CHAPTER 6
ROLE OF Cs⁺/Rb⁺ AS AN OBLIGATE NUTRITIONAL
REQUIREMENT IN A Cs⁺-R MUTANT OF THE
CYANOBACTERIUM NOSTOC MUSCORUM

Introduction

Growing pollution and consequent toxicity of natural environments are posing a serious threat to the existing ecosystems. There is a serious concern about the biological implications of pollution caused by ¹³⁷Cs⁺, arising from its continual discharge from nuclear industries into aquatic habitats in view of its high bioavailability and long half-life. Cyanobacteria and algae are the main primary producers of the aquatic ecosystems and the biological consequences of Cs⁺ pollution on these primary producers needs to be investigated and understood at ecological, physiological and molecular level. Previous studies have demonstrated adverse effects of Cs⁺ on the physiology and growth of cyanobacteria and microalgae that function as primary producers in the aquatic food chain (Williams and Swanson, 1958). Recent studies on the physiological reasons of Cs⁺ toxicity or lack of it to cyanobacteria and algae have shown that it results from cellular replacement of K⁺ by Cs⁺ associated with the inability of Cs⁺ to substitute functionally for K⁺ (Avery et al. 1991), that K⁺, Na⁺ or NH₄⁺ mitigates/eliminates it by preventing the entry and accumulation of Cs⁺ (Avery et al. 1992 a & b; Avery et al. 1993; Singh et al. 1994) and that it arises specifically from Cs⁺ inhibition of N₂-fixation in the cyanobacterium Nostoc muscorum (Singh et al. 1994).

Earlier studies have shown that Cs⁺ toxicity in Nostoc muscorum is diazotrophic specific and NH₄⁺-repressible and that one class of caesium resistant (Cs⁺-R) mutants of the cyanobacterium arise with about 50% impairment in their nitrogenase activity and diazotrophic growth (Singh et al. 1994).

In the present study, experiments have been carried out to analyse the nature and consequences of caesium resistance (Cs⁺-R) associated loss in cyanobacterial diazotrophy in the presence and absence of alkali cations such as Na⁺, K⁺, Cs⁺ and Rb⁺,
specifically the latter two cations. An evidence is presented here to show that Cs\(^+\)-R mutant has suffered genetic damage of pleiotropic nature adversely influencing its growth, oxygenic photosynthesis, chlorophyll \(a\) content, nitrogenase activity and osmotolerance specific to diazotrophic mode of growth and that Cs\(^+\) or Rb\(^+\) alone is nutritionally capable of repairing fully such cyanobacterial mutational pleiotropy.

Materials and Methods

Axenic clonal cultures of the parent *Nostoc muscorum* used in the present study were routinely grown and maintained in combined nitrogen free medium of Gerloff et al. (1950), as described in Chapter-2.

Composition of the nutrient medium

The original growth medium of Gerloff et al. (1950) was modified to make it free from Na\(^+\) and K\(^+\) for the experiments examining the role of Na\(^+\), K\(^+\) along with Rb\(^+\) or Cs\(^+\) in the cyanobacterial diazotrophic nutrition. Accordingly, Na\(_2\)SiO\(_3\), Na\(_2\)CO\(_3\) and K\(_2\)HPO\(_4\) were omitted from the original growth medium thus rendering the medium \(-Na^+\) (Sodium free) and \(-K^+\) (Potassium free). CaHPO\(_4\) and CaCO\(_3\) were added in place of CaCl\(_2\) and Na\(_2\)CO\(_3\) at equimolar concentration. Further, except for the experiments involving the study on the effect of individual cations on the diazotrophic growth medium of the parental strain, all other experiments have been conducted with the cultures of parent or mutant strains grown in modified Chu No.-10 medium containing 3mM NaCl, 5mM KCl, 0.0574mM CaHPO\(_4\) and 0.19mM CaCO\(_3\). Addition of 1mM NH\(_4\)Cl to the growth medium has been termed as NH\(_4^+\)-medium. The pH of all the media were adjusted to 7.5 using 1mM HEPES buffer.

Osmotic Survival studies

Osmotic survival studies were made on N\(_2\) or NH\(_4^+\)-nutrient plates containing increasing concentrations of sucrose as described in Chapter-2. The inoculum size per nutrient plate was 500 CFUs. Role of Cs\(^+\)/Rb\(^+\) in regulation of cyanobacterial osmotolerance was examined by scoring the osmotic survival characteristics in the presence or absence of either alkali cations.
Determination of Intracellular $^{137}\text{Cs}^+$

Effects of Rb$^+$ on the uptake and accumulation of Cs$^+$ in the cyanobacterial strains was examined as follows. The diazotrophically grown cultures were harvested and sampled in 10 mM HEPES buffer of pH 7.5. The various samples contained a fixed amount of radioactive Cs$^+$ (0.2 mM $^{137}\text{CsCl}$; specific activity 38.85 MBq mol$^{-1}$) against increasing concentration of cold RbCl. The samples thus prepared were incubated for 10 minutes, then harvested and examined for intracellular radiolable as described in Chapter-4. Similar method was employed to evaluate the influence of Na$^+$/K$^+$ on the uptake and accumulation of $^{137}\text{Cs}^+$.

Determination of intracellular $^{86}\text{Rb}^+$

Effect of Cs$^+$ on the uptake and accumulation of Rb$^+$ in the cyanobacterial strains was examined exactly as per the protocol given above for $^{137}\text{Cs}^+$ except that a fixed amount of radioactive Rb$^+$ (0.4 mM $^{86}\text{RbCl}$; specific activity 55.5 MBq mol$^{-1}$) was used against increasing concentrations of cold CsCl.

Growth, Chlorophyll-$\alpha$, Protein, Nitrogenase activity, GS activity, Oxygenic photosynthesis were estimated as described in Chapter-2.

Chemicals used

$^{137}\text{CsCl}$ and $^{86}\text{RbCl}$ were obtained from Board of Radiation and Isotope Technology (BRIT), India. All other chemicals used in the present study were purchased either from M/s. Sigma Chemical Co., USA or BDH Chemicals Co., India and were of analytical grade.

Results

The spontaneously occurring cyanobacterial Cs$^+$ resistant (Cs$^+$-R) mutant under diazotrophic growth condition arose with a frequency of 0.3-0.7×10$^{-7}$. The diazotrophic growth medium devoid of both Na$^+$ and K$^+$ was used as basal growth medium to analyse the nutritive role of various alkali cations on growth of parent Nostoc muscorum (Fig. 6.1). The optimal growth with individual cations occurred at 3mM NaCl, 5mM KCl or
Fig. 6.1
Effect of Na⁺ (3 mol m⁻³ NaCl), K⁺ (5 mol m⁻³ KCl), Rb⁺ (3 mol m⁻³ RbCl), Cs⁺ (1.5 mol m⁻³ CsCl), Na⁺ + K⁺ (3 mol m⁻³ NaCl + 5 mol m⁻³ KCl) and Na⁺ + Rb⁺ (3 mol m⁻³ NaCl + 3 mol m⁻³ RbCl) on the growth of parent *Nostoc muscorum* in diazotrophic medium.

(O), Control (no addition of alkali cations)

(•), Na⁺

(□), K⁺

(■), Rb⁺

(▲), Cs⁺

(▲), Na⁺ + K⁺

(×), Na⁺ + Rb⁺

Alkali cations were added the point marked by an arrow. Mean values from three independent experimental determinations are shown ± SEM, where these exceed the dimensions of the symbols.
Fig. 6.1
3mM RbCl. However, growth was always significantly better with Na⁺ and K⁺ or Na⁺ and Rb⁺ together, than with Na⁺, K⁺ or Rb⁺ alone. Cs⁺ (used as CsCl) on the other hand was extremely growth inhibitory and lethal at 1.5 mM. Neither Na⁺ (3mM NaCl) nor K⁺ (5mM KCl) was found to mitigate the Cs⁺ toxicity to the cyanobacterium. These findings demonstrate clearly the nutritive role of Rb⁺ like that of Na⁺ or K⁺ and inhibitory role of Cs⁺ in the cyanobacterial diazotrophic growth.

The Cs⁺⁻R mutant capable of growth in the presence or absence of 2mM CsCl in the diazotrophic growth medium was examined along with its parent for diazotrophic growth characteristics (Fig. 6.2). The mutant grew very slowly in Cs⁺ or Rb⁺ free diazotrophic growth medium and in quantitative terms, its estimated diazotrophic growth at the end of 12 days period was about 50% lower to that shown by the parental strain. Clearly, mutation to Cs⁺⁻R phenotype has resulted in about 50% impairment of the cyanobacterial diazotrophic growth. Interestingly, addition of 2mM, CsCl or 2mM RbCl to the diazotrophic growth medium caused almost complete absence of mutationally impaired diazotrophy. In other words, Cs⁺, the inhibitor of diazotrophic growth in the parent became a nutritional requirement for normal diazotrophy in the mutant strain. In addition, Rb⁺ was found effectively substituting for such Cs⁺ nutritional requirement. In comparison, Na⁺ (3mM NaCl) or K⁺ (5mM KCl) in the growth medium did not repair the mutational damage. Thus, it can be concluded that Cs⁺ or Rb⁺ is a specific nutritional requirement for restoration of normal diazotrophy in the Cs⁺⁻R mutant strain. It must be mentioned here that the parent and the mutant strains, both grew equally well in 1 mM NH₄Cl medium with or without Cs⁺ or Rb⁺. Evidently, cyanobacterial mutational damage is apparently diazotrophy specific and NH₄⁺ repressible.

Under diazotrophic growth condition without Cs⁺/Rb⁺ supplement, the mutant cyanobacterial samples looked greenish yellow. The question whether such Cs⁺ or Rb⁺ nutritional requirement is specific for chlorophyll-α content (Fig. 6.3) and nitrogenase activity (Fig.6.4) or for both under diazotrophic growth condition was further investigated. In these experiments the source of inocula for the two strains was NH₄⁺ grown cultures. As expected, nitrogenase activity of the two strains developed after a lag period of about 2 days, thereafter it increased differentially with a rate nearly 2 fold
Fig. 6.2
Growth of parent *Nostoc muscorum* in diazotrophic medium (0—0) and in diazotrophic medium containing 2 mol m\(^{-3}\) CsCl (Cs\(^+\), •——•) as well as of its Cs\(^+\)-R mutant strain in diazotrophic medium (□——□) and in diazotrophic medium containing 2 mol m\(^{-3}\) CsCl (Cs\(^+\), ■——■) or 3 mol m\(^{-3}\) RbCl (Rb\(^+\), ■——■). Cs\(^+\)/Rb\(^+\) were added at the point marked by an arrow. Mean values from three independent experimental determinations are shown ± SEM, where these exceed the dimensions of the symbols.
Fig. 6.2

Growth (O.D at 663 nm)

Time (days)

Cs^+

Cs^+/Rb^+
Chlorophyll \( a \) content in diazotrophic medium of the parent \textit{Nostoc muscorum} lacking \( \text{Cs}^+/\text{Rb}^+ \) (O—O) and of its \( \text{Cs}^+/\text{R} \) mutant strain lacking \( \text{Cs}^+/\text{Rb}^+ \) (□—□) or containing \( \text{Cs}^+/\text{Rb}^+ \) (■—■). The inocula for the experiments was 1 mol m \(^{-3}\) \text{NH}_4\text{Cl} grown cultures of either strain transferred to diazotrophic medium. \( \text{Cs}^+/\text{Rb}^+ \) were added at the point marked by an arrow. Mean values from three independent experimental determinations are shown \( \pm \) \text{SEM}, where these exceed the dimensions of the symbols.
Chlorophyll a content (mg L\(^{-1}\))

Time (days)

Fig. 6.3
Nitrogenase activity in diazotrophic medium of the parent *Nostoc muscorum* lacking Cs⁺/Rb⁺ (O----O) and of its Cs⁺-R mutant strain lacking Cs⁺/Rb⁺ (□-----□) or containing Cs⁺/Rb⁺ (■——■). The inocula for the experiments was 1 mol m⁻³ NH₄Cl grown cultures of either strain transferred to diazotrophic medium. Cs⁺/Rb⁺ was added at the point marked by an arrow. Mean values from three independent experimental determinations are shown ± SEM, where these exceed the dimensions of the symbols.
Fig. 6.4
higher in the parent than in the mutant strain. Addition of Cs\(^+\) or Rb\(^+\) restored nitrogenase activity of the mutant to almost parental level. Chlorophyll-\(a\) content like nitrogenase activity, also required Cs\(^+\) or Rb\(^+\) to maintain its normal level. Thus mutation to Cs\(^+\)\,-\(R\) phenotype appears to have adversely influenced the common cellular target essential for maintenance of normal nitrogenase activity and chlorophyll-\(a\) content in the cyanobacterium.

The cyanobacterial strains grew diazotrophically at the expense of oxygenic photosynthesis and ammonium assimilatory activity of GS. It was therefore natural to investigate the effect of Cs\(^+\)\,-\(R\) mutation on these two aspects and the role of Cs\(^+\) or Rb\(^+\) in such mutational damage. The results show that photosynthetic \(O_2\)-evolution in the parent decreased with increasing concentration of Cs\(^+\) and was zero at 2mM CsCl (Table 6.1). In comparison, none of the Rb\(^+\) concentrations influenced this process significantly. This does suggest that in parent while Cs\(^+\) is inhibitory to oxygenic photosynthesis, Rb\(^+\) is not. In comparison, oxygenic photosynthesis of the Cs\(^+\)\,-\(R\) strain was nearly 50% of the parent in the absence of Cs\(^+\) or Rb\(^+\). Gradual increase in concentration of Cs\(^+\) or Rb\(^+\) was found increasing the oxygenic photosynthetic activity of the mutant proportionately. In contrast, GS (biosynthetic) activity was not much adversely affected with increasing concentrations of Cs\(^+\) \(\text{or}\) Rb\(^+\) in the parent or mutant strain. The mutation to Cs\(^+\)\,-\(R\) phenotype thus seems to have adversely affected mainly oxygenic photosynthesis of the cyanobacterium.

The next series of experiments were conducted to examine the role of nitrogen source or Cs\(^+\)/Rb\(^+\) in the regulation of osmotolerance in the parent and Cs\(^+\)\,-\(R\) strains. As shown in Table 6.2, the osmotic survival rate of the parent in diazotrophic medium or NH\(_4\)\(^+\)-medium remained almost similar with increasing doses of sucrose and reached zero value with 250mM sucrose. Clearly, the nature of the nitrogen source or nutrition does not seem to influence the osmotolerance characteristics of the parent cyanobacterium. The osmotic survival pattern of Cs\(^+\)\,-\(R\) mutant in the NH\(_4\)\(^+\)-medium was almost similar to that of the parental strain under parallel condition. However, it was not so in diazotrophic medium lacking Cs\(^+\)/Rb\(^+\) where its percent survival decreased much faster with rise in sucrose concentration and reached a zero value at 200mM sucrose. Addition
Table 6.1

Effect of graded concentrations of Cs⁺ (CsCl) and Rb⁺ (RbCl) on photosynthetic O₂-evolution (mmol O₂ evolved g⁻¹ Chl a) and on GS (biosynthetic) activity (μ mol NADH oxidized g⁻¹ protein min⁻¹) in the parent and Cs⁺-R mutant strains of Nostoc muscorum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parent Strain</th>
<th></th>
<th>Cs⁺-R strain</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O₂ evolution</td>
<td>GS activity</td>
<td>O₂ evolution</td>
<td>GS activity</td>
</tr>
<tr>
<td>Control</td>
<td>610 ± 12.5</td>
<td>86 ± 8.2</td>
<td>292 ± 15.2</td>
<td>82 ± 6.4</td>
</tr>
<tr>
<td>+Cs⁺ (mol m⁻³)</td>
<td>0.25</td>
<td>436 ± 18.4</td>
<td>82 ± 6.7</td>
<td>316 ± 12.3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>313 ± 21.5</td>
<td>76 ± 5.8</td>
<td>412 ± 8.6</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>36 ± 2.3</td>
<td>72 ± 5.5</td>
<td>562 ± 17.4</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.0</td>
<td>75 ± 5.3</td>
<td>586 ± 6.3</td>
</tr>
<tr>
<td>+Rb⁺ (mol m⁻³)</td>
<td>1.0</td>
<td>590 ± 36.0</td>
<td>82 ± 5.2</td>
<td>422 ± 14.5</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>602 ± 21.3</td>
<td>79 ± 5.7</td>
<td>518 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>586 ± 29.4</td>
<td>82 ± 5.5</td>
<td>594 ± 36.6</td>
</tr>
</tbody>
</table>

Cultures of both the strains were grown diazotrophically with or without the various treatments for 72 h before using them for the various experimental determinations. Each reading is an average (± SEM) of three independent experimental determinations.
Table 6.2

Osmotic survival characteristics of the parent and Cs⁺-R mutant strains of *Nostoc muscorum* on diazotrophic \((N_2)\) medium and on \(NH_4^+\)-medium \((1 \text{ mol m}^{-3} \text{ NH}_4\text{Cl})\) supplemented with increasing concentrations of sucrose. The \(N_2\)-medium with or without \(\text{Cs}^+ / \text{Rb}^+ \) \((2 \text{ mol m}^{-3} \text{ CsCl or } 3 \text{ mol m}^{-3} \text{ RbCl})\) was used to score the survival of \(\text{Cs}^+-\text{R}\) strain in order to examine the role of \(\text{Cs}^+/\text{Rb}^+\) in its osmoprotection.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parent strain</th>
<th>(\text{Cs}^+-\text{R}) strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N_2) Medium</td>
<td>(NH_4^+) Medium</td>
</tr>
<tr>
<td>Basal medium</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>+ Sucrose ((\text{mol m}^{-3}))</td>
<td>100</td>
<td>65 (± 4.5)</td>
</tr>
<tr>
<td>100</td>
<td>42 (± 3.6)</td>
<td>45 (± 5.1)</td>
</tr>
<tr>
<td>200</td>
<td>0.0 (± 0.03)</td>
<td>0.5 (± 0.06)</td>
</tr>
</tbody>
</table>

The inoculum size per nutrient plate was about 500 colony forming units (CFU’s). The efficiency of survival on control medium was taken was 100%. Values in parentheses are ± SEM of three independent experimental determinations.
of Cs⁺/Rb⁺ in the diazotrophic medium resulted in restoration of the osmotolerance of the mutant strain to almost parental level. Rb⁺ like Cs⁺ also caused restoration of osmotolerance in the mutant. Thus, Rb⁺ is also required for repair of the osmotolerance in the mutant strain. The pattern of Cs⁺ or Rb⁺ uptake was also studied in the two strains grown diazotrophically as well as in the NH₄⁺ medium. DCCD at a concentration 20mg/L was used to pretreat the parental diazotrophic culture for 30 minutes in order to examine the energy dependence of Cs⁺ uptake. As shown in Fig. 6.5 and 6.6, the rate of Rb⁺ uptake in the parent. The uptake pattern of both the cations was similar in the mutant but was slightly higher compared to the parent. DCCD pretreated diazotrophic cultures of the parent lacked Cs⁺ uptake and accumulation. NH₄⁺-grown cultures of the parent similarly lacked Rb⁺ uptake and accumulation or Cs⁺ uptake and accumulation. NH₄⁺ grown cultures of the mutant also lacked Rb⁺ or Cs⁺ uptake and accumulation. DCCD pretreated diazotrophic cultures of the mutant like that of its parent were similarly deficient in Cs⁺ or Rb⁺ uptake and accumulation. Evidently, both cyanobacterial strains contain NH₄⁺-repressible DCCD sensitive Cs⁺ or Rb⁺ uptake process of more or less equal magnitude. In other words, mutation to Cs⁺-R phenotype does not seem to have altered the Cs⁺ or Rb⁺ uptake process in the diazotrophic cultures of the two cyanobacterial strains.

Experiments were also conducted to examine the influence of exogenous Cs⁺ on the uptake activity of Rb⁺ transport system and of exogenous Rb⁺ on the uptake activity of Cs⁺ transport system. As shown in Fig. 6.7 and 6.8, when both the cations are present simultaneously in the growth medium, uptake of one cation got inhibited progressively at increasing concentration of the other cation and vice-versa. These results do suggest that the two cations affect the uptake and accumulation of the other.

The effect of increasing concentrations of K⁺ (KCl) and Na⁺ (NaCl) on the intracellular content of Cs⁺ was also examined. As shown in Fig. 6.9 and 6.10, neither Na⁺ upto 5mM NaCl nor K⁺ upto 10mM KCl could influence the intracellular level of Cs⁺ in the parent or in the Cs⁺-R strain. However, with further rise in Na⁺ or in K⁺ concentrations there was a corresponding decline in Cs⁺ accumulation which became almost negligible at an external concentration of 20mM NaCl or 50mM KCl.
Fig. 6.5

Uptake of $^{137}\text{Cs}^+$ in parent *Nostoc muscorum* grown diazotrophically (O---O) and in 1 mol m$^{-3}$ NH$_4$Cl medium for 96 h (•---•) and of its $Cs^-\cdot$R mutant strain grown diazotrophically (□) and in 1 mol m$^{-3}$ NH$_4$Cl medium for 96 h (■). DCCD at a strength of 20 mg L$^{-1}$ was used to pretreat the parent strain(O---O) to examine the role of energy metabolism on $^{137}\text{Cs}^+$ uptake. Mean values from three independent experimental determinations are shown ±SEM, where these exceed the dimensions of the symbols.
Fig. 6.5
Fig. 6.6

Uptake of $^{86}$Rb$^+$ in parent *Nostoc muscorum* grown diazotrophically (O—O) and in 1 mol m$^{-3}$ NH$_4$Cl medium for 96 h (•—•) and of its Cs$^+$-R mutant strain grown diazotrophically (□) and in 1 mol m$^{-3}$NH$_4$Cl medium for 96 h (■). DCCD at a strength of 20 mg L$^{-1}$ was used to pretreat the parent strain (O----O) to examine the role of energy metabolism on $^{86}$Rb$^+$ uptake. Mean values from three independent experimental determinations are shown ±SEM, where these exceed the dimensions of the symbols.
Intracellular Rb⁺ (m mol/5 x 10⁹ CFU)

Time (min)

Fig. 6.6
Fig. 6.7

Influence of increasing concentrations of RbCl on the uptake of $^{137}\text{Cs}^+$ in parent *Nostoc muscorum* (O) and its $\text{Cs}^+\cdot R$ mutant strain (□). Diazotrophic cultures of the two strains grown with Cs$^+$ for 48 h were used in the present study. Mean values from three independent experimental determinations are shown ±SEM, where these exceed the dimensions of the symbols.
Fig. 6.7
Fig. 6.8

Influence of increasing concentrations of CsCl on the uptake of $^{86}$Rb$^+$ in parent *Nostoc muscorum* (O) and its Cs$^+$-R mutant strain (□). Diazotrophic cultures of the two strains grown with Cs$^+$ for 48 h were used in the present study. Mean values from three independent experimental determinations are shown ±SEM, where these exceed the dimensions of the symbols.
Fig. 6.8
Discussion

Diazotrophic growth results in the parent strain suggest that Rb\(^+\) can replace for K\(^+\) functionally. Parallel studies with the mutant suggest K\(^+\) cannot substitute functionally for Rb\(^+\) while Cs\(^+\) can do so very effectively. These findings imply a role of the cyanobacterial genetic determinant in controlling the specificity of nutritional interchangeability of alkali cations from K\(^+\)/Rb\(^+\) to Rb\(^+\)/Cs\(^+\) in the cyanobacterium. K\(^+\) is required by microbes as an enzyme activator, as an osmotic regulator and as regulator of internal pH (Booth 1985; Walderhaug et al. 1987). It is therefore essential to examine specifically the role of Rb\(^+\) in various known cellular functions of K\(^+\) by modern techniques of molecular biology before accepting or rejecting its biological functional equivalence to K\(^+\).

Cs\(^+\) toxicity to cyanobacterial diazotrophic cultures is because such cultures contain both active NH\(_4^+\)-repressible Cs\(^+\) transport system and Cs\(^+\) sensitive intracellular target(s). In cyanobacteria, mutational alteration of the NH\(_4^+\)-repressible Cs\(^+\) transport system has been shown to be one genetic mechanism of Cs\(^+\)-R phenotype (Avery et al. 1992). The other genetic mechanism of Cs\(^+\)-R phenotype has been the mutational acquisition of resistance by the Cs\(^+\) sensitive intracellular target against Cs\(^+\) (Singh et al. 1994). The present finding that Cs\(^+\)/Rb\(^+\) uptake and accumulation is NH\(_4^+\)-repressible in the parent as well as in the mutant strain while confirming earlier conclusion of Cs\(^+\) transport being NH\(_4^+\)-repressible, further shows that Rb\(^+\) transport like Cs\(^+\) transport is also NH\(_4^+\)-repressible in the cyanobacterium. Since the observed mutational frequency of the Cs\(^+\)-R phenotype falls within the range characteristic of a single mutational event in chromosomal genes, it is concluded that the present cyanobacterial Cs\(^+\)-R mutant is also a product of a single mutational event. Since the Cs\(^+\)-R mutant showed impaired diazotrophy, oxygenic photosynthesis, chlorophyll-\(a\) content and osmotolerance which are fully repairable by exogenous Cs\(^+\), it is concluded that the Cs\(^+\)-R mutant is a pleiotropic mutant and that its apparent resistance to Cs\(^+\) is because of the requirement of this cation for its normal diazotrophy. The mutational pleitropy is expressed only under diazotrophic growth condition and never under nitrate or ammonium nutrition growth condition thus suggesting the mutant phenotype to be the result of mutation in a genetic
Fig. 6.9

Influence of external Na\(^+\) (as NaCl) on the cellular level of \(^{137}\)Cs\(^+\) in parent *Nostoc muscorum* (O) and its Cs\(^+\)-R mutant strain (□). Mean values from three independent experimental determinations are shown ±SEM, where these exceed the dimensions of the symbols.
Fig. 6.9
determinant co-ordinating cyanobacterial oxygenic photosynthesis, N\textsubscript{2}-fixation and osmotolerance. Rb\textsuperscript{+} like Cs\textsuperscript{+} is found equally effective in physiological restoration of the cyanobacterial mutational pleiotropy. Since neither Na\textsuperscript{+}/K\textsuperscript{+} is found capable of repairing the Cs\textsuperscript{+}/Rb\textsuperscript{+} repairable mutational phenotype, the obvious inference is that Cs\textsuperscript{+}/Rb\textsuperscript{+} requirement cannot be substituted by Na\textsuperscript{+}/K\textsuperscript{+} in the cyanobacterium. The question is why chlorophyll-\textit{a} content declines in the absence of provision for Cs\textsuperscript{+}/Rb\textsuperscript{+} under diazotrophic growth condition can simply be explained on the basis of the dependence of diazotrophic growth of the mutant on exogenous Cs\textsuperscript{+}/Rb\textsuperscript{+}. Since the cyanobacterial Cs\textsuperscript{-}\textit{R} mutant seems to be a product of single mutational event in a genetic determinant of regulatory nature that controls oxygenic photosynthesis, N\textsubscript{2}-fixation and osmotolerance, it would be interesting to find out how such a regulatory genetic determinant co-ordinates the three major cyanobacterial processes. In recent years, PII protein has been shown to modulate photosynthetic carbon and nitrogen metabolism in unicellular non-diazotrophic cyanobacteria (Allen, 1992; Tsinoremas et al. 1991).

Osmoremedial bacterial mutations where mutant phenotypes are expressed in medium of low osmolarity but not expressed in medium of elevated osmolarity are already known (Csonka, 1989). Some bacterial mutants also show specific requirement for NaCl to correct their mutational defects (Kohno and Roth, 1979). The present osmosensitive pleotropic cyanobacterial mutant belongs to a different category in that, it requires specifically Cs\textsuperscript{+}/Rb\textsuperscript{+} at low non-osmotic concentrations for the repair of osmosensitive mutant phenotype. Since Cs\textsuperscript{+}/Rb\textsuperscript{+} also repairs the other phenotypes of the pleotropic cyanobacterial mutant, it is logical to infer that such specific requirement of Cs\textsuperscript{+}/Rb\textsuperscript{+} results from their corrective role in the mutant phenotype.

A knowledge of how monovalent (alkali) cations influence each other’s transport, accumulation and toxicity in cyanobacteria would be extremely useful in understanding the cyanobacterial mechanism(s) involved in regulation of their alkali cation nutrition and toxicity. It has been shown recently, that Li\textsuperscript{+}/Na\textsuperscript{+} stimulates K\textsuperscript{+} uptake and accumulation and Li\textsuperscript{+}, Na\textsuperscript{+} or K\textsuperscript{+} inhibit Cs\textsuperscript{+} uptake and accumulation by individually influencing the activity of K\textsuperscript{+} transport system in \textit{Synechocystis PCC} 6803 (Avery et al. 1991).
Fig. 6.10

Influence of external K⁺ (as KCl) on the cellular level of ¹³⁷ Cs⁺ in parent Nostoc muscorum (O) and its Cs⁺-R mutant strain (□). Mean values from three independent experimental determinations are shown ±SEM, where these exceed the dimensions of the symbols.
Fig. 6.10
Cs⁺ toxicity in cyanobacterium has been suggested to result from cellular replacement of K⁺ by Cs⁺ which cannot substitute functionally for K⁺ in cyanobacterial physiology (Avery et al. 1991, 1992 a&b). The present finding that K⁺ is unable to repair the Cs⁺/Rb⁺ repairable mutant phenotype in the cyanobacterium implies that K⁺ also cannot substitute functionally for Cs⁺/Rb⁺ in cyanobacterial diazotrophic physiology. Since Cs⁺ is found to be more efficient in inhibiting Rb⁺ uptake and accumulation than Rb⁺ in inhibiting Cs⁺ uptake and accumulation and since Na⁺ or K⁺ at many fold higher concentration, also inhibits Cs⁺ uptake and accumulation, it is suggested that Na⁺, K⁺ and Rb⁺ individually or in combination would determine the intracellular level of Cs⁺ in cyanobacterial population growing under Cs⁺ polluted habitats. The one most obvious ecological implications of the present finding is that, Cs⁺ requiring mutants of the given type likely to arise spontaneously in Cs⁺ polluted habitats would not survive in such habitats rich in Na⁺/K⁺ because of the inhibitory effect of the latter on the availability of exogenous Cs⁺ to the cyanobacterial mutant. The other implication of present work concerns the application of such Cs⁺/Rb⁺ requiring cyanobacterial mutants in identification and removal/recovery of Cs⁺/Rb⁺ from habitats rich in either.