On the distribution of ligands within the asymmetric chaperonin complex, $GroEL_{14}\cdot ADP_7\cdot GroES_7$

Alexander S. Girshovich[a,*], Elena S. Bochkareva[a], Matthew J. Todd[b], George H. Lorimer[b]

[a]*Department of Biochemistry, Weizmann Institute of Science, Rehovot, 76100, Israel*
[b]*Central Research and Development Department, Dupont Co., Experimental Station, Wilmington, DE 19880-0402, USA*

Received 7 April 1995; revised version received 27 April 1995

Abstract In the presence of MgATP or MgADP the *E. coli* chaperonin proteins, GroEL and GroES, form a stable asymmetric complex with a stoichiometry of two $GroEL_7$:one $GroES_7$: seven MgADP. The distribution of the ligands between the two heptameric GroEL rings is crucial to our understanding of the mechanism of chaperonin-assisted folding, being either *cis* (i.e. $[GroEL_7\cdot MgADP_7\cdot GroES_7]$-$[GroEL_7]$) or *trans* (i.e. $[GroEL_7\cdot MgADP_7]$-$[GroEL_7\cdot GroES_7]$. On the basis of cross-linking experiments with 8-azido-ATP and the heterobifunctional reagent, *N*-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), it was suggested that GroES and MgADP are bound to the same GroEL ring which resists proteinase K digestion [Nature 366 (1993) 228–233]. However, we find that the SPDP-promoted cross linking of GroES and GroEL occurs in the absence of Mg^{2+}, ADP or ATP, which are required for the formation of the asymmetric complex. Cross-linking is shown to occur only when the SPDP-modified GroES is co-precipitated with GroEL by trichloracetic acid. Furthermore, there are structural grounds for questioning whether SPDP can crosslink, in a physiologically relevant manner, an amino group of GroES with any of the cysteinyl groups of GroEL.

Key words: Chaperonin; GroEL; GroES; Crosslinking

1. Introduction

The *E. coli* chaperonin proteins GroEL and GroES form asymmetric and symmetric complexes in the presence of MgADP or MgATP [1–7]. GroEL consists of two rings of seven subunits each [8] while GroES is a single ring of 7 subunits [9]. The asymmetric complex is particularly stable, consisting of a double-ringed $[GroEL_7]$-$[GroEL_7]$ particle to which a single ring of $GroES_7$ and seven molecules of MgADP are tightly bound [10,11]. There may be additional Mg^{2+} ions, but their role is not understood at present [12]. The bound ligands $GroES_7$ and MgADP only slowly exchange ($t_{1/2}\sim 5$ h), although a single round of ATP hydrolysis at the seven remaining active sites on GroEL is sufficient to discharge both ligands [13].

A question pertinent to the mechanism of chaperonin assisted folding concerns the distribution of ligands in the asymmetric complex. Although more complex distributions involving nucleotide binding to seven sites on both rings of GroEL have not yet been formally ruled out, a more likely scenario is that all seven MgADP are tightly bound to the same GroEL ring. Positively cooperative interactions between the subunits of one ring and negatively cooperative interactions between the two rings would favor the latter [14]. Given this asymmetric distribution of the nucleotide, it follows that the single GroES ring in the asymmetric complex either binds *cis*, $[GroEL_7\cdot MgADP_7\cdot GroES_7]$ -$[GroEL_7]$ (i.e. to the same ring to which the MgADP is bound) or *trans*, $[GroEL_7\cdot MgADP_7]$-$[GroEL_7\cdot GroES_7]$ (i.e. to the distal GroEL ring).

Recently Martin et al. [15] reported experiments (Figures 3 and 4 of their paper) which purport to resolve this issue in favour of the *cis* configuration. Their conclusion is based on specifically cross-linking the ligands, GroES and 8-azido-ADP to one of the two rings of GroEL. Digestion of the asymmetric complex by proteinase K is believed to clip 16 amino acids from the C-terminus of the GroEL subunits from one or other of the two rings [2,15], and can thus be used to distinguish one GroEL ring from the other. Martin et al. [15] reported that both crosslinked ligands are bound to the GroEL ring which does not undergo digestion with proteinase K and that, consequently, the ligands are distributed in the *cis* configuration. In order to crosslink ^{3}H-labeled GroES to GroEL in the asymmetric complex, use was made of the heterobifunctional reagent, *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP). This reagent might be expected to cross link one or more amino groups of GroES, which has no cysteinyl residues, with one (or more) of the three cysteinyl residues of GroEL, if the reactive amino group(s) on GroES come close enough to the cysteines of GroEL in the asymmetric complex. For reasons given below, we believe that the SPDP crosslinking experiment is flawed and that the distribution of the ligands in the asymmetric complex remains unresolved.

2. Experimental

GroEL and GroES were purified by methods that have been described elsewhere [10,11,16]. ^{3}H-GroES was prepared in Rehovot by reductive methylation as previously described [14]. Radiochemically-pure [^{35}S]GroES was prepared in Wilmington by metabolic labelling as previously described [13]. SPDP was obtained from Pierce Chem. Co. (Rockford, Illinois). Other details are described in the figure legends.

3. Results and discussion

Structural considerations prompted us to re-examine the SPDP-promoted crosslinking of GroES to GroEL. This analysis was conducted independently in Rehovot by ASG and ESB and in Wilmington by MJT and GHL. When following the procedures described by Martin et al. [15], we also observe the formation of a novel species ($M_r\sim 70$ kDa), as a result of SPDP-

*Corresponding author. Fax: (972) (8) 344 105.
E-mail: csgirsh@weizmann.weizmann.ac.il

SSDI 0014-5793(95)00479-3

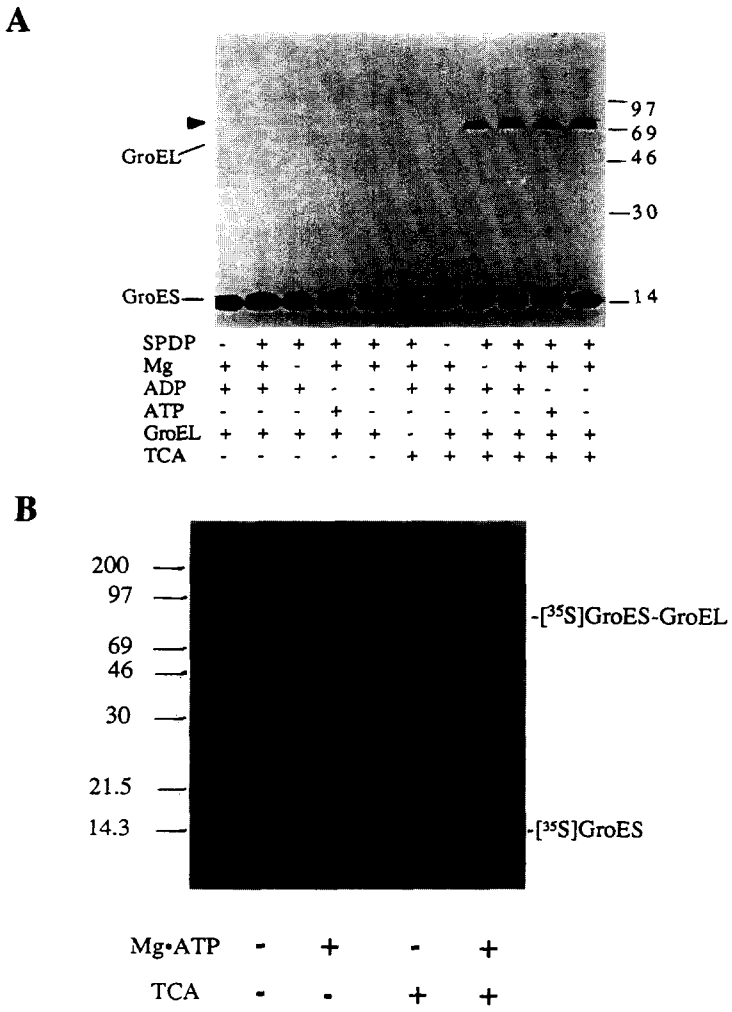

Fig. 1. Conditions for crosslinking (A) [^{3}H]- and (B) [^{35}S]GroES to GroEL. Positions of the 70 kDa cross-linked species, labeled GroES and molecular mass markers (in kDa) are indicated. The procedures and concentrations of the components and buffers are identical to that published in ref. [15], except 5 mM iodoacetamide was replaced by *N*-ethylmaleimide (NEM) which can also be used to alkylate the thiol groups of GroEL [18] (Fig. 1A). Some experiments (e.g. Fig. 1B) were carried out with iodoacetamide. After treatment with SPDP followed by glycine quench, the radiolabeled GroES was incubated with GroEL in the presence of different additions as indicated below. Then, each sample was divided in two aliquots. One was precipitated with TCA, the pellet was dissolved in SDS-sample buffer containing NEM or iodoacetamide and analyzed by non-reducing SDS-PAGE as in [15]. The other aliquot was treated with alkylating reagent, mixed with SDS-sample buffer and loaded directly on to the gel.

crosslinking, using either chemically modified [15] (Fig. 1A) or metabolically labeled GroES (Fig. 1B). The same species could also be detected by Western blotting using unlabeled GroES (ASG and ESB, unpublished data). In the case of the ^{35}S-labeled GroES, an additional cross-linked species at approximately 190 kDa was observed (Fig. 1B). This presumably contains two or more GroEL subunits. The appearance of cross linked species did not depend upon the presence of ADP, ATP or Mg^{2+} ions, components which are absolutely essential for the formation of the asymmetric complex. We therefore examined the experimental protocol more closely.

The procedure of Martin et al. [15] included precipitation of the proteins with trichloracetic acid prior to SDS-PAGE. In their original description of SPDP, Carlsson et al. [17] noted that, in contrast to aliphatic disulphides, 2-pyridyl disulphides increase their electrophilicity at acidic pH, due to protonation of the ring nitrogen atom (pK 2–3). Thus, crosslinking via thiol-disulfide exchange, can be expected to occur under acidic conditions. Accordingly, we excluded precipitation with trichloracetic acid from the protocol and loaded the samples (mixed with SDS lacking β-mercaptoethanol) directly on to the SDS-PAGE gel. Under such conditions, no crosslinked species were observed. The trichloracetic acid-dependent formation of the 70 kDa cross-linked species, required the presence of only GroEL and SPDP-modified GroES (Fig. 1). Since the 70 kDa product did not form under conditions of specific GroEL-GroES interaction, we conclude that its formation is the result of non-specific crosslinking which only occurs at acidic pH, after denaturation with trichloracetic acid.

Recent crystallographic analysis of GroEL has defined the positions of the three cysteinyl residues C138, C458 and C519 [8]. C458 and C519 are located in the equatorial domain, while C138 is located in the loop connecting the last N-terminal α-helix of the equatorial domain with the first N-terminal α-

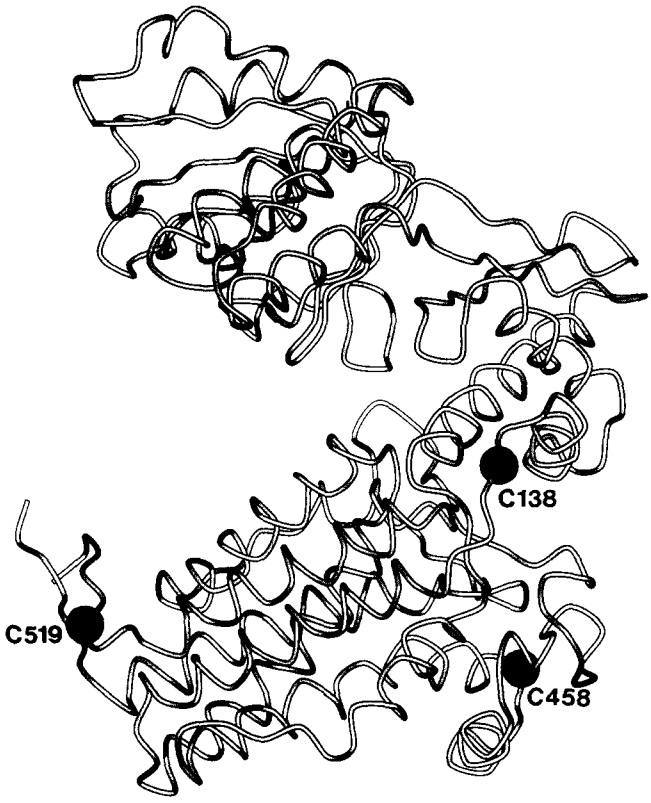

Fig. 2. (A) A C_α-tracing of GroEL showing the location of the three cysteinyl residues within (or in the case of C138, very close to) the equatorial domain. (Courtesy of Kerstin Braig, Yale University).

helix of the intermediary domain (Fig. 2) [8]. Electron microscopy shows that, in forming the asymmetric complex, the apical domain of GroEL undergoes a substantial outward re-orientation by as much as 60°, with the GroES being positioned atop the apical domain at a distance of some 50–60Å from the upper surface of the equatorial domain (Fig. 3) [2–4]. The available evidence suggests that the gross structural re-arrangement is limited to the apical domain, with perhaps some more modest, hinge-like movement of the intermediate domain. It would therefore seem improbable that a hetero-bifunctional cross-linker such as SPDP, which can span a distance of 6.8 Å, could cross-link an amino group on GroES with a cysteinyl group on GroEL at least 50Å distant, as the Martin et al. [15] result implies.

In conclusion, the SPDP-promoted crosslinking of GroEL and GroES is an artefact of trichloracetic acid precipitation. Consequently, the distribution of ligands in the asymmetric complex, whether *cis* or *trans*, remains unresolved.

Acknowledgements: We thank Kerstin Braig, Yale University, for providing us with Fig. 2 and Johannes Buchner, University of Regensburg, for the images necessary to construct Fig. 3.

References

[1] Saibil, H., Zhang, D., Wood, S. and auf der Mauer, A. (1991) Nature 353, 25–26.
[2] Langer, T., Pfeifer, G., Martin, J., Baumeister, W. and Hartl, F.U. (1992) EMBO J. 11, 4757–4765.
[3] Chen, S., Roseman, A.M., Hunter, A.S., Wood, S.P., Burston, S.G., Ranson, N.A., Clarke, A.R. and Saibil, H.R. (1994) Nature 371, 261–264.
[4] Schmidt, M., Rutkat, K., Rachel, R., Pfeifer, G., Jaenicke, R., Viitanen, P.V., Lorimer, G.H. and Buchner, J. (1994) Science 265, 656–659.
[5] Azem, A., Kessel, M. and Goloubinoff, P. (1994) Science 265, 653–656.
[6] Harris, J.R., Plückthun, A. and Zahn, R. (1994) J. Struct. Biol. 112, 216–230.
[7] Llorca, O., Marco, S., Carrascosa, J.L. and Valpuesta, J.M. (1994) FEBS Lett. 345, 181–186.

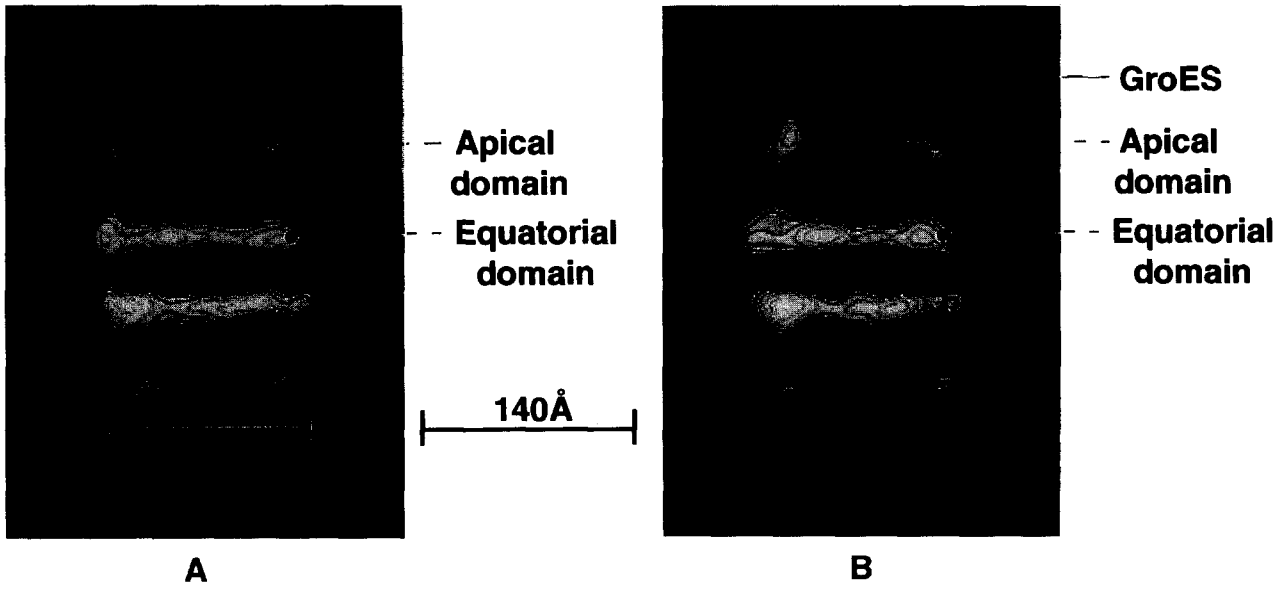

Fig. 3. A diagram derived from the outer contours of sections of (A) GroEL alone and (B) the asymmetric GroEL-GroES complex, showing the outward rotation of the apical domains of GroEL to interact with GroES. This movement places GroES at a distance of >50Å from the upper surface of the equatorial domain of GroEL, where the three cysteinyl residues C138, C458 and C519, are located (see Fig. 2). Since the heterobifunctional cross-linker SPDP can span a distance of 6.8Å, a physiologically meaningful crosslinking of one or more of the amino groups of GroES with any of the three cysteines of GroEL appears improbable. (This figure is adapted from Fig. 2 of ref. [4]).

[8] Braig, K., Otwinowski, Z., Hedge, R., Boisvert, D.C., Joachimiak, A., Horwich, A.L. and Sigler, P.B. (1994) Nature 371, 578–586.
[9] Weaver, A.J., Landry, S.J. and Deisenhofer, J. (1993) Biophys. J. 64, Abstract No. Th-PM-G7.
[10] Bochkareva, E.S., Lissin, N.M., Flynn, G.C., Rothman, J.E., and Girshovich, A.S. (1992) J. Biol. Chem. 267, 6796–6800.
[11] Todd, M.J., Viitanen, P.V. and Lorimer, G.H. (1993) Biochemistry 32, 8560–8567.
[12] Azem, A., Diamond, S. and Goloubinoff, P. (1994) Biochemistry 33, 6671–6675.
[13] Todd, M.J., Viitanen, P.V. and Lorimer, G.H. (1994) Science 265, 659–666.
[14] Bochkareva, E.S. and Girshovich, A.S. (1994) J. Biol. Chem. 269, 23869–23871.
[15] Martin, J., Mayhew, M., Langer, T. and Hartl, F.U. (1993) Nature 366, 228–233.
[16] Horovitz, A., Bochkareva, E.S., Kovalenko, O. and Girshovich, A.S. (1993) J. Mol. Biol. 231, 58–64.
[17] Carlsson, J.A., Drevin, H. and Axen, R. (1978) Biochem. J. 173, 723–737.
[18] Mendoza, J.A. and Horowitz, P.M. (1992) J. Protein Chem. 11, 589–594.