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Ligands regulate GroEL thermostability

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Abstract ***Escherichia coli*** **heat-shock proteins GroEL and GroES stimulate (in an ATP-dependent manner) the folding of various proteins. In this study scanning microcalorimetry was applied to investigate GroEL thermostability in the presence of its ligands. Mg^{2+} and K^+ ions stabilize while ADP destabilizes the GroEL molecule against the action of temperature. Furthermore, ADP essentially increases the number of binding sites for the hydrophobic probe (ANS) and the number of GroEL SH-groups accessible to Ellman's reagent as well as the accessibility of the protein to the action of trypsin. The interaction of GroEL with GroES in the presence of Mg^{2+}-ADP eliminates the destabilizing effect of ADP on the GroEL molecule against the action of temperature and Ellman's reagent but does not change its hydrophobicity and accessibility to trypsin.**

Key words: GroEL; GroES; Scanning microcalorimetry; Hydrophobic probe binding; Reactivity of SH-groups

1. Introduction

Molecular chaperones assist in the intracellular transport and folding of many proteins [1,2]. The best studied chaperone is the *Escherichia coli* heat shock protein GroEL, also called hsp60 and chaperonin [3,4]. GroEL is an oligomeric protein containing 14 identical 57.3 kDa subunits [3], which are arranged in two stacked seven-membered rings to form a cylinder [5]. Each GroEL subunit is folded into three distinct domains [5]. It was shown that GroEL interacts with partially folded protein chains both in vivo and in vitro [6–8]. The nucleotides (ATP and ADP) in the presence of Mg^{2+} affect the interaction of GroEL with protein targets and strongly decrease the binding reaction constants [9]. Moreover, GroEL possesses a K^+-dependent ATPase activity [10]. GroEL requires another *Escherichia coli* heat shock protein, GroES (hsp10), to promote protein folding [11,12]. GroES is a seven-membered ring composed of seven identical 10 kDa subunits [13,14] and in the presence of Mg-ADP or Mg-ATP it forms a stable complex with GroEL [11,15].

Elucidation of the effect of the ligand on the structure of GroEL is one of the main ways to understand GroEL-assisted protein folding. However, there are only few experimental data available. An electron microscopic study [16] has shown that nucleotide binding changes GroEL quaternary structure. Formation of the GroEL-GroES complex increases the ATP binding constant [17], but inhibits ATPase activity [6,10,11]. Mg^{2+} and K^+ stabilize the GroEL structure against the action of urea [18], while ADP and ATP destabilize GroEL against the proteolytic action of proteinase K [19].

Here we report the effects of Mg^{2+}, K^+, ATP, ADP and co-chaperonin GroES on the thermostability and conformation of GroEL in solution. Scanning microcalorimetry, reactivity of SH-groups, hydrophobic probe (ANS) binding and limited trypsinolysis were used to test GroEL's conformation and stability in solution.

2. Materials and methods

2.1. Materials

GroEL and GroES were purified after expression in *Escherichia coli* of the corresponding plasmid according to published protocols [8,12].

2.2. Preparation of protein solutions

All samples were prepared in 50 mM HEPES, pH 7.6 (buffer A), or in 50 mM HEPES, pH 7.6, containing 100 mM KCl, 10 mM $MgAc_2$ (buffer B). Protein concentrations were 1.8 mg/ml, 0.01 mg/ml, 0.5 mg/ml and 0.1 mg/ml for scanning microcalorimetry, fluorometric experiments, limited proteolysis and SH-group reactivity, respectively.

2.3. Physical methods

Scanning microcalorimetry experiments were carried out using a differential scanning microcalorimeter DASM-4 (Russia) with a 0.49 ml cell volume at a scan rate of 1 deg/min. Fluorescence measure-

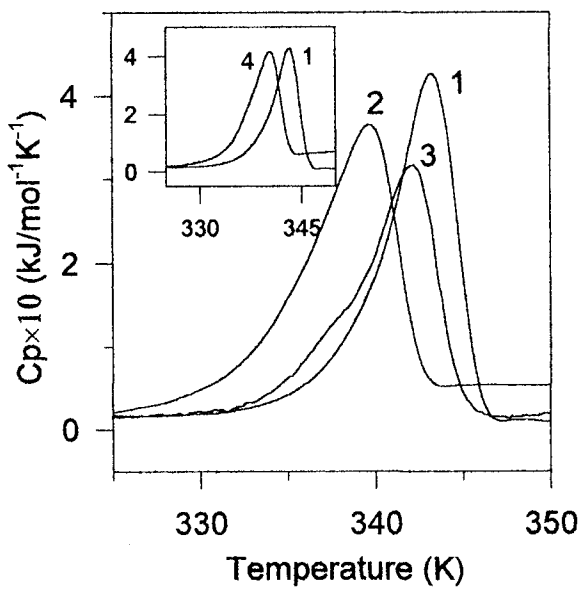

Fig. 1. Temperature dependence of GroEL partial heat capacity in the presence of various ligands: 1, GroEL, buffer B; 2, GroEL, buffer B, 2 mM ADP; 3, GroEL, buffer B, 2 mM ADP and GroES (GroEL and GroES molar concentrations 1:1.5). The inset shows the effect of Mg^{2+} and K^+ on the temperature dependence of GroEL partial heat capacity: 1, GroEL, buffer B; 4, GroEL, buffer A.

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Abbreviations: ANS, 8-anilino-naphthalene-1-sulfonate; $MgAc_2$, magnesium acetate

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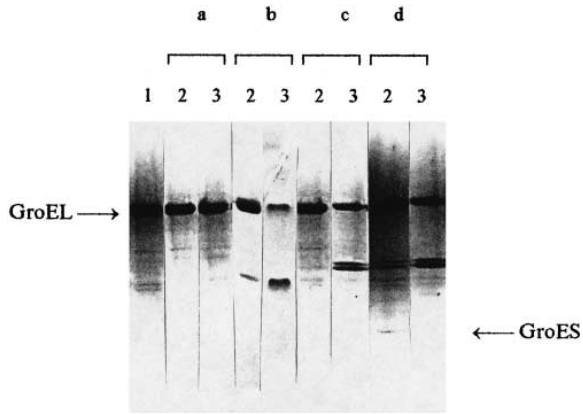


Fig. 2. Kinetics of GroEL cleavage with trypsin in the presence of ligands: a, GroEL, buffer A or buffer B; b, GroEL, buffer B, 2 mM ADP; c, GroEL, buffer B, 2 mM ATP; d, GroEL, buffer B, 2 mM ADP and GroES ([GroEL]:[GroES] = 1:1.5). Temperature, 23°C. Lane 1, without trypsin; lanes 2 and 3, incubation with trypsin for 10 and 90 min.

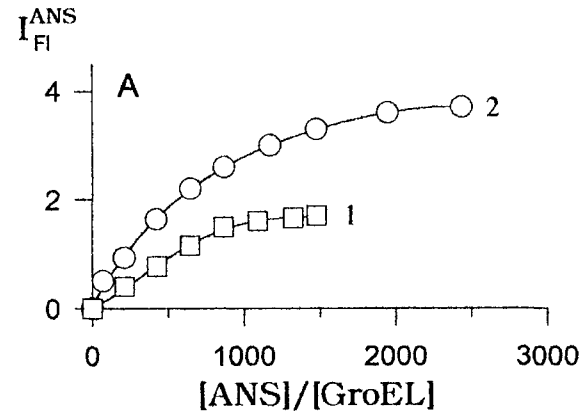
ments with ANS were made with spectrofluorimeter SPF-1000 CS (Aminco, USA) with excitation at 390 nm and emission at 480 nm. The reactivity of the GroEL SH-groups against Ellman's reagent was measured with the Carry-219 (USA) spectrophotometer.

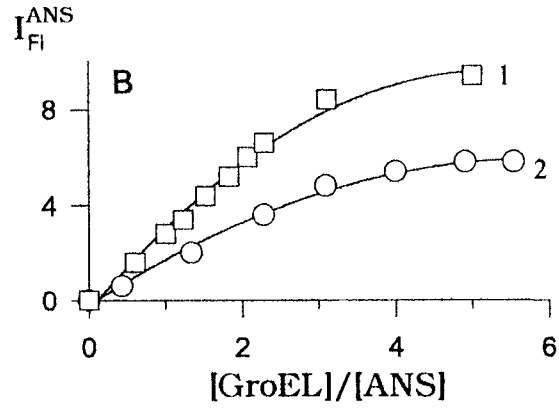
2.4. Experimental procedures

Kinetics of GroEL cleavage with trypsin was measured by incubation of the protein (0.5 mg/ml) with trypsin (molar ratio GroEL:trypsin was 5:1). Trypsinolysis was inhibited by the addition of 0.3 M Tris-HCl, pH 7.0, containing 50% glycerol, 10% SDS, 5% β-mercaptoethanol and 2 mM Na_2EDTA, and the samples were analyzed by PAGE. The number of GroEL SH-groups accessible for modification with Ellman's reagent was determined using the published method of Ellman [20]. Titration experiments with ANS were done in a 2 ml volume cuvette and the dilution was corrected. The number of binding sites for ANS was estimated by routine Scatchard procedure [21].

3. Results and discussion

Fig. 1 shows the temperature dependence of the GroEL heat capacity in the presence of the protein ligands. The calorimetric and the effective (van 't Hoff) enthalpies calculated from these curves are presented in Table 1. First, the ligands affect the temperature of the thermal transition of GroEL with no influence on the enthalpy values. Second, Mg^{2+} and K^+ stabilize, while ADP destabilizes the structure of GroEL under the action of temperature. Third, the binding of GroES





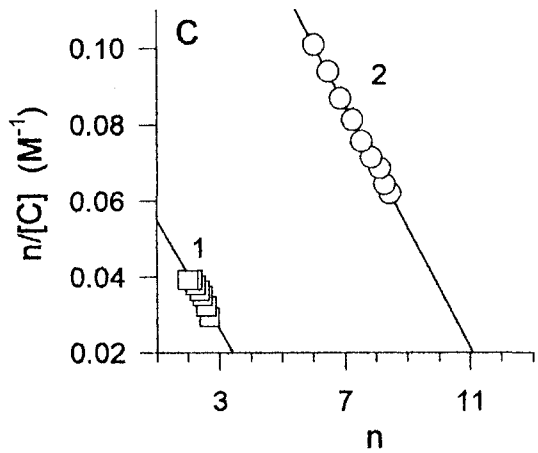


Fig. 3. Fluorescent hydrophobic probe (ANS) binding with GroEL: A, titration of GroEL with ANS; B, ANS titration with GroEL; C, Scatchard plots (n, mol of ANS bound per mol of GroEL, [C], concentration of ANS). 1, in the presence of ADP; 2, without ADP. Buffer B. Temperature, 23°C.

Table 1
Parameters of GroEL heat denaturation in the presence of various ligands

Object, conditions	T_d (K)	ΔH^{cal} (kJ/mol)	$\Delta H^{cal\ a}$ (kJ/mol)	ΔH^{eff} (kJ/mol)	R[b]
GroEL, buffer A	340 ± 0.5	16000 ± 3000	1140 ± 215	720 ± 100	1.6 ± 0.3
GroEL, buffer B	343 ± 0.5	16000 ± 3000	1140 ± 215	700 ± 100	1.6 ± 0.3
GroEL, buffer B, 2 mM ADP	339 ± 0.5	18000 ± 3000	1290 ± 215	760 ± 100	1.7 ± 0.3
GroES, buffer B, 2 mM ADP	349 ± 0.5	1200 ± 250	170 ± 40	610 ± 100	0.28 ± 0.05
GroEL, buffer B, 2 mM ADP and GroES[c]	342 ± 0.5	19000 ± 3000	1360 ± 215	790 ± 100	1.7 ± 0.3

[a]Per subunit.
[b]$R = \Delta H^{cal\ a}/\Delta H^{eff}$.
[c]Essential differences of GroEL and GroES in molecular mass, calorimetric enthalpy and temperature of heat capacity maximum allowed us to ignore the contribution of GroES.

Table 2
Physico-chemical characteristics of GroEL in the presence of ligands

Object, conditions	Number of ANS binding sites (per GroEL molecule)	Proteolytic degradation	Number of accessible SH-groups (per subunit)
GroEL, buffer A or B	3.5 ± 0.5	–	1
GroEL, buffer B, 2 mM ADP	11 ± 1	+	2
GroEL, buffer B, 2 mM ADP and GroES	11 ± 1[a]	+	1

[a]Corrected value for ANS binding with GroES.

eliminates the destabilizing effect of ADP on the GroEL structure. Furthermore, the ratio $\Delta H^{cal}/\Delta H^{eff}$ (van 't Hoff coefficient) per subunit is more than 1.1 (characteristic value for a two-state thermal denaturing transition [22]). This means that each GroEL subunit apparently contains more than one cooperative thermodynamic block [22]. The absence of a change of the GroEL enthalpy values upon binding of the ligands suggests the absence of essential intrasubunit structural rearrangements of the protein.

Several physico-chemical techniques were applied to elucidate local conformational changes of GroEL upon ligand binding. Fig. 2 represents trypsin digestion of GroEL both with and without ATP, ADP and GroES. The data demonstrate that nucleotide binding essentially increases the rate of GroEL degradation by trypsin and confirm the results with proteinase K [19]. The binding of GroES in the presence of Mg-ADP does not change the accessibility of GroEL to trypsin in comparison with that in the presence of Mg-ADP alone. The destabilizing effect of ADP on the GroEL structure against the action of trypsin seems to be due to local alterations of the protein intrasubunit structure (probably near the ADP binding sites). The binding of GroES does not affect these local ADP-dependent GroEL intrasubunit structural changes, but apparently affects GroEL conformational mobility resulting in an increase of GroEL stability against the action of temperature (see Fig. 1 and Table 1). This result is confirmed by hydrophobic probe (ANS) binding. Fig. 3 demonstrates the ANS titration experiment for GroEL with and without Mg-ADP. The results for the GroEL-GroES complex in the presence of ADP as well as for GroEL in the absence of ligands are shown in Table 2. The following conclusions may be drawn from these data. First, the GroEL molecule alone has some hydrophobic sites exposed to the solvent. Second, ADP binding essentially increases the number of GroEL sites accessible for ANS binding, i.e. ADP disturbs the structure of GroEL. Third, GroES binding in the presence of ADP does not change the accessibility of the GroEL molecule to the hydrophobic probe. Another method confirms the presence of ADP-dependent conformational changes in GroEL. The reactivity of the GroEL SH-groups against Ellman's reagent increases from 1 to 2 per subunit of the three SH-groups in the protein's primary structure [23] upon ADP binding (Table 2). Formation of the GroEL-GroES complex eliminates the effect of ADP on the reactivity of the GroEL SH-groups (Table 2), but this may be explained by steric protection of the SH-groups in the GroEL-GroES complex.

The data presented here demonstrate that the nucleotide ligands decrease the stability and cause local conformational changes of GroEL in solution. On the other hand, Mg^{2+} and K^+, as well as the co-chaperonin GroES apparently affect the conformational mobility of GroEL and increase the stability of the protein against the action of temperature.

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