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On the chaperonin activity of GroEL at heat-shock temperature

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Abstract

The studies of GroEL, almost exclusively, have been concerned with the function of the chaperonin under non-stress conditions, and little is known about the role of GroEL during heat shock. Being a heat shock protein, GroEL deserves to be studied under heat shock temperature. As a model for heat shock in vitro, we have investigated the interaction of GroEL with the enzyme rhodanese undergoing thermal unfolding at 43 °C. GroEL interacted strongly with the unfolding enzyme forming a binary complex. Active rhodanese (82%) could be recovered by releasing the enzyme from GroEL after the addition of several components, e.g. ATP and the co-chaperonin GroES. After evaluating the stability of the GroEL–rhodanese complex, as a function of the percentage of active rhodanese that could be released from GroEL with time, we found that the complex had a half-life of only one and half-hours at 43 °C; while, it remained stable at 25 °C for more than 2 weeks. Interestingly, the GroEL–rhodanese complex remained intact and only 13% of its ATPase activity was lost during its incubation at 43 °C. Further, rhodanese underwent a conformational change over time while it was bound to GroEL at 43 °C. Overall, our results indicated that the inability to recover active enzyme at 43 °C from the GroEL–rhodanese complex was not due to the disruption of the complex or aggregation of rhodanese, but rather to the partial loss of its ATPase activity and/or to the inability of rhodanese to be released from GroEL due to a conformational change.

Keywords: GroEL complex; Rhodanese; Chaperone; Heat shock temperature; Stability; Refolding

1. Introduction

The *Escherichia coli* chaperonins or GroE proteins are GroEL and GroES and represent a subclass of molecular chaperones that have been extensively shown to facilitate the folding of many polypeptides (Martin & Hartl, 1997). GroEL stabilizes incompletely folded intermediates through the formation of a binary complex with those species. Substrate polypeptides re-

Abbreviations: ATP, adenosine triphosphate; βME, β-mercaptoethanol; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Th T, thioflavin T

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main bound to GroEL in inactive forms that subsequently can be released into active states upon addition of K^+, Mg^{2+}, ATP, and sometimes GroES (Hayer-Hartl, Weber, & Hartl, 1996; Rye et al., 1999). In contrast to the interaction of GroEL with unfolded proteins, less is known about the interaction between chaperonins and native proteins subjected to elevated temperatures. GroEL appears to be stable under conditions that lead to the accumulation of denatured protein. When *E. coli* is exposed to moderately high temperatures, the concentration of GroEL is dramatically increased. Such an increase suggests a greater requirement for GroEL for the protection of native cellular proteins from thermal denaturation.

Although, several studies have shown that GroEL prevents the aggregation of native proteins at elevated temperatures (Grallert, Rutkat, & Buchner, 1998; Hartman, Surin, Dixon, Hoogenraad, & Hoj, 1993; Holl-Neugebauer, Rudolph, Schmidt, & Buchner, 1991; Lawton & Doonan, 1998; Look & Harding, 1997; Martin, Horwich, & Hartl, 1992; Mendoza, Lorimer, & Horowitz, 1992; Smith & Fisher, 1995), the direct formation of a complex between GroEL and proteins undergoing thermal denaturation has not been directly demonstrated at heat shock temperatures. Previously, we showed that when rhodanese was thermally unfolded in the presence of an excess of GroEL, at elevated temperatures, the enzyme was prevented from irreversible aggregation by apparently binding to GroEL in the form of a complex, since the recovery of active rhodanese was observed when ATP and GroES were quickly added to the sample (Mendoza et al., 1992b).

Here, we directly demonstrate, by velocity sedimentation, the formation of a functional complex between GroEL and rhodanese when a mixture of these proteins was incubated at 43 °C. After evaluating the stability of the GroEL–rhodanese complex, as a function of the percentage of active rhodanese that could be released from GroEL with time, we found that the complex had a half-life of only one and half-hours at 43 °C; while, it remained stable at 25 °C for more than 2 weeks.

Interestingly, the GroEL–rhodanese complex remained intact and only 13% of its ATPase activity was lost during its incubation at 43 °C. Further, rhodanese underwent a conformational change over time while it was bound to GroEL at 43 °C. Overall, our results indicated that the inability to recover active enzyme at 43 °C from the GroEL–rhodanese complex was not due to the disruption of the complex or aggregation of rhodanese, but rather to the partial loss of its ATPase activity and/or to the inability of rhodanese to be released from GroEL due to a conformational change.

2. Materials and methods

2.1. Proteins purification

All the reagents used were of analytical grade and were obtained from Sigma Co. The chaperonins, GroEL and GroES were purified as described (Clark, Ramanathan, & Frieden, 1998) from lysates of *E. coli* cells bearing the multicopy plasmid pGroESL (Clark, Hugo, & Frieden, 1996) that were kindly provided by Dr. Carl Frieden. After purification, the chaperonins were dialyzed against 50 mM Tris–HCl, pH 7.6, and 1 mM dithiothreitol. Then, glycerol 10% (v/v) was added and the proteins were rapidly frozen in liquid nitrogen and stored at −70 °C. Previous to their utilization, the chaperonins were dialyzed against 50 mM Tris–HCl, pH 7.6 and kept at 4 °C. The protomer concentrations of GroEL and GroES were estimated by their absorbance at 280 nm using extinction coefficients of 12,200 M^{-1} cm^{-1} for GroEL (Fisher, 1992), and 3440 M^{-1} cm^{-1} for GroES (Viitanen et al., 1990), and molecular masses of 57,259 and 10,368 Da, respectively (Hemmingsen et al., 1988). Rhodanese was purified as previously described (Kurzban & Horowitz, 1991). The purified enzyme was stored at −70 °C as a crystalline suspension in 1.8 M ammonium sulfate. Rhodanese concentration was determined spectrophotometrically using a molecular mass of 33 kDa (Ploegman et al., 1978) and an absorption coefficient of 57,750 M^{-1} cm^{-1}.

2.2. Formation of the GroEL–rhodanese complex at 43 °C

Rhodanese (109–486 nM) was incubated in 50 mM Tris–HCl buffer, pH 7.6, in the absence or presence of 109 nM GroEL (14-mer) for 60 min at 43 °C in a total volume of 200 μL. GroEL was pre-incubated at 43 °C for 15 min before adding rhodanese, so that when rho-