

Stabilization of a compact conformation of monomeric GroEL at low temperature by adenine nucleotides

Nikolai M. Lissin^{a,b} and Sean M. Hemmingsen^b

^a*Institute of Protein Research, Academy of Sciences of Russia, 142292 Pushchino, Moscow Region, Russian Federation* and ^b*Plant Biotechnology Institute, National Research Council Canada, 110 Gymnasium Place, Saskatoon, Saskatchewan, S7N 0W9, Canada*

Received 8 April 1993

E. coli GroEL chaperonin monomers, isolated after urea-induced dissociation of GroEL₁₄, undergo cold denaturation below 5°C. Above 5°C, these monomers undergo MgATP-dependent self-assembly. We have demonstrated a conformational transition at 0°C induced by interaction of monomeric GroEL with adenine nucleotides. This conformation has a dramatically decreased Stokes radius and enhanced resistance to trypsin but it is slightly less compact than the conformation of monomers at 23°C in the absence of MgATP and it is not capable of spontaneous self-assembly. A second, temperature-dependent conformational change with a transition at about 5°C is required for GroEL to undergo oligomerization.

Molecular chaperone; Chaperonin; GroEL; Self-assembly; Protein folding; ATP

1. INTRODUCTION

GroEL, the *E. coli* prototype of the ubiquitous chaperonin-60 proteins [1], interacts transiently with newly synthesized polypeptides in their non-native conformations [2] and functions both in vivo and in vitro as an ATP-driven molecular chaperone [3–5] required for transmembrane translocation of some polypeptides and for the folding and assumption of the correct quaternary structures of others (for review see [6–10]). GroEL functions as a homo-tetradecameric particle (GroEL₁₄) composed of 60 kDa polypeptide subunits [11–14]. An ATPase [11], GroEL exhibits positive cooperativity in the binding and hydrolysis of ATP [15,16] in the presence of the co-chaperonin, GroES, a heptameric protein [17]. Since GroEL functions are believed to be essential for the assembly of many proteins in *E. coli* [1], it is of particular importance to understand the formation of its own structure.

A recent study described the denaturation of GroEL₁₄ by guanidine-HCl [18]. Refolding of the monomers in the absence of ATP resulted in the spontaneous restoration of secondary structure, but oligomerization did not occur and chaperonin function was not restored. We have demonstrated previously that GroEL₁₄ dissociates in vitro in the presence of 3.5–4.0

M urea into monomers which are capable of MgATP-dependent self-assembly and reformation of native GroEL₁₄. These oligomers, in turn, ‘self-chaperone’ the assembly of additional GroEL₁₄ [19]. From their effect on oligomerization at 23°C, it was inferred that adenine nucleotides stabilized an assembly-competent state of the GroEL monomers [19]. We report here a transition caused by interaction of monomeric GroEL with adenine nucleotides at 0°C that results in a conformation that is distinct from the monomeric state at 23°C in the presence or absence of MgATP.

2. MATERIALS AND METHODS

GroEL was isolated from *E. coli* HB101 bearing pGroE4 plasmid as described previously [16,19], except the sucrose gradient centrifugation step was replaced by chromatography on a DEAE-Toyopearl HW 650 M column (Toyo Soda) with a linear 0–0.7 M NaCl gradient in 20 mM Tris-HCl, pH 8.0, 1 mM β-ME. Monomeric GroEL was prepared for each experiment by incubating the protein in 4 M urea (20 min on ice) followed by purification of the monomers by chromatography at 4°C on a Superose 6 HR 10/30 column (Pharmacia FPLC system) in buffer A (40 mM triethanolamine-acetate, pH 7.5, 100 mM potassium acetate, 0.1 mM EDTA, 1 mM DTT). Analytical size-exclusion chromatography was executed similarly but the column was placed in an ice jacket (except as indicated otherwise) and the DTT was omitted. Other additives in the buffer were as indicated. Concentrations of native and monomeric GroEL were determined spectrophotometrically at λ_{max} 276.5 nm using values of A_{1%¹cm}⁰ of 0.25 and 0.21, respectively [19].

Trypsin-digestion experiments [20] were performed as follows. Samples containing 2.4 μM monomeric GroEL were pre-incubated for 20 min on ice in buffer A with additives as indicated. After the addition of bovine pancreatic trypsin (Sigma; Type XIII), the samples were incubated for 10 min on ice then mixed with SDS-electrophoresis sample buffer [21] and frozen immediately in liquid nitrogen. All samples were heated for 5 min at 100°C then electrophoresed under

Correspondence address: N.M. Lissin, CNRS, CBBM4, 31 Chemin Joseph Aiguier, PB71 – 13402 Marseilles Cedex 9, France. Fax: (33) 9171 7896.

Abbreviations. EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; β-ME, β-mercaptoethanol; ATP-γ-S, adenosine 5'-O-(3-thiotriphosphate).

standard conditions on a 10–20% gradient SDS-polyacrylamide gel [2] with subsequent Coomassie G-250 staining.

3. RESULTS

The results of size-exclusion chromatography of monomeric GroEL are presented in Fig. 1. At room temperature and in the absence of adenine nucleotides, monomeric GroEL behaved like a protein of about 60 kDa (profile 1), but when the chromatography was performed at 0°C monomeric GroEL exhibited an increase in apparent molecular mass of greater than 3-fold (profile 2). Since the behaviour of folded or denatured proteins during size-exclusion chromatography reflects their Stokes radii [22], and since a significant loss of secondary structure was indicated by circular dichroism (CD) spectroscopy, it was concluded previously that monomeric GroEL was in a partially unfolded state below 5°C [19]. Surprisingly, when monomeric GroEL was subjected to chromatography at 0°C in buffer A containing 10 mM magnesium acetate and 0.2 mM ATP

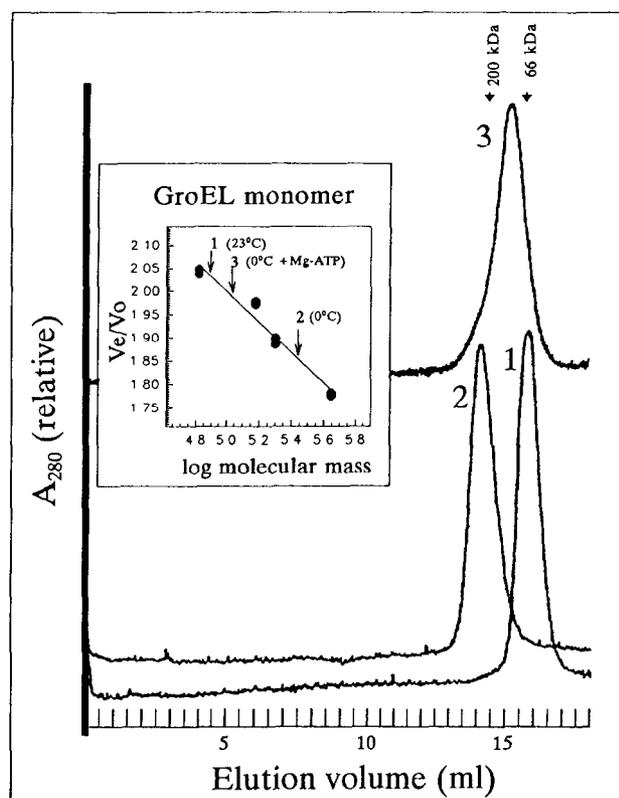


Fig. 1. Size-exclusion chromatography of monomeric GroEL on Superose 6 (Pharmacia) under different conditions. Chromatography was performed in buffer A (see section 2) at 23°C (1) or 0°C (2) or at 0°C in buffer A containing 10 mM magnesium acetate and 0.2 mM ATP (3). Elution volumes of β -amylase (200 kDa) and bovine serum albumin (66 kDa) are indicated with arrows. The inset is a semi-logarithmic plot of the relative elution volumes against apparent molecular mass under the same conditions. Protein standards (Sigma) shown on the plot were (left to right): bovine serum albumin, alcohol dehydrogenase, β -amylase, apoferritin. Chromatographic behaviours of the standards were determined both at 23°C and 0°C.

Mg ²⁺ (10mM)	—	+	+	+	+	—	+	+	+
ATP mM	—	—	5	—	—	5	.5	.05	0.05
ADP mM	—	—	—	5	—	—	—	—	—
ATP- γ -S mM	—	—	—	—	5	—	—	—	—
Trypsin	—	+	+	+	+	+	+	+	+

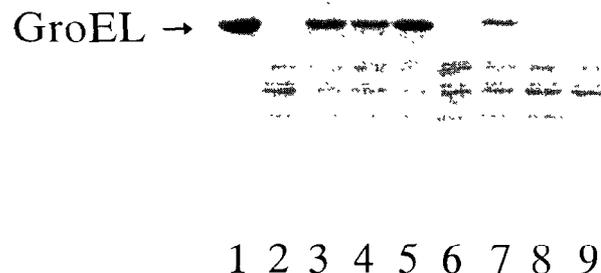


Fig. 2. Protease sensitivity of monomeric GroEL at 0°C in the absence or presence of adenine nucleotides. Purified GroEL monomers were subjected to digestion with trypsin at 0°C and analysed by SDS-PAGE as described in section 2. The additions made to each sample are indicated above each lane.

(MgATP), the peak was observed to have shifted close to its original location (profile 3), suggesting that a more compact structure had been restored. The chromatographic profile of the GroEL monomer observed under these conditions was noticeably asymmetric (profile 3). This asymmetry persisted when samples were pre-incubated for 30 min on ice in the presence of 10 mM magnesium acetate and 5 mM ATP before the chromatography (not shown), indicating that the compact state was in an equilibrium with a population of more unfolded molecules. When the chromatography buffer lacked either Mg²⁺ or ATP, the monomers adopted the unfolded conformation (as in profile 2) whether or not they had been pre-incubated with MgATP (not shown). This observation suggests both that the observed effect results from interaction of monomers with magnesium adenine nucleotides and that the interaction is fully reversible.

We used a trypsin digestion-based test, which was proven to be sensitive to protein conformation [20], to obtain independent evidence of the observed stabilization of a compact state of monomeric GroEL by MgATP (Fig. 2). Monomeric GroEL, present at 2.4 μ M, was completely converted to digestion products after incubation with 0.0075 μ M trypsin for 10 min at 0°C in the presence of 10 mM magnesium acetate lacking ATP (lane 2) or 5 mM ATP lacking magnesium acetate (lane 6). This high sensitivity of monomeric GroEL to proteolysis under these conditions is consistent with an unfolded conformation. Strikingly enhanced resistance to cleavage by trypsin was observed, however, upon incubation of the protein in the presence

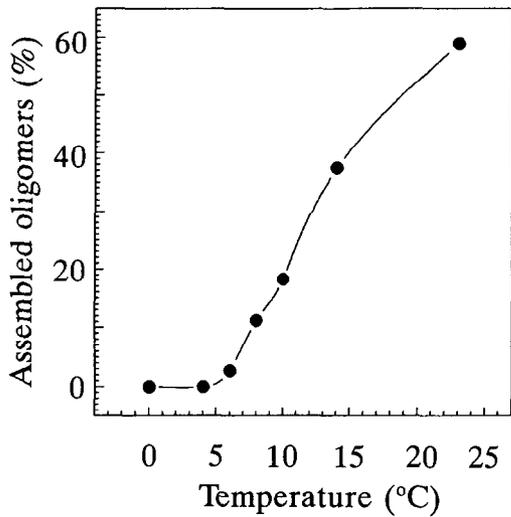


Fig. 3. Temperature dependence of the self-assembly of GroEL. Samples containing $2.6 \mu\text{M}$ monomeric GroEL were incubated at different temperatures for 1 h in the presence of 10 mM magnesium acetate and 5 mM ATP. The products of each incubation were analysed by chromatography on a Superose 6 column at 0°C . The extent to which self-assembly occurred was calculated as the ratio of UV-absorbance (280 nm) due to GroEL₁₄ to total UV-absorbance due to GroEL₁₄ plus monomeric GroEL. Oligomers were not detected after incubation of the samples in the absence of ATP (not shown).

of MgATP as well as MgADP or MgATP- γ -S (lanes 3–5). Similar effects were observed for each of these three nucleotides, although slightly less stabilization was apparent in the case of ADP. A gradually decreasing stabilization effect was observed with decreased ATP concentration in the range from 5 mM to $5 \mu\text{M}$ (compare lanes 3 and 7–9).

Prolonged incubation of monomeric GroEL at 0°C in the presence of MgATP did not cause its self-assembly [19]. Since we have found that adenine nucleotides stabilize a compact conformation of monomeric GroEL at 0°C , we have re-examined the self-assembly of GroEL monomers at different temperatures. As expected, at temperatures above 5°C , increased self-assembly was obtained as the temperature was increased to 23°C (Fig. 3). At 0°C or 4°C , however, no detectable oligomers were formed after incubation of $2.6 \mu\text{M}$ monomeric GroEL for 1 h (Fig. 3). Extended incubations (up to 40 h) under similar conditions failed to yield detectable assembly of GroEL₁₄.

4. DISCUSSION

Adenine nucleotide-induced or temperature-induced conformational transitions in monomeric GroEL are summarized in Fig. 4. We have shown previously [19] that MgATP (or, with lower efficiency, non-hydrolysable MgATP-analogs or MgADP) promoted oligomerization of GroEL at 23°C . This observation suggested a nucleotide-dependent change in monomer conforma-

tion (transition I). On the other hand, in the absence of adenine nucleotides, monomeric GroEL has been shown to undergo a fully reversible unfolding at low temperature (cold denaturation [23]) with the transition point at about 5°C (transition II) [19]. We have now demonstrated an adenine nucleotide-dependent conformational transition in monomeric GroEL at 0°C (transition III) which leads to a decreased Stokes radius and to enhanced resistance to proteolysis. The observed stabilization of a compact conformation at relatively low ATP concentrations (0.2 mM or lower) indicates that GroEL in its monomeric form possesses detectable affinity for the nucleotides at 0°C . This is particularly striking bearing in mind its unfolded state at this temperature, and it suggests that the binding of adenine nucleotides to a small population of more 'native' molecules causes the equilibrium to shift towards the compact conformation. This interaction is easily convertible *in vitro*. A nucleotide-stabilized state is, evidently, the only state of monomeric GroEL that can exist at low temperature *in vivo* given the physiological concentrations of free Mg^{2+} and ATP in the cell.

Compared to the unfolded conformation at 0°C , the nucleotide-stabilized conformation of monomeric GroEL at 0°C is much more similar in compactness to monomeric GroEL at 23°C . Nevertheless, even after extended incubations at 4°C , self-assembly did not occur. Assembly of GroEL₁₄ can be achieved at temperatures below 5°C if the concentration of monomeric GroEL is increased by 4-fold (not shown). The plot of extent of self-assembly vs. temperature (Fig. 3) clearly suggests that adenine nucleotide-stabilized monomers behave differently below and above about 5°C . This temperature corresponds to the midpoint of a transition identified by CD spectroscopy of monomers in the absence of adenine nucleotides [19]. Taken together, these observations indicate that an additional temperature-

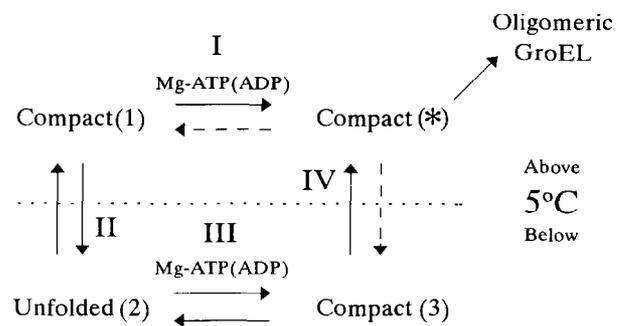


Fig. 4. The effect of adenine nucleotides and temperature on the conformation of monomeric GroEL *in vitro*. Four states of monomeric GroEL are indicated: Compact (*), Compact (1), Unfolded (2) and Compact (3). The conformational transitions proposed to result from the presence or absence of adenine nucleotides, MgATP (ADP), at temperatures above or below 5°C (dotted line) are indicated by Roman numerals. The arrows indicate the reversibility of the transitions. The dotted arrows indicate two transitions that have not been directly demonstrated.

induced change (transition IV) is required to achieve an assembly-competent state (Compact *). Native GroEL₁₄ exhibits a higher content of secondary structure than that exhibited by 'folded' monomers [19] and each subunit in the assembled protein possesses obvious two-domain topology, as revealed by electron microscopy [13,14]. The final conformational transition in monomeric GroEL which leads to the formation of tetradecameric particles that are active as molecular chaperones remain to be determined. Direct study of the ATP-induced effects on the conformation of monomers is complicated if the conditions used support the spontaneous oligomerization process. It is anticipated that the nucleotide-stabilized intermediate of monomeric GroEL at 0°C described in this paper will serve as a useful model for further studies.

Acknowledgements: We thank Vladimir Uversky and Gennady Semisotnov for helpful discussions. N.M.L. was the recipient of a fellowship from the Eastern Europe Fellowship Program of the National Research Council Canada.

REFERENCES

- [1] Hemmingsen, S.M., Woolford, C., van der Vies, S.M., Tilly, K., Dennis, D.T., Georgopoulos, C.P., Hendrix, R.W. and Ellis, R.J. (1988) *Nature* 333, 330-334.
- [2] Bochkareva, E.S., Lissin, N.M. and Girshovich, A.S. (1988) *Nature* 336, 254-257.
- [3] Ellis, R.J. (1987) *Nature* 328, 378-379.
- [4] Pelham, H.R.B. (1986) *Cell* 46, 959-961.
- [5] Rothman, J.E. and Kornberg, R.D. (1986) *Nature* 322, 209-210.
- [6] Ellis, R.J. and Hemmingsen, S.M. (1989) *Trends Biochem. Sci.* 14, 339-342.
- [7] Rothman, J.E. (1989) *Cell* 59, 591-601.
- [8] Ellis, R.J. (1990) *Sem. Cell Biol.* 1, 1-9.
- [9] Zeilstra-Ryalls, J., Fayet, O. and Georgopoulos, C. (1991) *Annu. Rev. Microbiol.* 45, 301-325.
- [10] Ellis, R.J. and van der Vies, S.M. (1991) *Annu. Rev. Biochem.* 60, 321-347.
- [11] Hendrix, R.W. (1979) *J. Mol. Biol.* 129, 375-392.
- [12] Hohn, T., Hohn, B., Engel, A., Wurtz, M. and Smith, P.R. (1979) *J. Mol. Biol.* 129, 359-373.
- [13] Hutchinson, E., Tichelaar, W., Hofhaus, G., Weiss, H. and Leonard, K.R. (1989) *EMBO J.* 8, 1485-1490.
- [14] Zwickl, P., Pfeifer, G., Lottspeich, F., Kopp, F., Dahlmann, B. and Baumeister, W. (1990) *J. Struct. Biol.* 103, 197-203.
- [15] Gray, T.E. and Fersht, A.R. (1991) *FEBS Lett.* 292, 254-258.
- [16] Bochkareva, E.S., Lissin, N.M., Flynn, G.C., Rothman, J.E. and Girshovich, A.S. (1992) *J. Biol. Chem.* 267, 6796-6800.
- [17] Chandrasekhar, G.N., Tilly, K., Woolford, C., Hendrix, R. and Georgopoulos, C. (1986) *J. Biol. Chem.* 261, 12414-12419.
- [18] Price, N.C., Kelly, S.M., Thomson, G.J., Coggins, J.R., Wood, S. and auf der Mauer, A. (1993) *Biochim. Biophys. Acta* 1161, 52-58.
- [19] Lissin, N.M., Venyaminov, S.Yu. and Girshovich, A.S. (1990) *Nature* 348, 339-342.
- [20] Nakayama, N., Arai, N., Kaziro, Y. and Arai, K. (1984) *J. Biol. Chem.* 259, 88-96.
- [21] Laemmli, U.K. (1970) *Nature* 227, 680-685.
- [22] Corbett, R.J.T. and Roche, R.S. (1984) *Biochemistry* 23, 1888-1894.
- [23] Privalov, P.L. (1989) *Rev. Biophys. Biophys. Chem.* 18, 47-69.