Interaction of 4,4′-Dithiodipyridine with Cys458 Triggers Disassembly of GroEL*

(Received for publication, March 16, 1999, and in revised form, April 28, 1999)

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Chaperonin GroEL, consisting of two seven-subunit rings stacked back-to-back, is disassembled by interaction of 4,4′-dithiodipyridine (DTP) with Cys458 located close to the intersubunit contacts within and between the rings. The thiol group of Cys458 is inaccessible to the probe being buried into the pocket locked by segment Asn477–Asn487. Flexibility of this segment is proposed to induce the “open” state of the pocket and accommodate the bulky probe inside so that the consequential irreversible shifts in the pocket constituents disassemble GroEL. This scheme is supported by the finding that DTP-induced disassembly of GroEL is facilitated by ATP, which specifically stimulates a local shift of the segment Asn477–Asn487 into solution.

The Escherichia coli heat-shock protein GroEL belongs to the Hsp60 (cpn60, chaperonin) family of molecular chaperones mediating the correct folding and assembly of newly synthesized proteins (1, 2). The crystal structure of GroEL (3, 4) shows a porous cylinder (137 Å in diameter and 146 Å in height) of 14 subunits made of two heptameric rings stacked back-to-back. Stability of the GroEL assembly is determined primarily by the equatorial domains, which provide most of the side-side contacts between subunits within the rings and all of the contacts across the equatorial plane that hold the two rings together (5). We report here that attack of thiolic probe DTP at the Cys458 triggers disassembly of GroEL via irreversible stepwise and local conformational shifts.

EXPERIMENTAL PROCEDURES

Wild type and mutant GroELs were prepared as described by us earlier (6–8). AMPPNP and DTP were obtained from Sigma. Disassembly of GroEL was started by mixing 5 μl of 1.25 mM GroEL oligomers (0.052 mM cysteine residues) in the buffer, containing 45 mM Tris-HCl pH 7.6, 120 mM KCl, and 0.15 mM EDTA, with 2.5 μl of 15 mM DTP in 10% acetonitrile. After incubation for 40 min at 25 °C, the GroEL forms were separated by nondenaturing polyacrylamide gel electrophoresis as described earlier (6, 7). The effect of AMPPNP was tested using the same protocol, except that 1 mM DTP was used and the buffer contained 10 mM MgCl2 (in order to promote the nucleotide binding to GroEL) plus or minus 2 mM AMPPNP. At different time periods of incubation at 25 °C, the reaction was stopped by addition of equal volume of the buffer containing 50 mM dithiothreitol, 50 mM EDTA/Tris, pH 7.5, and 30% sucrose. After electrophoresis and Coomasie Blue staining, the GroEL bands were quantitated by scanning densitometry (model GS-690 Imaging Densitometer, Bio-Rad). Notes: (i) 5% acetonitrile and DTP pretreated with dithiothreitol have no effect on stability of GroEL oligomeric structure (data not shown); (ii) AMPPNP is used instead of ATP/γS (9), because the latter contains the thiol group interacting with DTP.

RESULTS AND DISCUSSION

Each subunit of GroEL contains three cysteines (Cys138, Cys458, and Cys519), which are nonessential for assembly of GroEL, although the replacement of them by serine slightly destabilizes the GroEL oligomeric state (6, 10). All of cysteines react with the relatively small thiol-directed probe N-ethylmaleimide, whereas only two of them are titrated by minute incubation with the larger probe DTP (11). Here we show that longer (0.5–1 h) incubation with DTP (5 mM) leads to a complete disassembly of GroEL (Fig. 1). This result depends evidently on the reaction of DTP with a low reactive thiol and not with other groups because, as reviewed by Brocklehurst (12), DTP possesses essentially absolute specificity for thiols reacting via the disulfide-thiol interchange reaction.

In order to identify the cysteine residue modification of which by DTP results in disassembly of GroEL, all three cysteines in GroEL have been individually replaced by serine and tested. Fig. 1 shows that the Cys138 → Ser and Cys519 → Ser mutants (C138S and C519S, respectively) and also the Cys138 → Ser, Cys458 → Ser double mutant (C138S, C519S) of GroEL are disassembled by DTP similarly to that for the wild type GroEL. In contrast, the Cys458 → Ser mutant (C458S) is stable to the action of DTP. This result demonstrates that disassembly of GroEL is caused by the attack of DTP at the Cys458.

As it follows from the crystal structure of GroEL (3, 4), the Cys458 belongs to equatorial domain of GroEL and is located at the intersubunit interface within the rings and close to the ring-ring contact area. The carboxyl group of Cys458, via hydrogen bond, stabilizes the intersubunit contacts within the rings (3), whereas the thiol group is inaccessible, being buried into the pocket (Fig. 2). The bottom of the pocket incorporates the small helix Pro462–Gly471 (H17, ref. 4), which is the site of interaction between rings. In addition, a hydrogen bond between Glu461, adjacent to this helix, and Arg452 is the major bond stabilizing the ring-ring association. In other words, via Glu461 the underface of the pocket is in direct contact with another ring and affects the stability of the assembly. The walls of the pocket are constructed from the side chains of Glu461, Glu463, Tyr465, and Val466 exposed to the solvent (at the left) and from the β-turn fragment Leu31–Arg40 (at the right). The side chain of Lys39 blocks the entrance into the pocket from the side of its right-handed neighboring subunit within the ring. The β-loop Val5–Thr50 adjacent to the β-turn fragment is the constituent of the interface that provides most of the contacts between equatorial domains within the ring. Finally, the pocket is covered by the segment Asn475–Asn487 projected to solvent from outer surface of GroEL.

Thus, Cys458 is located in a focus of the GroEL quaternary structure, where nearly all structural elements surrounding its thiol group are involved in formation of intersubunit contacts.
within and between the rings and contribute dominantly to stability of the assembly. The thiol group of Cys 458 is buried into the pocket and a priori inaccessible for the bulky external probe. However, the results of the biochemical experiments presented here indicate the accessibility of the thiol group to DTP, raising the possibility of the "open pocket" conformation. It is the primary suggestion that the displacement of the flexible segment Asn475–Asn487, which covers the pocket, can provide the penetration of DTP inside.

Comparison of the overall size of the DTP molecule (~12 Å) with the distances between the thiol group of Cys 458 and the proximal residues (Table I) shows clearly that the interior of the pocket is too small to accommodate the probe. Consequently, the placement of DTP within the pocket will be accompanied by the essential conformational changes in the vicinity of the thiol group. Specifically, the sterical hindrance in this area will lead to the DTP-induced shift of the β-turn fragment Leu31–Arg36 in vicinity of the major intersubunit interface within the ring as green. The figure is prepared from the coordinate file 1GRL and 1DER using the program GRASP (13).

Table I

| Distance | Residue          |
|----------|------------------|
| 4.60     | Pro33-CG         |
| 4.63     | Pro33-CD         |
| 4.32     | Lys34-CB         |
| 4.56     | Lys34-CG         |
| 4.03     | Lys33-NG         |
| 3.44     | Ile454-O         |
| 4.97     | Ile454-CG2       |
| 3.96     | Val455-O         |
| 5.30     | Val455-CG2       |
| 5.18     | Tyr458-OH        |
| 3.59     | Ala460-CB        |
| 4.80     | Ala460-O         |
| 4.65     | Glu483-OE2       |

Fig. 1. Effect of 5 mM DTP on disassembly of the wild type (wt), C138S, C458S, and C519S single mutants and C138S,C519S double mutant of GroEL at 25 °C as determined by nondenaturing polyacrylamide gel electrophoresis. GroELp and GroELm are GroEL particle and monomer, respectively. Note: the monomeric form of the GroEL (C138S) mutant shows a lower mobility compared with that for other mutants (see also Ref. 6).

Fig. 2. Molecular surface of the GroEL area around Cys458 illustrating the thiol-containing pocket. The color scheme shows the surface of the loop Asn475–Asn487 covering the pocket as red, the surface of the helix H17 (Pro462–Gly471) at the bottom involved in the ring-ring interactions as blue, and the surface containing the β-turn fragment Leu31–Arg36 in vicinity of the major intersubunit interface within the ring as green. The figure is prepared from the coordinate file 1GRL and 1DER using the program GRASP (13).

Fig. 3. Effect of 2 mM AMPPNP on disassembly of the wild type GroEL (solid line) and the C138S,C519S double mutant GroEL (dotted line) induced by 1 mM DTP at 25 °C. The absence or presence of AMPPNP are shown by open or closed circles, respectively. The result of incubation of GroEL with the nucleotide alone is indicated by an x.

Based on this scheme, it is interesting to check the effect of ATP on the DTP-induced disassembly of GroEL. According to Boisvert et al. (9), ATP binds to the equatorial domain of GroEL close the thiol-containing pocket. In particular, the distance between the thiol group and the amide of Ala460 donating a hydrogen bond to adenine-N1 of the ATP molecule, is only 3.6 Å (Table I). The binding of ATP5•S (nonhydrolyzable analog of ATP) is shown to induce conformational changes limited by...
equatorial domain of GroEL, specifically including a noticeable shift of the segment Gly\textsuperscript{472}–Gly\textsuperscript{492} (9). Since this segment covers the thiol-containing pocket, we expected that the ATP binding will make the thiol group more accessible to the chemical probe. To examine this version, we have tested the effect of AMPPNP (nonhydrolyzable analog of ATP similar to ATP\textsubscript{γS}) on the DTP-induced disassembly of GroEL. As seen in Fig. 3, the action of the nucleotide is crucial: under the described conditions, the 40-min incubation with 1 mM DTP alone shows a slight disassembling effect (~10%), whereas the addition of AMPPNP results in ~80–90% disassembly of GroEL (the wild type and double mutant GroEL are tested). An ability of the adenine nucleotide to facilitate the DTP-promoted disassembly of GroEL argues in favor of the proposed disassembly mechanism.

To summarize, our data show that disassembly of GroEL is caused by the attack of DTP at Cys\textsuperscript{458}, the thiol group of which \textit{a priori} seems sterically inaccessible to the external probe. From here, the requirements of flexibility in the constituents surrounding the thiol group and ability of them to move outward are the key determinants for the DTP attack to take a place. A well known high internal cooperativity in the GroEL assembly makes these motions, although local, to occur in a concerted manner for all of the GroEL subunits within and between the rings with dramatic affecting the overall GroEL assembly.

\textit{Note Added in Proof—}The related paper of E. A. Jai and P. M. Horowitz (1999) \textit{J. Protein Chem.} \textbf{18}, 387–396 has been published recently.

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