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The ATPase activity of GroEL is supported at high temperatures by divalent cations that stabilize its structure

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Abstract

Previously, we reported that the ATPase activity of GroEL that requires potassium and magnesium was highly temperature dependent in the 25–60 °C range. Here, we report that the monovalent cations, rubidium and ammonium were able to fully substitute for potassium; while the divalent cations manganese, cobalt, and nickel supported the ATPase activity of GroEL albeit to a lesser degree than magnesium. ATPase activities with manganese, cobalt, and nickel were 64%, 41%, and 29%, respectively, of the maximum activity (100%) when utilizing magnesium. Interestingly, the ability of all the cations to support the GroEL ATPase activity was somewhat consistent over the entire 25–60 °C range. Maximum ATPase activities were observed at 49 °C. Here, the influence of these cations on the thermal denaturation of GroEL was also monitored using bisANS binding as an indication of the exposure of hydrophobic surfaces during thermal denaturation of GroEL. Maximum exposure of hydrophobic surfaces on GroEL alone or in the presence of each of the monovalent cations was determined to occur at 65 °C. However, the maximum exposure of hydrophobic surfaces on GroEL in the presence of magnesium, manganese, cobalt or nickel was found to occur at 71 °C indicating that GroEL is significantly stabilized against thermal denaturation by these divalent cations.

Abbreviations: ATP – adenosine triphosphate; BisANS – 1,1′-bi (4-anilino) naphthalene-5, 5′-disulfonic acid, dipotassium salt.

Introduction

The *E. coli* chaperonins or GroE proteins, GroEL and GroES, represent a subclass of molecular chaperones that have been shown to facilitate the folding of many polypeptides (Martin & Hartl 1997; Fenton & Horwich 1997). Previously, it has been reported that GroEL prevents aggregation of native proteins at elevated temperatures by stabilizing incompletely unfolded intermediates through the formation of a binary complex with those species (Holl *et al.* 1991; Martin *et al.*1992; Mendoza *et al.* 1992; Hartman *et al.* 1993; Grallert *et al.* 1998; Hook & Harding 1997; Lawton & Doonam 1998). Substrate polypeptides remain bound to GroEL in inactive forms that subsequently can be released into active states upon addition of ATP and sometimes GroES (Hayer-Hartl *et al.* 1996; Rye *et al.* 1999; Bhattacharyya & Horowitz 2002). GroEL possesses an ATPase activity that requires potassium and magnesium (Gray & Fersht 1991; Viitanen *et al.* 1990; Todd *et al.* 1993). We have reported that the GroEL ATPase activity was highly temperature dependent in the 25–60 °C range (Mendoza *et al.* 1996), and maximum activity was observed at 48 °C. Further, the ATPase activity of GroEL remained unaffected for 3 h at 48 °C (unpublished data). Since the release of many GroEL-bound polypeptides requires ATP hydrolysis, it was concluded that a faster rate of ATP hydrolysis by

GroEL at higher temperatures might allow the chaperonin to function more efficiently in mediating the folding of other polypeptides (Mendoza *et al.* 1996). Also, it was suggested that heat-shock proteins, in general, might have an increased activity under stressful conditions, e.g., under high temperatures. Similar temperature dependence was previously reported for the ATPase activity and autophosphorylation of the heat-shock protein dnaK (McCarty & walker 1991).

Previously, it has been shown that the monovalent cations rubidium and ammonium, but not sodium, lithium or cesium were able to substitute for potassium in supporting the chaperonin's ATPase activity at 25 °C (Viitanen *et al.* 1990). It has also been reported that divalent cations can induce the exposure of GroEL hydrophobic surfaces and strengthen GroEL hydrophobic binding interactions (Brazil *et al.* 1998). However, none of these cations were tested at higher temperatures, such as physiological, heat-shock temperatures or even at higher temperature. Therefore, the present study examines the ability of GroEL to hydrolyze ATP at elevated temperatures substituting potassium with rubidium or ammonium, and magnesium with one of several divalent cations and the effect of divalent cations on the stabilization of the chaperonin against thermal denaturation.

Here, we report that the monovalent cations, rubidium and ammonium were equally effective as potassium; while the divalent cations manganese, cobalt, and nickel were not as effective as magnesium in supporting the ATPase activity of GroEL. Interestingly, the partial ability of these cations to support the GroEL ATPase activity was somewhat consistent over the entire 25–60 °C temperature range. Maximum ATPase activities were observed at 49 °C. ATPase activities with manganese, cobalt and nickel were 64%, 41%, and 29%, respectively, of the maximum effects observed when utilizing potassium and magnesium with GroEL at 49 °C. Also, we report that the presence of all the aforementioned divalent cations, but not the monovalent, afforded protection to GroEL against thermal unfolding at elevated temperatures as it was monitored by bisANS binding as an indication of the exposure of hydrophobic surfaces during thermal denaturation of GroEL.

Materials and methods

Analytical grade ammonium chloride, potassium chloride, rubidium chloride, magnesium chloride, manganese chloride, cobalt chloride, and nickel chloride were obtained from Sigma Co. (St. Louis, MO). GroEL was purified as described (Clark *et al.* 1998), from lysates of *E. coli* cells bearing the multicopy plasmid pGroESL (Clark *et al.* 1996) that were kindly provided by Dr. Carl Frieden. After purification, the chaperonin was dialyzed against 50 mM Tris-HCl, pH 7.6, and 1 mM dithiothreitol. Then, glycerol was added at a final concentration of 10% [v/v] and the protein was rapidly frozen in liquid nitrogen and stored at −70 °C. Previous to its utilization, the chaperonin was dialyzed against 50 mM Tris-HCl, pH 7.6 and kept at 4 °C. The protomer concentration of GroEL was estimated by its absorbance at 280 nm using an extinction coefficient of 12,200 M^{-1} cm^{-1} (Fisher 1992), and a molecular mass of 57,259 Da (Hemmingsen *et al.* 1988).

GroEL (2.91 μM; protomer) was incubated in a buffer containing 50 mM Tris-HCl, pH 7.6, 2 mM KCl (or other monovalent cation) and 2 mM $MgCl_2$, (or other divalent cation) at the desired temperature. After a 5-min incubation, the mixture was made 1 mM in ATP by the addition of 5 μl of 0.1 M ATP in a final volume of 500 μl, to initiate the hydrolysis reaction. Periodically, 120 μl aliquots were removed and mixed with 3 ml of the 360-3 diagnostic reagent (Sigma), which forms a complex with released phosphate, and the absorbance at 340 nm was read at 25 °C using a Shimadzu spectrophotometer.

Fluorescence of bisANS was measured using an SLM Aminco Series 2 spectrofluorometer using an excitation wavelength of 396 nm. For the temperature dependence of the bisANS fluorescence intensity, the sample was heated under constant stirring at a rate of 1 °C/3 min to allow complete equilibration of the sample. Fluorescence intensity of the sample was recorded every 3 °C using an emission wavelength of 494 nm. Each sample contained 2.91 μM GroEL (protomers) and 20 μM bis-ANS and one of the following salts, NaCl, KCl, RbCl, NH_4Cl, $MgCl_2$, $MnCl_2$ $CoCl_2$ and $NiCl_2$ (2–10 mM). The maximum exposure to hydrophobic surfaces obtained with 2 mM concentration of above metals, therefore final results presents with 2 mM concentration of each metal.

Results and discussion

Figure 1 shows the temperature-dependence of the ATPase activity of GroEL in the presence of potassium and magnesium (circles). In the presence of these two

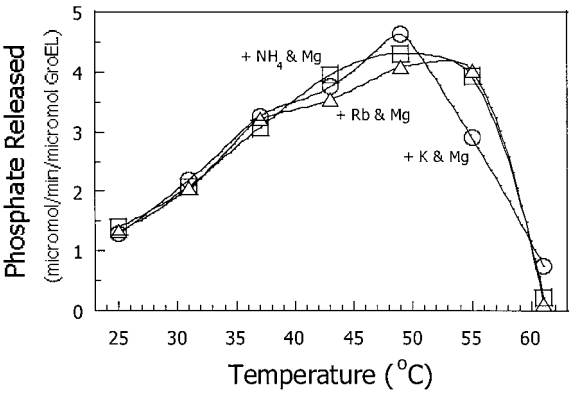

Fig. 1. Temperature dependence of the ATPase activity of GroEL in the presence of magnesium and one monovalent cation in the 25–60 °C range. GroEL (2.91 μM; protomer) was incubated at the indicated temperature with 50 mM Tris-HCl, pH 7.6, 2 mM $MgCl_2$, and either 2 mM KCl (circles), 2 mM RuCl (triangles) or 2 mM NH4Cl (squares), and the ATPase activity determined.

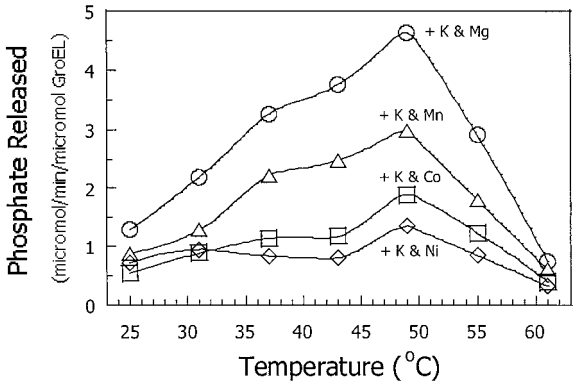

Fig. 2. Temperature dependence of the ATPase activity of GroEL in the presence of potassium and one divalent cation in the 25–60 °C range. GroEL (2.91 μM; protomer) was incubated at the indicated temperature with 50 mM Tris-HCl, pH 7.6, 2 mM KCl and either 2 mM $MgCl_2$ (circles), 2 mM $MnCl_2$ (triangles), 2 mM $CoCl_2$ (squares) or 2 mM $NiCl_2$ (diamonds), and the ATPase activity determined.

cations, the rate of ATP hydrolysis was similar to that previously observed (Mendoza *et al.* 1996). The maximum ATPase activity was observed with 2 mM potassium and magnesium. Therefore for all mono- and divalent cations the same concentration 2 mM was used. The ATPase activity in GroEL with 2 mM of potassium and 2 mM of magnesium was used as a positive control to compare the ability of GroEL to hydrolyze ATP in the presence of other cations at different temperatures.

The temperature-dependence of the ATPase activity of GroEL in the presence of magnesium and either one of the following monovalent cations: rubidium (triangles) or ammonium (squares) is shown in Figure 1. As observed previously (Viitanen *et al.* 1990), rubidium and ammonium were able to substitute for potassium in supporting the chaperonin's ATPase activity at 25 °C. As the temperature was increased, the ATPase activity of GroEL was highly stimulated in the presence of these cations. Surprisingly, the ability of rubidium and ammonium to support the chaperonin's ability to hydrolyze ATP over the 25 to 55 °C temperature range was similar to that of potassium. Cesium and lithium did not support the chaperonin's ATPase activity at any temperature in the 25 to 60 °C range, while sodium had a very small stimulating effect only at elevated temperatures (not shown). Our results with rubidium and ammonium strongly suggested that these cations bind to GroEL in a similar manner as potassium. Although potassium's role is not clearly known, it has recently been reported that potassium has cooperative effects with magnesium on the release of a bound protein from GroEL (Clark *et al.* 1999).

Binding of magnesium or other divalent cations causes a conformational change in GroEL (Gibbons & Horowitz 1996; Mendoza & Del Campo1996; Jai & Horowitz 1999) that leads to the exposure of hydrophobic surfaces on the chaperonin (Brazil *et al.* 1998). In addition, binding of magnesium to GroEL leads to the stabilization of the chaperonin's oligomeric structure (Azem *et al.* 1994, 1998). Thus, we tested the ability of other divalent cations, such as manganese or calcium to support GroEL's ATPase activity at elevated temperatures. Previously, only manganese was shown to support the ATPase activity of GroEL (Diamant *et al.* 1995a). Although manganese was found to be only half as effective as magnesium in supporting the ATPase activity of GroEL, it was found to be more effective in supporting the GroEL-assisted refolding of urea denatured malate dehydrogenase as compared to magnesium (Diamant *et al.* 1995b). However, the effect of manganese or other divalent cations on the structure and function of GroEL were not examined at elevated temperatures. Therefore, the temperature dependence of the ATPase activity of GroEL in the presence of potassium and one of several divalent cations was examined.

Figure 2 shows the ATPase activities of GroEL in the 25–60 °C range in the presence of potassium and one of the following divalent cations: magnesium, which was used as a control (circles), manganese (triangles), cobalt (squares), and nickel (diamonds).

Table 1. Temperature at which maximum bisANS fluorescence was observed.

Sample	Temperature (°C)
GroEL	65
GroEL + Potassium	65
GroEL + Rubidium	65
GroEL + Ammonium	65
GroEL + Magnesium	71
GroEL + Manganese	71
GroEL + Cobalt	71
GroEL + Nickel	71

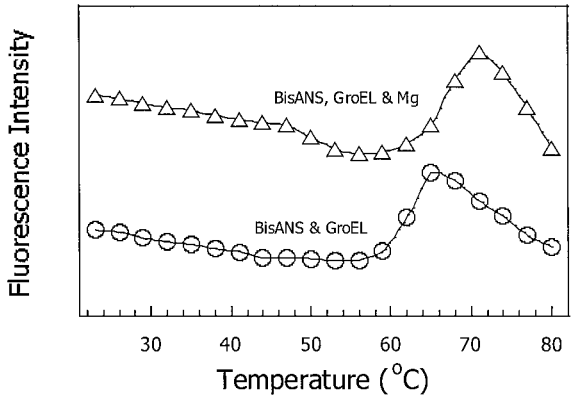

Fig. 3. Fluorescence emission intensity of bisANS in the presence of GroEL or GroEL and magnesium. Bis ANS (53 μM) was incubated with GroEL alone (circles) or with GroEL and 2 mM $MgCl_2$ (triangles) at the indicated temperature with 50 mM Tris-HCl, pH 7.6 in a final volume of 3 ml and the bisANS fluorescence intensities measured. The final GroEL concentration was 2.91 μM (protomer).

Surprisingly, none of the divalent metals was able to fully substitute for magnesium in supporting the chaperonin's ATPase activity at any of the temperatures. Interestingly, the partial ability of these cations to support the GroEL ATPase activity was somewhat consistent over the entire 25–60 °C temperature range. Maximum ATPase activities were observed at 49 °C. GroEL's ATPase activities with manganese, cobalt and nickel were 64%, 41% and 29%, respectively, of the maximum effects observed when utilizing potassium and magnesium at 49 °C. Although it was shown previously that zinc was able to strengthen the hydrophobic binding interactions of GroEL (Brazil *et al.* 1998), this divalent cation, as well as, calcium did not support the chaperonin's ATPase activity at any of these temperatures (not shown). The effects of cobalt or nickel on the structure of GroEL have not been reported. Our results with these two cations suggested that they might induce a conformational change in GroEL similar to that brought about by magnesium, thus facilitating the binding and hydrolysis of ATP.

To further understand the impact of the substituted cations, we examined their effect on the stability of the chaperonin against temperature. Thus, we used the hydrophobic reporter probe, bisANS, to monitor the exposure of hydrophobic surfaces on GroEL in the presence of these cations. BisANS is a hydrophobic probe that is virtually non-fluorescent in an aqueous buffer, but becomes highly fluorescent when it is bound to hydrophobic surfaces on proteins that are exposed to the solvent (Rosen & Weber 1969). Previously, GroEL was shown, by circular dichroism and scanning microcalorimetry, to undergo a thermal unfolding transition centered at about 67 °C (Mendoza *et al.* 1991; Lissin *et al.* 1990; Surin *et al.* 1997). Magnesium has been shown to affect the conformational mobility of GroEL and increase the stability of the protein against temperature denaturation (Surin *et al.* 1997). Here, the fluorescence of bisANS was utilized to track changes in the exposure of hydrophobic surfaces on GroEL during the thermal unfolding of the chaperonin in the presence of each of the cations that were able to support the ATPase activity at elevated temperatures. The maximum fluorescence intensity for the probe in the presence of GroEL alone was observed at 65 °C (Figure 3, circles). Therefore, we used this temperature as a control to compare against other temperatures at which maximum fluorescence intensities were observed in the presence of GroEL and each of the examined cations. Maximum fluorescence intensities in the presence of GroEL and either potassium, rubidium or ammonium were also found to occur at 65 °C (Table 1), indicating that these monovalent cations had no apparent effect on the exposure of hydrophobic surfaces on GroEL during the thermal unfolding of the chaperonin. However, as shown in Figure 3, maximum fluorescence intensity in the presence of GroEL and magnesium (triangles) was observed to occur at a significantly higher temperature (71 °C) than in the presence of GroEL alone (circles). Remarkably, as indicated in Table 1, maximum fluorescence intensities in the presence of GroEL and the divalent cations that were shown to substitute for magnesium in supporting the ATPase activity of GroEL (manganese, cobalt or nickel,) were also observed at 71 °C, indicating that the binding of each of them led

to the stabilization of the chaperonin against thermal denaturation.

In summary, our results show that, at elevated temperatures, potassium, rubidium and ammonium fully support the ATPase activity of GroEL with no effect on the thermal stability of GroEL with these monovalent cations. Our results also show that, the divalent cations seem to support the ATPase activity of GroEL at elevated temperatures and also to protect GroEL against heat stress. Therefore, we concluded that the ATPase activity of GroEL could be supported not only by magnesium, but also by other divalent cations whose binding also leads to the stabilization of the chaperonin against thermal denaturation.

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