeast. This Mg^{2+} transport system is physiologically important for this organism although the benefit of the sensitivity of this system to polyamines is still unclear.

In any case, further studies will be required to attain an understanding of the mechanism(s) and functions of this polyamine-sensitive Mg^{2+} transport system. Isolation of mutants resistant to spermine in a low-Mg^{2+} medium is now in progress.

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