Mapping the Transition State of the Allosteric Pathway of GroEL by Protein Engineering

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A striking example of an allosteric protein is the chaperonin GroEL which facilitates protein folding in vivo and in vitro in an ATP-regulated manner.[1] GroEL consists of 14 identical subunits that form two stacked heptameric rings with a central cavity.[2] Each subunit consists of three domains: (i) an apical domain that forms the opening of the central cavity (ii) an equatorial domain that contains an ATP binding site and forms all the inter-ring and many of the intra-ring contacts and (iii) an intermediate domain that connects the apical and equatorial domains.[2] GroEL has a weak, K^+-dependent[3] ATPase activity which is cooperative with respect to ATP[4] and K^+ ions.[5] Binding of ATP to GroEL causes large and concerted tertiary conformational changes which lead to vertical opening of the rings and twisting of the apical domains in the plane of the ring.[6] A nested model for cooperativity in ATP hydrolysis by GroEL was recently put forward.[7] In this model, each ring is in equilibrium between a tense (**T**) state, with high affinity for nonfolded proteins and low affinity for ATP, and a relaxed (**R**) state with low affinity for nonfolded proteins and high affinity for ATP.[7,8] Recent cryo-EM studies on the Arg197 → Ala GroEL mutant[9] have provided structural evidence in support of the nested model.[10]

Virtually nothing is known about the kinetic pathway of allosteric transitions in GroEL. Here, it has been studied by combining protein engineering methods with data analysis based on the Brönsted plot. This plot relates, for a series of *i* modified proteins, the rate constants (k_i) of a process with the respective equilibrium constants (K_i) of that process, as follows:

$$\log k_i = \log(\mathrm{A}) + \beta \log K_i \qquad (1)$$

where log(A) is a constant and β is a measure of how close the transition state of the process is to either the reactants ($\beta = 0$) or the products ($\beta = 1$). For example, by measuring rate and equilibrium constants of allosteric transitions, one can determine, using Brönsted plots, whether the transition state of the reaction is **T**-like or **R**-like. Such an approach was previously shown to be useful in the study of protein-folding reactions and enzyme catalysis.[11]

Recently, we introduced the mutation Phe44 → Trp into GroEL (which has no tryptophan residues) to follow the binding of ATP and the resulting conformational changes by monitoring time-resolved changes in fluorescence.[12] In the present study, the mutation Phe44 → Trp was introduced[13] as a probe into a series of GroEL single mutants Asp196 → Ala, Arg197 → Ala, Arg501 → Ala, and Thr522 → Ala, each with altered allosteric properties as determined by previous steady-state kinetic measurements.[9,14] These mutants were chosen so that (i) their allosteric constants span a wide range of values and that (ii) different regions of the GroEL molecule would be probed.

One interaction within subunits and two intra-ring interactions between subunits were investigated. The mutation Arg501 → Ala breaks a salt-bridge with Glu409 in the equatorial domain of the same subunit. The mutation Thr522 → Ala removes a hydrogen bond between Thr522 in the equatorial domain of one subunit and Asp41 in the equatorial domain of an adjacent subunit in the same ring. The mutation Arg197 → Ala breaks a salt-bridge between Arg197 in the apical domain and Glu386 in the intermediate domain of an adjacent subunit. The mutation Asp196 → Ala probes the same region but without disrupting the Arg197-Glu386 salt-bridge or other pairwise interactions.

Initial rates of ATP hydrolysis by the various GroEL mutants were measured as a function of ATP concentration as described[15] (representative data are shown in Figure 1A for the Phe44 → Trp, Asp196 → Ala mutant). The data were fitted to the Hill equation and to an equation based on the nested model[7] yielding estimates for the values of the Hill coefficients, the allosteric equilibrium constants, L_1 (= [TR]/[TT]) and L_2 (= [RR]/[TR]), and the ATP binding constant which was found to be about 5 μM for all the mutants. The mutation Phe44 → Trp was found to have little effect on the allosteric properties of wild-type GroEL[7] and the previously characterized single mutants.[9,14] Observed rate constants of ATP-induced conformational changes of GroEL were measured as a function of ATP concentration (representative data for the Phe44 → Trp, Asp196 → Ala mutant are shown in Figure 1B) as described.[12] Values of the Hill coefficients and the forward (**T** → **R**) rate constants of conformational changes of GroEL were obtained by fitting the data to an equation derived,[12] assuming that ATP binding occurs much faster than the binding-induced conformational changes.[16]

The kinetic and equilibrium data for the allosteric transitions of the GroEL mutants were combined using the Brönsted relation. A plot of the logarithms of the rate constants of the allosteric transition, in the absence of ATP, against the logarithms of the respective allosteric equilibrium constants yields a very good straight line ($r = 0.99$) with a slope, β, of 0.17 for all of the

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(1) For recent reviews see: (a) Fenton, W. A.; Horwich, A. L. *Protein Sci.* **1997**, *6*, 743−760. (b) Ranson, N. A.; White, H. E.; Saibil, H. R. *Biochem. J.* **1998**, *333*, 233−242. (c) Horovitz, A. *Curr. Opin. Struct. Biol.* **1998**, *8*, 93−100.

(2) Braig, K.; Otwinowski, Z.; Hegde, R.; Boisvert, D. C.; Joachimiak, A.; Horwich, A. L.; Sigler, P. B. *Nature* **1994**, *371*, 578−586.

(3) Viitanen, P. V.; Donaldson, G. K.; Lorimer, G. H.; Lubben, T. H.; Gatenby, A. A. *Biochemistry* **1990**, *29*, 5665−5671.

(4) (a) Gray, T. E.; Fersht, A. R. *FEBS Lett.* **1991**, *292*, 254−258. (b) Bochkareva, E. S.; Lissin, N. M.; Flynn, G. C.; Rothman, J. E.; Girshovich, A. S. *J. Biol. Chem.* **1992**, *267*, 6796−6800. (c) Jackson, G. S.; Staniforth, R. A.; Halsall, D. J.; Atkinson, T.; Holbrook, J. J.; Clarke, A. R.; Burston S. G. *Biochemistry* **1993**, *32*, 2554−2563.

(5) Todd, M. J.; Viitanen, P. V.; Lorimer, G. H. *Biochemistry* **1993**, *32*, 8560−8567.

(6) Roseman, A. M.; Chen, S.; White, H.; Braig, K.; Saibil, H. R. *Cell* **1996**, *87*, 241−251.

(7) Yifrach, O.; Horovitz, A. *Biochemistry* **1995**, *34*, 5303−5308.

(8) (a) Staniforth, R. A.; Burston, S. G.; Atkinson, T.; Clarke A. R. *Biochem. J.* **1994**, *300*, 651−658. (b) Yifrach, O.; Horovitz, A. *J. Mol. Biol.* **1996**, *255*, 356−361.

(9) Yifrach, O.; Horovitz, A. *J. Mol. Biol.* **1994**, *243*, 397−401.

(10) White, H. E.; Chen, S.; Roseman, A. M.; Yifrach, O.; Horovitz, A.; Saibil, H. R. *Nat. Struct. Biol.* **1997**, *4*, 690−694.

(11) Fersht, A. R.; Matouschek, A.; Serrano, L. *J. Mol. Biol.* **1992**, *224*, 771−782.

(12) Yifrach, O.; Horovitz, A. *Biochemistry* **1998**, *37*, 7083−7088.

(13) Construction of the Phe44 → Trp single mutant and the Phe44 → Trp, Arg197 → Ala double mutant has been described.[12] The Phe44 → Trp, Asp196 → Ala double mutant was generated as before[12] using single-stranded DNA containing the gene for the Phe44 → Trp GroEL mutant and the oligonucleotide: 5′-GGTAGCCACGGGCGAACTGCATAC-3′. The other double mutants were obtained by subcloning. Expression and purification of GroEL were achieved as described [Inbar, E.; Horovitz, A. *Biochemistry* **1997**, *36*, 12276−12281].

(14) Aharoni, A.; Horovitz, A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 1698−1702.

(15) Horovitz, A.; Bochkareva, E. S.; Kovalenko, O.; Girshovich, A. S. *J. Mol. Biol.* **1993**, *231*, 58−64. All of the ATPase reactions and rapid mixing experiments were carried out at 25 °C in 50 mM Tris-HCl buffer containing 10 mM $MgCl_2$, 10 mM KCl, and 1 mM dithiothreitol (pH 7.5).

(16) Hammes, G. G.; Schimmel, P. R. *J. Phys. Chem.* **1966**, *70*, 2319−2324.

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Published on Web 12/08/1998

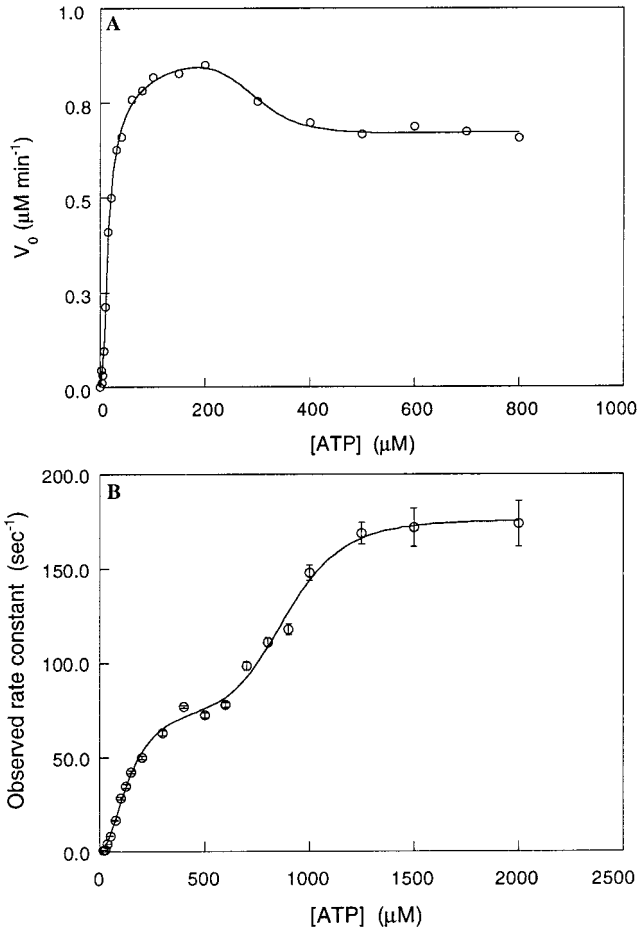

Figure 1. Steady-state and transient kinetic data for the ATP binding-induced allosteric transitions of the Phe44 → Trp, Asp196 → Ala GroEL mutant. Initial rates of ATP hydrolysis (A) were measured as described.[3,15] The data were fitted to an equation based on the nested model.[7] The final GroEL concentration in the ATPase reactions was 25 nM. Observed rate constants, k_{obs}, of the ATP-induced conformational changes (B) were measured, and the data were fitted as described.[12] The final GroEL concentration in the stopped-flow experiments was 0.25 μM.

mutants except Phe44 → Trp, Arg197 → Ala GroEL for which the data does not lie on the line (Figure 2). This result indicates that the transition-state of the reaction has a **T**-like structure in the regions of the molecule probed, including the Arg501−Glu409 salt-bridge which may be involved in vertical opening of the rings. Previous Brönsted analysis of the quaternary structural change of human hemoglobin at various ligation states and pH values showed that the transition state of that reaction has an **R**-like structure.[17]

The data for the Phe44 → Trp, Arg197 → Ala GroEL mutant ($\beta = 0.62$) indicates, hoewever, that the Arg197−Glu386 intersubunit salt-bridge is perturbed in the transition state. Owing to GroEL's ring structure, twisting of the apical domains, which leads to large changes in the intersubunit interfaces, is expected to occur in a concerted manner.[18] Our finding that values of the Hill coefficients for ATP hydrolysis determined for the various mutants under steady-state conditions are very similar to those determined from the transient kinetic data (Figure 3, slope = 0.89) provides

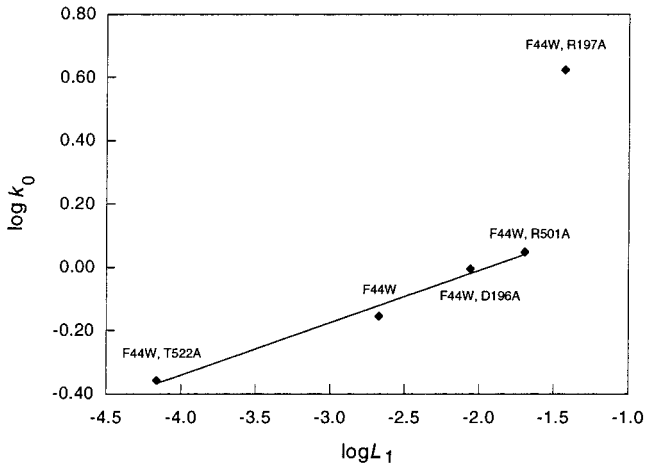

Figure 2. Plot of the logarithm of rate constants of the allosteric transitions of different GroEL mutants against the logarithm of the respective allosteric equilibrium constants. Values of the allosteric equilibrium constants, L_1, were determined as before.[7] Values of the forward rate constants, k_0, were obtained as described.[12] Single-letter notation for amino acids is used.

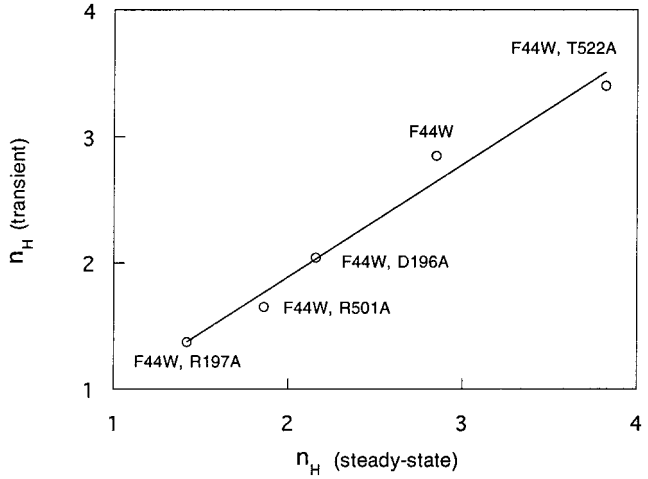

Figure 3. Plot of the Hill coefficients for the observed rate constants of conformational changes of different GroEL mutants against their respective Hill coefficients for ATP hydrolysis. Values of Hill coefficients for ATP hydrolysis were obtained by fitting data of initial rates of ATP hydrolysis as a function of ATP concentration to the Hill equation. Values of Hill coefficients for the observed rate constants of conformational changes were obtained by fitting data of observed rate constants of conformational changes as a function of ATP concentration as before.[12] Single-letter notation for amino acids is used.

evidence that the allosteric transitions of individual rings are indeed concerted. Taken together, our results suggest that in the transition state of the allosteric reaction of GroEL there is some concerted twisting of the apical domains which reduces affinity for protein substrates and precedes vertical opening of the rings. These results are in agreement with recent theoretical simulations of GroEL using normal-mode analysis.[18]

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(17) Eaton, W. A.; Henry, E. R.; Hofrichter, J. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4472−4475.
(18) Ma, J.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 8502−8507.