ATP Induces Non-identity of Two Rings in Chaperonin GroEL*

(Received for publication, July 14, 1994)

Elena S. Bochkareva and Alexander S. Girshovich

From the Department of Structural Biology, Weizmann Institute of Science, Rehovot 76100, Israel

For its function, the Escherichia coli chaperonin GroEL requires the presence of ATP and co-chaperonin GroES. We have observed that ADP displays a two-step inhibition of GroEL-dependent ATP hydrolysis, wherein one-half of the GroEL ATPase sites is strongly inhibited by ADP while the other half is affected very mildly. It is suggested that interaction with ATP induces structural and functional differences between two initially identical rings in GroEL (inter-ring negative cooperativity) and that the subsequent binding of GroES occurs to the ring that is occupied first by ATP in a positively cooperative manner.

The Escherichia coli heat-shock protein GroEL is a molecular chaperone of the chaperonin family that interacts transiently and ATP dependently with various newly synthesized proteins before they fold into the native form (2) and mediates their correct folding and assembly (for reviews, see Refs. 3–7). The molecular mechanism for its action is still unclear, and a high resolution three-dimensional structure of it is not yet available. GroEL is an oligomeric protein consisting of 14 identical subunits (8, 9), each with a molecular mass of 57.3 kDa (10). GroEL has 14 ATP-binding sites, presumably one site per subunit (11), and possesses a moderate ATPase activity (8). ATP bound to GroEL is in contact with the Cys-137 residue (12).

For successful folding and assembly of some proteins, GroEL requires the presence of the co-chaperonin GroES, which comprises a single ring of seven identical subunits (13) of a molecular mass of 10.4 kDa each (10). In the presence of Mg2+ ions and ATP (13) and, also, ADP or nonhydrolyzable ATP analogues (11), GroES 7-mer forms a 1:1 stoichiometric complex with GroEL 14-mer and inhibits its ATPase activity (13–15). GroEL hydrolyzes ATP with a positive cooperativity, and GroES acts as an allosteric effector by increasing the cooperativity (11, 15, 16). It has been suggested that GroES acts as a "coupling" factor linking the hydrolysis of ATP by GroEL to the release of the target protein in a form that is committed to the native state (14). According to negatively stained electron microscopy (8, 9, 17) and x-ray crystallography at a resolution of 8.9 Å (18), GroEL is a cylindrical structure (its height and diameter are both around 130 Å) that possesses an unusual 7-fold symmetry and is comprised of two heptameric rings stacked "base-to-base." GroEL binds GroES asymmetrically as a cap to either of the two end surfaces of its double ring whereas the opposite end surface is markedly perturbed (17, 19). The binding of only one GroES to GroEL is surprising since the base-to-base stacking of two identical rings in GroEL implies that its two end surfaces should also be identical. Hence, it has been proposed that ATP-promoted binding of GroES to either end surface of the GroEL cylinder induces allosterical inhibition of the GroES binding to the opposite surface of GroEL (15, 19, 20).

Here we suggest that asymmetric ATP-promoted binding of GroES to GroEL is a consequence of non-identity of two stacked rings in GroEL caused by its interaction with ATP.

EXPERIMENTAL PROCEDURES

Proteins, Chemicals, and Buffers—GroEL and GroES were purified from E. coli TG2 cells (11, 21). [γ-32P]ATP and NaB[3H]14 were from Amersham Corp. Buffer A consisted of 10 mM MgCl2, 100 mM KCl, 20 mM Tris-HCl, 0.1 mM EDTA, and 1 mM dithiothreitol (pH 7.5).

Effect of ADP and ATPyS on Initial Rate of ATP Hydrolysis by GroEL—The reaction was started by mixing 10 μl of 0.8 mM [γ-32P]ATP with 30 μl of 0.2 μM GroEL (14-mer) in buffer A without or in the presence of 0.3 μM GroES (7-mer) and various concentrations of ADP or ATPyS. After mixing, both solutions were incubated at 25 °C for 5 min. The reaction was carried out at the same temperature and terminated at different time intervals from 1 to 6 min after the start by removal of 8-μl aliquots to 1.5-ml Eppendorf tubes containing 70 μl of 1 m perchloric acid and 1 mM sodium orthophosphate, cooled on ice. The subsequent quantitative analysis of the [32P]Pi released was carried out as described previously (21). Control reactions were run under all conditions in the absence of GroEL, and extracted orthophosphate was subtracted as background. The [32P]Pi background was constant and equals about 1.5 × 104 cpm, whereas incubation in the presence of GroEL for 2 or 4 min gave about 2.5 or 5 × 104 cpm of the extracted orthophosphate, respectively. Note that initial rates were measured when <10% of [γ-32P]ATP was hydrolyzed.

1H Labeling of GroES—The labeling was carried out using the reductive methylation procedure (22) by treatment of 40 μM GroES (7-mer) with 5 mM formaldehyde and 0.5 mM of NaB[3H]14 in 95 μl of 0.2 mM sodium borate (pH 9.0) for 25 min on ice followed by gel filtration through Sephadex G-25 superfine in buffer A. The specific radioactivity of the [3H]GroES preparation was about 1.8 × 106 cpm/μg of protein.

The ATP Concentration Dependence of Interaction of GroES with GroEL—1.8 μM GroEL (14-mer) was incubated with 2.4 μM [3H]GroES (7-mer) in 40 μl of buffer A containing different concentrations of ATP (4 μM–8 mM) for 10 min at 25 °C. Then, GroES bound to GroEL was separated by centrifugation through 1.4 ml of 5–20% sucrose gradient in buffer A containing the same concentrations of ATP as in the samples (Beckman TL100 centrifuge, TLS 55 rotor, 50,000 rpm for 150 min at 4 °C). 0.1-ml fractions were collected and counted.

RESULTS AND DISCUSSION

As we have shown previously (11), in the presence of GroES only half of the 14 ATP-binding sites of GroEL have a strong affinity for ADP and can bind and hydrolyze ATP with a positive cooperativity. It has been suggested that two initially identical rings in GroEL become functionally non-identical within the GroEL-GroES complex, and only one of them is able to interact with ATP (or ADP) in a positively cooperative manner (11). This suggestion has been further supported by the observation that hydrolysis of ATP by the GroEL-GroES complex occurs in an asymmetric manner in which one of the rings of GroEL is completely inhibited, while the other hydrolyzes ATP with significantly altered kinetic properties (15). All of these and other (19) data have led to the generally accepted conclusion that functional non-identity of two rings in GroEL is

* The abbreviation used is: ATPyS, adenosine 5'-3-O-(thio)triphosphate.

† To whom correspondence should be addressed. Tel.: 972-8-342-152; Fax: 972-8-344-105; E-mail: csgirsh@weizmann.weizmann.ac.il.
ATP hydrolysis by GroEL was also tested in the presence of ATPase sites (corresponding to one of the two rings) shows a ticatorly does not. This result is consistent with the ability of ADP, which coincides with that for ATP hydrolysis by GroEL in the presence of GroES (nH = 3.1), that some structural rearrangements of the intersubunit interactions in GroEL 14-mer should be induced by binding of substrate molecules. Moreover, these ATP-induced rearrangements in GroEL were detected experimentally (17, 21, 26). Hence it seems likely that a positively cooperative binding and/or hydrolysis of ATP by either of the two rings in GroEL induces some changes in intersubunit interactions that are transmitted to the other ring and diminish its initially equivalent affinity for ADP (so-called negative cooperativity or half-of-the-sites reactivity (27, 28)). In other words, because of the interaction with ATP two initially identical rings in GroEL become structurally and functionally different.

CONCLUSION

The following scenario can be suggested, which describes how two identical rings of GroEL become non-identical. An initial cause of this change is the interaction of GroEL with ATP, which occurs with a positive cooperativity within the rings and with a negative cooperativity between them. The binding and/or hydrolysis of ATP by GroEL induces some structural changes making the rings different. Either of them, which are

FIG. 1. Effect of adenine nucleotides (ADP and ATPγS) and GroES on the initial rate of hydrolysis of [γ-32P]ATP by GroEL. Inset shows the Dixon plot, where 1/V is the reciprocal initial rate of ATP hydrolysis.

FIG. 2. The ATP concentration dependence of interaction of [3H]GroES with GroEL. Inset shows the Hill plot. Note that the maximal yield of the GroES-GroEL complex, corresponding to equimolar binding of GroES 7-mer to GroEL 14-mer, is observed, also, at the increase of ATP concentration up to 5 mM (in the sample and in a sucrose gradient) and does not change if centrifugation is carried out at 25 °C.
occupied first with ATP in a positively cooperative manner, binds GroES. Due to the inter-ring negative cooperativity, the ATP-binding sites of the other ring show the depressed affinity for adenine nucleotides and GroES. Thus, an asymmetric binding of GroES to GroEL can be a consequence of non-identity of two stacked rings in GroEL, which arises from its interaction with ATP.

REFERENCES
1. Deleted in proof
2. Bochkareva, E. S., Lissin, N. M. & Girshovich, A. S. (1988) Nature 336, 254-257
3. Ellis, R. J. & van der Vies, S. M. (1991) Annu. Rev. Biochem. 60, 321-347
4. Getting, M. J. & Sambrook, J. (1992) Nature 355, 33-43
5. Georgopoulos, C. & Welch, W. J. (1993) Annu. Rev. Cell Biol. 9, 601-644
6. Hendrick, J. P. & Hartl, F-U. (1993) Annu. Rev. Biochem. 62, 349-384
7. Becker, J. & Craig, E. A. (1994) Eur J. Biochem. 216, 11-23
8. Hendrick, J. P. & Hartl, F-U. (1993) Annu. Rev. Cell Biol. 9, 601-634
9. Bochkareva, E. S., Horovitz, A. & Girshovich, A. S. (1994) J. Biol. Chem. 269, 44-46
10. Chandrasekhar, G. H., Tilly, K., Woolford, C., Hendrix, R. & Georgopoulos, C. (1988) J. Biol. Chem. 261, 12414-12419
11. Vitanen, P. V., Lubben, T. N., Reed, J., Goloubinoff, P., O'Keefe, D. P. & Lorimer, G. H. (1990) Biochemistry 29, 5665-5671
12. Tolk, M. J., Vitanen, P. V. & Lorimer, G. H. (1993) Biochemistry 32, 8660-8667
13. Gray, T. E. & Fersht, A. R. (1991) FEBS Lett. 252, 254-258
14. Saibil, H. R., Zheng, D., Roseman, A. M., Hunter, A. S., Watson, H. M. & Chen, J. (1995) Curr. Biol. 5, 925-933

20. Ishii, N., Taguchi, H., Sumi, M. & Yoshida, M. (1992) FEBS Lett. 299, 169-174
21. Horovitz, A., Bochkareva, E. S., Kovalenko, O. A. & Girshovich, A. S. (1993) J. Mol. Biol. 231, 56-64
22. Girshovich, A. S., Bochkareva, E. S. & Ovchinnikov, Y. A. (1981) J. Mol. Biol. 151, 229-243
23. Jackson, G. S., Staniforth, R. A., Halseall, D. J., Atkinson, T., Holbrook, J. J., Clarke, A. R. & Burston, S. G. (1993) Biochemistry 32, 2554-2563
24. Masts, J., Wyman, J. & Changeux, J.-P. (1965) J. Mol. Biol. 12, 81-118
25. Koshland, D. E., Jr., Nemethy, G. & Filmer, D. (1966) Biochemistry 5, 805-835
26. Benneys, F. & Ganen, A. A. (1992) J. Biol. Chem. 267, 1657-1664
27. Levitakis, A. & Koshland, D. E., Jr. (1969) FEBS Symp. 19, 267-270
28. Long, C. W., Levitakis, A. & Koshland, D. E., Jr. (1970) J. Biol. Chem. 245, 82-87