High resolution surface structure of *E. coli* GroES oligomer by atomic force microscopy

Jianxun Mou, D.M. Czajkowsky, Sitong (Jun) Sheng, Rouya Ho, Zhifeng Shao*

Department of Molecular Physiology and Biological Physics, University of Virginia School of Medicine, Box 449, Charlottesville, VA 22908, USA

Received 27 December 1995; revised version received 22 January 1996

Abstract Using atomic force microscopy (AFM) in aqueous solution, we show that the surface structure of the oligomeric GroES can be obtained up to 10 Å resolution. The seven subunits of the heptamer were well resolved without image averaging. The overall dimension of the GroES heptamer was 8.4±0.4 nm in diameter and 3.0±0.3 nm high. However, the AFM images further suggest that there is a central protrusion of 0.8±0.2 nm high and 4.5±0.4 nm in diameter on one side of GroES which displays a profound seven-fold symmetry. It was found that GroEL could not bind to the adsorbed GroES in the presence of AMP-PNP and Mg^{2+}, suggesting that the side of GroES with the central protrusion faces away from the GroEL lumen, because only one side of GroES was observed under these conditions. Based on the results from both electron and atomic force microscopy, a surface model for the GroES is proposed.

Key words: Atomic force microscopy; GroES; GroEL; Resolution

1. Introduction

Escherichia coli GroES, a heat shock protein, is a member of the chaperonin 10 (cpn 10) family, and is encoded for by a common operon with another chaperonin GroEL [1,2]. Both GroEL and GroES have been extensively studied [3–13] and are found involved in assisting the folding of other proteins both in vivo and in vitro [14–17]. Each monomer of GroES has a nominal molecular weight of 10 kDa [2], and the GroES is normally found in an oligomeric configuration of seven subunits [2,13]. Although no specific functions have been identified with the GroES heptamer alone, it has been firmly established with both electron microscopy (EM) and biochemical methods that, in the presence of ATP or ADP, the GroES heptamer can form a stable complex with the GroEL tetradecamer [5,8,18–20]. Upon the binding of GroES, the rate of ATP hydrolysis of GroEL is strongly affected [4,6,7,9,12,21], but the mechanism has not been well understood [14–17]. However, it is convincingly demonstrated that the folding of non-native state proteins by GroEL requires the participation of GroES and ATP under 'non-permissive' conditions, under which spontaneous folding could not occur [9,21,22]. Therefore, GroES plays a critical role in the folding process in vivo.

Although the structure of the GroEL tetradecamer has been solved to 2.8 Å resolution [23], the structure of GroES has only been elucidated by EM with negatively stained specimens [2,18]. Even with two-dimensional crystals, the GroES heptamer was only resolved as a ring of 7–8 nm, and the individual subunits were not discernible [18]. Because of the variable conformations observed by EM, it was even suggested that GroES could have a flexible structure or a symmetry other than the seven-fold [18]. Since the atomic force microscopy (AFM) has been shown to be capable of obtaining high resolution surface structures of several oligomeric bacterial proteins (for recent reviews, see [24–26]), we have applied this method to determine the surface structure of GroES under aqueous solutions. We show that the subunit structure can be clearly resolved in the AFM images without image processing/averaging. Surface structures beyond the subunits were also resolved, demonstrating a surface resolution of 10 Å or so, which is much higher than that from EM images. In combination with the results from EM, a model for the GroES is also proposed.

2. Materials and methods

E. coli GroES and GroEL were obtained from Sigma Chemicals (St. Louis, MO), which were purified from overexpression in *E. coli*. The purity of both GroES and GroEL was better than 95% (SDS-PAGE). The lyophilized powder was reconstituted to 0.25 mg/ml for GroES (25 mM Tris, 75 mM KCl, 0.5 mM DTT and 1.25% trehalose, pH 7.5) and 1 mg/ml for GroEL (50 mM Tris, 150 mM KCl, 10 mM $MgCl_2$, 1 mM DTT and 2.5% trehalose, pH 7.5) with deionized water. All chemicals used were reagent grade, and 18 MΩ deionized water was used to prepare the solutions. For structural studies, 10 μl of GroES solution was either directly applied to a freshly cleaved mica surface, or injected into a solution (~200 μl) of 1% ammonium molybate and 0.2% PEG1450 at pH 6.0 covering a mica surface. After incubation at room temperature for 5–30 min, the specimen was washed for several times with deionized water to remove the excess GroES. A uniform coverage of the mica surface was normally achieved at this point with both methods. To further stabilize the specimen for high resolution AFM imaging, the specimen was fixed with 2% glutaraldehyde for 30 s. To avoid any possible structural alteration or damage, the specimen was never dried. For the study of GroEL binding, GroES was first allowed to adsorb to a mica surface with the above methods. After the specimen was examined with the AFM, the excess GroES was removed with extensive washing. GroEL (~0.3 μg) was then injected into the solution (~200 μl, 10 mM $MgCl_2$, 2.5 mM AMP-PNP, 10 mM KCl and 10 mM Tris at pH 8.0) covering the specimen surface. After incubation at room temperature for 30 min, the excess GroEL was washed off with the same buffer, and the specimen was subsequently fixed with 2% glutaraldehyde. According to the published results [8,18–20,27], the binding occupancy should be nearly 100% under these conditions, which was confirmed by EM in our laboratory as well (data not shown). All AFM images were obtained with a NanoScope II AFM (Digital Instruments, Santa Barbara, CA) in a home made fluid cell with the contact mode at room temperature. Commercial cantilevers with oxide sharpened tips and a nominal spring constant of 0.06 N/m (Digital Instruments, Santa Barbara, CA) were directly used without further processing. The typical scanning speed was 5–14 Hz and the probe force was maintained below 0.5 nN. The piezo scanner was calibrated with a grid of known dimensions. All images shown are the original data without processing.

*Corresponding author. Fax: (1) (804) 982-1616.

SSDI S0014-5793(96)00112-3

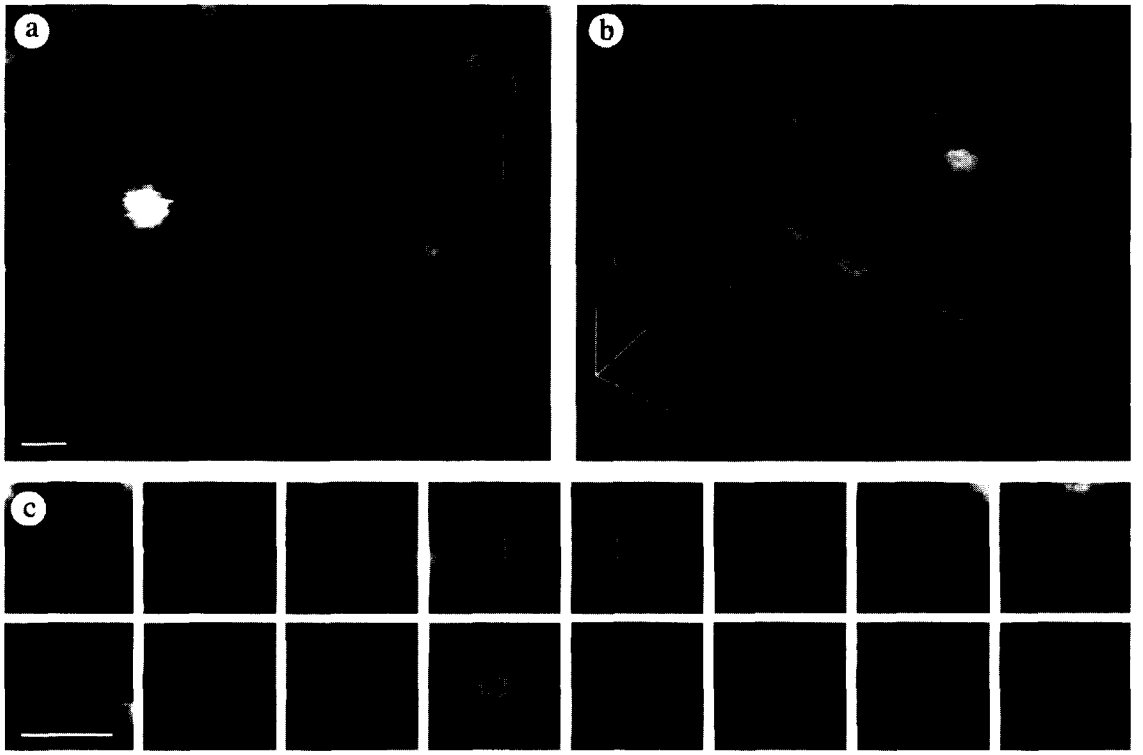

Fig. 1. AFM images of GroES oligomers adsorbed on mica surface and subsequently fixed with glutaraldehyde. These images were obtained in deionized water, and the adsorbed GroES was fairly stable that repeated scans at sub-nN probe forces did not cause observable structural changes or damages. These results can be reproducibly obtained following the procedure discussed in the text. (a) In this image, each GroES heptamer is clearly resolved without any image processing. The specimen is closely packed, but without apparent long range order. The seven-fold symmetry is seen more profoundly at the center of the heptamer, with a small central depression. The height of these molecules, measured from the edge of several aggregation patches, is 3.0 ± 0.3 nm. The overall diameter is 8.4 ± 0.4 nm. The central 'pore' has a nominal diameter of 1.1 ± 0.3 nm, surrounded with a 0.8 ± 0.2 nm high and 4.5 ± 0.4 nm diameter protrusion. It can be seen that the protrusion is formed with seven 'domains' of $1.7 \times 0.8 \times 0.8$ nm, encompassing a volume of 1100 Å^3. This volume corresponds to a mol.wt. of 460 Da, if 2.4 Å^3 is used per Da. The less than 10 Å width of these domains directly measured from the original image suggests that the resolution is quite high. However, since the specimen was chemically fixed, the extent of structural alteration has not been assessed to this resolution with other methods, due to the limited surface resolution achievable with other techniques. A comparison with that of X-ray diffraction will help to establish this limit. If indeed these are the true surface corrugations, the AFM will be an effective method for structural studies at a resolution higher than that of EM for similar specimens. At 10 Å resolution, useful surface features regarding to the function of many macromolecules may be revealed. (b) A surface plot at a smaller scale. In this image, each GroES is clearly shown as a somewhat domed surface with a small 'crown' at the center. (c) 16 representative individual GroES oligomers. All scale bars = 10 nm.

3. Results and discussion

Incubation at room temperature, GroES was found to adsorb to the freshly cleaved mica surface strongly, and after these molecules were adsorbed, they could not be washed off with deionized water. However, it was more difficult to achieve a uniform coverage on the mica surface, which would be required for high resolution AFM [26]. As we found, in addition to the incubation time and buffer conditions, the concentration of GroES also had profound effect on the surface coverage. With these experiments, a concentration of 10–100 μg/ml was found most appropriate. However, imaging the adsorbed GroES in the buffers or deionized water with AFM only achieved a fairly low resolution, even with perfectly uniform, closely packed samples. In these AFM images, individual GroES oligomers could be resolved, but the individual subunits were not discernible, indicating a resolution in the range of 4–5 nm. When the adsorbed GroES was briefly fixed with glutaraldehyde, the resolution of the AFM images was significantly improved. With a good quality AFM tip, not only could the seven-fold symmetry be resolved without image processing, but surface features beyond the subunits also became discernible. A typical AFM image of GroES heptamer is shown in Fig. 1a. It should be emphasized that the image shown is the original data without any additional processing. It is seen that the specimen was nearly closely packed, but no long range order was found, indicating that two-dimensional crystals were not formed under these conditions. Although the resolution in AFM is not well defined and is strongly dependent on the nature of the specimen [26,28], the smallest surface features resolvable in these images suggest a spatial resolution of 10 Å or better. For example, the end of the 'spike' near the central channel has a width of 8–10 Å, representing one of the highest resolution AFM images achieved so far [28–30], which is significantly higher than that achieved with EM of two-dimensional crystals [18]. Based on these images, the GroES heptamer can be described as having a dome surface with a central protrusion (crown), which can be better appreciated in a surface plot (see Fig. 1b). Measurements show that the GroES has an outer diameter of 8.4 ± 0.4 nm, with a central protrusion (crown) of 0.8 ± 0.2 nm high. It is seen that this central protrusion is formed from seven elongated domains with a maximum diameter of 4.5 ± 0.4 nm, and a central depression of 1.1 ± 0.3 nm, perhaps representing a

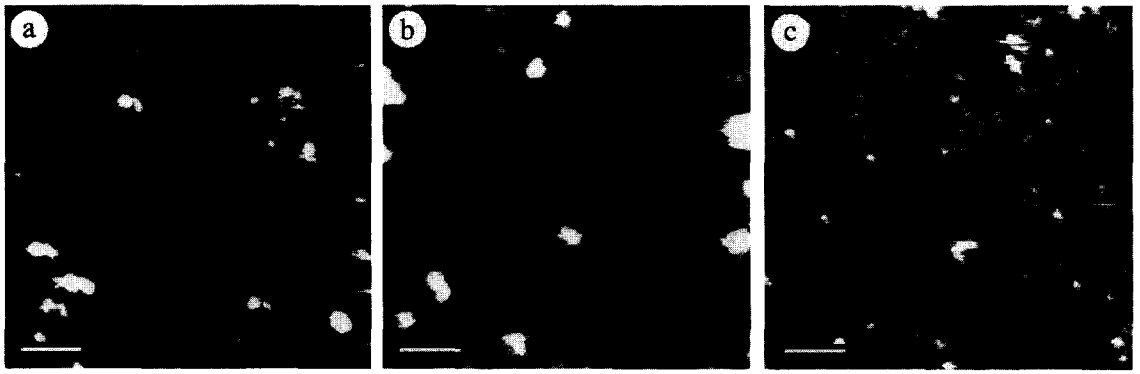

Fig. 2. The AFM was also used to determine whether GroEL could bind to the adsorbed GroES in the presence of 2.5 mM AMP-PNP and 10 mM $MgCl_2$. (a) A uniform coverage of GroES is seen in this image. The size of each GroES is about 8–9 nm, consistent with other measurements. With direct adsorption, only the side with the protrusion was found facing up when the specimens were fixed *after* adsorption. (b) After incubation with GroEL in AMP-PNP and Mg^{2+} for 30 min with GroES already adsorbed to a mica surface without fixation, no substantial GroEL binding was found, even though in solution, most GroES was bound with GroEL under these conditions after an incubation as short as 15 min, as shown by EM. Some GroES was lost due to the repeated washing with various buffers. Fixation was performed after GroEL incubation. (c) If the same amount of GroEL was allowed to adsorb to a mica surface without pre-adsorption of GroES under these conditions, a uniform coverage was formed within 30 min. In this image, most GroEL was found in the up-right orientation. The central channel of the GroEL is about 4.5 nm with an outer diameter of 13–14 nm, essentially the same as that from X-ray diffraction. This image is clearly different from that of GroES with or without fixation. These results seem to suggest that the side of GroES with the central protrusion (crown) is not the side that binds to GroEL, although the effect of the substrate below GroES is uncertain. However, this interpretation would be consistent with the structure deduced from EM, where the concave side of GroES was found facing the lumen of GroEL. Scale bars = 50 nm.

channel. The domains forming the protrusion were estimated with the dimensions of $1.7\times0.8\times0.8$ nm. If 2.4 $Å^3$ is used as the volume required per unit mol.wt., this volume represents a mol.wt. of 460 Da, a very respectable minimum detectable mass for non-crystalline specimens. 16 representative individual GroES oligomers are collected in Fig. 1c to show structural details. A close examination of these images suggests that lateral distortion is more than apparent, perhaps due to the dragging effect of a scanning tip, although true structural heterogeneity for such specimens is also possible because the molecules were not packed into a crystal. Such distortion makes it difficult to apply the method of image averaging, well developed for low contrast EM images. Moreover, the excellent signal-to-noise ratio in the AFM images rendered additional processing unnecessary. From this point of view, a 2D crystal may be more amenable to such treatment [28].

It was also interesting that all AFM images so far obtained with this approach showed essentially the same surface structure, suggesting that GroES was adsorbed with a preferential orientation, unless one is willing to consider that the two sides of the GroES have identical surface structures down to 10 Å scale. To image the other side, we have experimented with different buffer conditions and chemical additives, and so far failed to have the GroES adsorbed in the other orientation.

Based on this structure, it is tempting to speculate that the side of GroES with a central protrusion would be the side that binds to GroEL and the protrusion could fit into the opening of the GroEL channel, because the diameter of the GroEL channel is also about 4.5 nm [23]. However, preliminary experiments seem to suggest otherwise. When GroEL in 2.5 mM AMP-PNP and 10 mM $MgCl_2$ at pH 8.0 was incubated with GroES (before fixation) already adsorbed to a mica surface, no GroEL binding was found after incubation for 30 min at room temperature. The results are shown in Fig. 2. Adsorbed GroES was clearly resolved in Fig. 2a, forming a uniform coverage of the mica surface. The lateral dimension of the adsorbed molecules was consistent with that from the fixed samples (Fig. 1a). In Fig. 2b, we show the same specimen after incubation with GroEL which was subsequently fixed with glutaraldehyde after the excess GroEL was washed away with a buffer containing AMP-PNP and Mg^{2+}. No GroEL was found on the surface and the GroES was still well resolved, although some of the GroES appeared lost due to the repeated exchange of buffers. This is in sharp contrast with Fig. 2c, where the same amount of GroEL was directly incubated with a mica surface without the pre-adsorbed GroES. In this image, the GroEL was resolved with a diameter of 13.2 ± 0.8 nm and a clear central pore. Even the subunits were somewhat discernible, indicating that if there had been any GroEL binding with the adsorbed GroES, they should be resolvable. Since with EM, most GroES were found bound with GroEL under these conditions in solution, this result seems to suggest that the side of GroES with the central protrusion (crown) is not the side containing the high binding sites with GroEL. However, we cannot entirely rule out the possibility that the substrate had some inhibiting effect on the binding of GroEL. We may also point out that this interpretation appears consistent with the structure found by EM. In

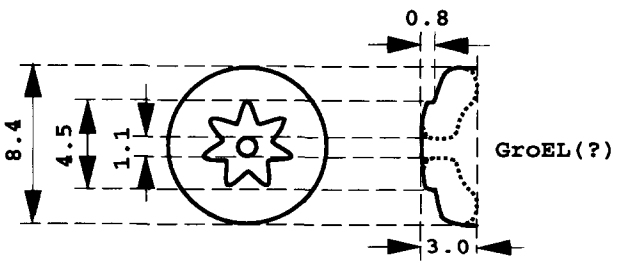

Fig. 3. Combining the observation from EM that the side of GroES facing the lumen of GroEL was concave and the result from AFM that the surface with a crowned dome did not bind GroEL, a surface model of GroES can be described. All relevant dimensions are included (in nm). The central channel is speculative, and the indicated GroEL binding side needs to be confirmed with other methods.

most EM images of GroES/GroEL complexes, the GroES appeared to be a domed cap at the end of GroEL, indicating that the surface of GroES facing the GroEL lumen is concave [8,17,19]. Therefore, the observed GroES surface with the AFM should be the surface facing away from the GroEL. If the structure of GroES remains the same when dissociated from GroEL, a surface model of GroES can be proposed based on these results which is summarized in Fig. 3. In this model, the central channel is only speculative, and the surface contour of the GroEL binding side is inferred from EM results.

In summary, we have presented the surface structure of GroES with details down to about 10 Å level, the highest resolution so far published with GroES. The resolution of the central protrusion (crown) in the GroES heptamer was not quite expected for the AFM, and intriguing in view of the small size of each monomer. However, the final confirmation of these fine surface structures must await the availability of the X-ray structure [31]. Only when compared with the X-ray model, can we assess the effect of chemical fixation and compression, and determine to what extent the surface structure is faithfully reproduced by the AFM. If these structural details are validated, the AFM will be a quite generally applicable method for structural determination at high resolution when crosslinkers, not necessarily glutaraldehyde, are introduced. Such high surface resolution may be sufficient for the identification of specific features in determining the orientation of certain macromolecules in a complex or on a membrane surface. We also demonstrated that the AFM can be a useful method for studying the interaction of other macromolecules to those adsorbed to the surface in situ, although the effect of the substrate in such studies needs further evaluation. With this method, it is suggested that the high affinity binding sites for GroEL appear to be on the concave side of GroES, a conclusion that needs to be confirmed by GroEL/GroES co-crystals.

4. Note added in proof

After this manuscript was submitted, the crystal structure of GroES oligomer was published [32]. Preliminary examination indicates that the surface structure by AFM is nearly identical to that of the X-ray model in both dimensions and morphology.

Acknowledgements: We thank Drs. A.V. and A.P. Somlyo for their continued interest in AFM. The work was supported by grants from National Institutes of Health (PO1-HL48807 and RO1-RR07720) and National Science Foundation (BIR-9115655).

References

[1] Tilly, K., Murialdo, H. and Georgeopoulos, C. (1981) Proc. Natl. Acad. Sci. USA 78, 1629–1633.
[2] Chandrasekhar, G.N., Tilly, K., Woolford, C., Hendrix, R. and Georgopoulos, C. (1986) J. Biol. Chem. 261, 12414–12419.
[3] Martin, J., Langer, T., Boteva, R., Schramel., A., Horwich, A. and Hartl, F.-U. (1991) Nature 352, 36–42.
[4] Jackson, G.S., Staniforth, R.A., Halsall, D.J., Atkinson, T., Holbrook, J.J., Clarke, A.R. and Burston, S.G. (1993) Biochemistry 32, 2554–2563.
[5] Azem, A., Kessel, M. and Goloubinoff, P. (1994) Science 265, 653–656.
[6] Todd, M.J., Viitanen, P.V. and Lorimer, G.H. (1994) Science 265, 659–666.
[7] Gray, T.E. and Fersht, A.R. (1991) FEBS Lett. 292, 254–258.
[8] Langer, T., Pfeifer, G., Martin, J., Baumeister, W. and Hartl, F.-U. (1992) EMBO J. 11, 4757–4756.
[9] Schmidt, M., Buchner, J., Todd, M.J., Lorimer, G.H. and Viitanen, P.V. (1994) J. Biol. Chem. 269, 10304–10311.
[10] Kawata, Y., Nosaka, K., Hongo, K., Mizobata, T. and Nagai, J. (1994) FEBS Lett. 345, 229–232.
[11] Crouy-Chanel, A., Yaagoubi, A.E., Kohiyama, M. and Richarme, G. (1995) J. Biol. Chem. 270, 10571–10575.
[12] Landry, S., Zeilstra-Ryalls, J., Fayet, O., Georgeopoulos, C. and Gierasch, L.M. (1993) Nature 364, 255–258.
[13] Zondlo, J., Fisher, K.E., Lin, Z., Ducote, K.R. and Eisenstein, E. (1995) Biochemistry 34, 10334–10339.
[14] Hendricks, J.A. and Hartl, F.-U. (1993) Annu. Rev. Biochem. 62, 349–384.
[15] Lorimer, G.H. (1994) Structure 2, 1125–1128.
[16] Landry, S.J. and Gierasch, L.M. (1994) Annu. Rev. Biophys. Biomol. Struct. 23, 645–669.
[17] Hartl, F.U. and Martin, J. (1995) Curr. Opin. Struct. Biol. 5, 92–102.
[18] Harris, J.R., Pluckthun, A. and Zahn, R. (1994) J. Struct. Biol. 112, 216–230.
[19] 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.
[20] Schmidt, M., Rutkat, K., Rachel, R., Pfeifer, G., Jaenicke, R., Viitanen, P., Lorimer, G. and Buchner, J. (1994) Science 265, 656–659.
[21] Todd, M.J., Viitanen, P.V. and Lorimer, G.H. (1993) Biochemistry 32, 8560–8567.
[22] Bochkareva, E.S. and Girshovich, A.S. (1992) J. Biol. Chem. 267, 25672–25675.
[23] Braig, K., Otwinowski, Z., Hegde, R., Boisvert, D.C., Joachimiak, A., Horwich, A.L. and Sigler, P.B. (1994) Nature 371, 578–586.
[24] Hansma, H.G. and Hoh, J. (1994) Annu. Rev. Biophys. Biomol. Struct. 23, 115–128.
[25] Bustamante, C., Erie, D. and Keller, D. (1994) Curr. Opin. Struct. Biol. 4, 750–760.
[26] Shao, Z. and Yang, J. (1995) Q. Rev. Biophys. 28, 195–251.
[27] Engel, A., Hayer-Hartl, M.K., Goldie, K.N., Pfeifer, G., Hegerl, R., Muller, S., da Silva, A.C.R., Baumeister, W. and Hartl, F.U. (1995) Science 269, 832–836.
[28] Schabert, F. and Engel, A. (1994) Biophys. J. 67, 2394–2403.
[29] Mou, J., Yang, J. and Shao, Z. (1995) J. Mol. Biol. 248, 507–512.
[30] Yang, J., Mou, J. and Shao, Z. (1994) FEBS Lett. 338, 89–92.
[31] Weaver, A.J., Landry, S.J. and Deisenhofer, J. (1993) Biophys. J. 64, A350.
[32] Hunt, J.F., Weaver, A.J., Landry, S.J., Gierasch, L. and Deisenhofer, J. (1996) Nature 379, 37–45.