We investigated the effects of high hydrostatic pressure in the range of 1–3 kilobars on tetradecameric GroEL, heptameric GroES, and the GroEL-GroES complex. Unlike GroEL monomers formed by urea dissociation, which can be reassembled back to the tetradecamer, the pressure-dissociated monomers do not reassemble readily. This indicates an alteration of their native structures, an example of conformational drift. Pressure versus time profiles and kinetics of the dissociation of both GroEL and GroES at fixed pressures were monitored by light scattering. Unlike GroEL, GroES monomers do reassociate readily. Reaction conditions were varied by adding ATP, Mg$^{2+}$, ADP, AMP-PNP, and KCl. At any individual pressure, the dissociation process is governed by both thermodynamics and kinetics. This leads to the decrease in the yield of monomers at lower pressures. In the presence of Mg$^{2+}$ and KCl, GroEL is stable up to 3 kilobars. The presence of either ATP or ADP but not AMP-PNP leads to GroEL dissociation at lower pressures. Interestingly, the GroEL-GroES complex is very stable in the range of 1–2.5 kilobars. However, the addition of ADP destabilizes the complex, which dissociates completely at 1.5 kilobars. The results are rationalized in terms of different degrees of cooperativity between individual monomers and heptameric rings in the GroEL tetradecamer. Such allosteric interactions leading to the alteration of quaternary structure of GroEL in the absence of chemical denaturants are important in understanding the mechanism of chaperonin-assisted protein folding by the GroEL-GroES system.

The bacterial chaperonin GroEL and its co-chaperonin GroES are multimeric proteins that assist folding of other proteins by preventing misfolding and aggregation. Their quaternary structures are crucial to the mechanism of chaperonin-assisted protein folding. These two proteins are coexpressed from a common GroE operon in *Escherichia coli* (1–3). Mutational studies have demonstrated that both chaperonins are essential for protein folding *in vivo* (4–6). GroEL is a single rotationally symmetric ring of seven identical 10-kDa subunits with a dome-shaped architecture (7). GroEL is a tetradecamer (14-mer) of 57-kDa subunits arranged in two seven-membered rings stacked back to back to yield a cylindrical structure. There are no tryptophan residues, and each subunit contains three cysteines, Cys$^{138}$, Cys$^{458}$, and Cys$^{519}$. The x-ray crystal structures of GroES (7), GroEL (8), GroEL fully complexed with 14 ATPyS$^3$ molecules (9), and the GroEL-GroES-(ADP)$_7$ complex (10) are available in the literature. The GroEL crystal structure demonstrates that each monomer is folded into three distinct domains. First, the apical domain faces the solvent and forms the opening to the central channel and is the peptide-binding site; second, a highly helical equatorial domain is the ATP binding site and forms the inter- and intraring contacts; and third, a hingelike intermediate domain links the apical and equatorial domains. The GroEL-assisted protein folding reaction cycle consists of a number of sequential reactions, i.e. (i) binding of the polypeptide at the apical domains of the cis-ring; (ii) binding of seven molecules of ATP and GroES, forming a stable cis assembly and freeing the tightly bound polypeptide into an “Anfinson’s cage” where it folds; (iii) hydrolysis of ATP; and (iv) release of ADP, GroES, and the folded polypeptide (10–12). In a recent investigation involving a GroEL containing binding-defective mutant apical domains, Horwich et al. (13) demonstrated that binding of polypeptides to the apical domain of GroEL requires a minimum of three binding-proficient apical domains for stringent substrate proteins, such as malate dehydrogenase and Ribisco, while only two were required for binding a less stringent substrate such as rhodanese (13). In addition to GroES and ATP, the presence of Mg$^{2+}$ and K$^+$ is also necessary for the GroEL-assisted folding (14–18). The role of ATP is important both as an energy source and an allosteric effector. It has been suggested that ATP binding displays both intrinsically positive cooperativity and interrings negative cooperativity (19, 20).

The conformational changes attributed to the binding of Mg$^{2+}$, ADP, and AMP-PNP with GroEL have been investigated from the stability of such complexes as assessed by urea dissociation, followed by both light scattering and intrinsic tyrosine fluorescence (21). The results indicate that the stabilities decrease in the following order: GroEL-Mg complex > GroEL > GroEL-Mg-AMP-PNP complex > GroEL-Mg-ADP complex. The binding of ATP has been suggested to destabilize the quaternary structure of GroEL (22). From labeling of the three cysteines (Cys$^{138}$, Cys$^{458}$, and Cys$^{519}$) of the GroEL 14-mer, it was demonstrated that the binding of adenine nucleotides induces specific changes in the conformation of the protein oligomer (23). It is also interesting to note that labeling at Cys$^{458}$ by fluorescein 5-maleimide (23) or 4,4’-dithiopyridine (24) leads to the disassembly of GroEL. These conformational changes

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due to the binding of nucleotides regulate the exposure of hydrophobic surfaces on the 14-mer that have been suggested to be important for the binding of protein and GroES during assisted folding by GroEL.

High hydrostatic pressure techniques are increasingly used as tools to study dissociation and unfolding of protein aggregates (25–28) in the absence of externally added chaotropes. The effects of pressure on proteins are generally reversible. The important theories behind this technique and excellent experimental details can be found in several edited books and monographs (29, 30–34). At pressures lower than 4–5 kbar, oligomeric proteins or protein assemblies generally undergo reversible dissociation (35) with denaturation (25, 35, 36). The resulting monomers may undergo conformational drifts away from their conformations in the oligomer and, therefore, may not reassociate rapidly upon depressurization. The application of higher hydrostatic pressure can cause many single chain proteins to denature. A combination of moderate pressure and low concentrations of chaotropes has been found to be suitable for studying the unfolding of proteins (37, 38) and for the recovery of proteins from aggregates (39). In addition to providing information on the nature of physical forces involved in the dissociation of oligomeric chaperonins, elucidation of the dissociation mechanism would provide insights into whether such structures would withstand high pressure in bacteria under the depths of the ocean and still be functional for assisting protein folding.

Three causes have been suggested for the pressure-induced dissociation of oligomeric proteins (25). The first cause is due to imperfect van der Waals contact between the participating monomers and the restriction of amino acid residues approaching too close to each other due to the repulsion of their of their electronic clouds. Such repulsion leads, even with optimal close packing, to creation of small “free volumes” or “dead spaces” (25, 36). Therefore, upon the application of hydrostatic pressure, these small volumes will disappear because of better packing of the solvent against each dissociated subunit/monomer or the unfolded peptide chain. A second cause is due to the existence of salt linkages at the interfaces of monomers/subunits of oligomers, which upon dissociation are exposed and solvated, causing a decrease in volume of the system as a result of solvent electrostriction (25). A third cause, which is less well established, is the solvation of nonpolar groups at boundaries of contact between the monomers in the oligomer (25).

In an earlier investigation, we reported that high hydrostatic pressure can dissociate GroEL tetradecamers (40). After depressurization, the monomers reassociated back to the oligomer very slowly with a $t_{1/2}$ of 150 h at 25 °C. The dissociation and association reactions were facilitated by Mg-ATP only if it was present during pressurization. From their reassociation properties, it has been demonstrated that the monomers formed by pressure dissociation of the 14-mer are different from those formed by the action of 2.5 M urea (40).

In the present investigation, we have studied the effects of high hydrostatic pressure on GroES and GroEL in both the absence and presence of Mg$^{2+}$ and adenine nucleotides and on the isolated complex GroEL-GroES-(ADP)$_7$. Although other divalent cations such as Ca$^{2+}$ are known to stabilize macromolecular assemblies (41, 42), the role of Mg$^{2+}$ as a functional ligand is unique for the GroEL-GroES system (14–18). The results are rationalized in terms of different degrees of cooperativity between individual monomers and heptameric rings in the GroEL tetradecamer.

### MATERIALS AND METHODS

GroEL and GroES were purified as described previously (43, 44). The GroEL-GroES-(ADP)$_7$ complex was prepared and isolated according to the method of Lorimer et al. (19). Briefly, 10 μM GroEL (14-mer) was added to 20 μM GroES (7-mer) in 50 mM Tris, pH 7.8, 5.0 mM MgCl$_2$, 1.0 mM dithiothreitol, 0.5 mM KCl, 0.1 mM EDTA, and 100 μM ATP. The total reaction volume was 100 μl. After 1 min of reaction, 20 μl of 50% glycerol was added to the mixture; the complex was isolated from excess unbound nucleotide and excess GroES using a Sephacryl S-300 gel filtration column (1.0 × 17 cm; bed volume, 13.3 ml). The elution buffer was the same as the reaction buffer but lacking EDTA and nucleotide. The fractions corresponding to the complex were pooled and quantified by Bradford protein determination.

The buffer solutions used in the investigation were filtered through 0.2-μm surfactant-free cellulose acetate membrane syringe filters (Nalgene). The 640-nm polystyrene microspheres (latex beads) were from Polysciences, Inc. (Warrington, PA). Tris buffer is suitable for pressure experiments because of the small pK dependence upon hydrostatic pressure (45). SDS was from Bio-Rad.

### High Pressure Experiments—
The high pressure cell and photon counting spectrophotometer were from ISS Inc. (Champaign, IL). The stainless steel alloy cell with quartz windows can be pressurized up to 3 kbar. Protein samples for the experiments were filled in quartz bottles (1-ml volume) with pressure caps (provided by ISS). These bottles are placed in the metal bottle holder and immersed in the pressurizing fluid (spectroscopic grade ethanol). The high pressure generator was from Advanced Pressure Products (Ilhaca, NY). The pressure generator is electronically controlled and programmable to obtain pressure gradients. The temperature of the high pressure cell was maintained by a circulating water bath. Two independent computers controlled the Advanced Pressure Products pressure generator and ISS spectrofluorometer. The pressure gradients were controlled by computer using a program written for the Advanced Pressure Products software. The pressure was increased in 0.1-kbar increments and held for 1 min between the successive steps. The generated data were imported to Origin software (version 6; Micrsoft Software, Northampton, MA) and analyzed. Kinetics experiments were done after the protein sample in the pressure cell (from ISS) was equilibrated (30 min) to the desired temperature. After equilibration, the fluorometer recording was turned on, followed by the pressure machine. Protein dissociation was followed by monitoring scattering at 400 nm (excitation and emission slits were 2 mm each). To reach the desired pressure, the rate of pressurization was controlled through the Advanced Pressure Products software. In typical experiments, to reach pressures of 1 kbar required 1 min, 2 kbar required 3 min, and 3 kbar required 4 min at a pump speed of 2.0. This introduces severe limitations on following kinetics that would occur in less than the time taken to achieve the target pressure. In some instances, we were able to pressure and depressurize much faster than the indicated times, but in most cases, rapid pressure change caused damage by shattering the quartz windows or sample bottles. To ensure that the intensity changes were not contributed by dimension changes of the cell due to high pressure, controls were run with latex beads in the sample bottle. The scattering intensity increased slightly under the pressure gradient and then reversed back to the original intensity upon depressurization (data not shown).

Kinetics experiments were done after the protein sample in the pressure cell (from ISS) was equilibrated (30 min) to the desired temperature. After equilibration, the fluorometer recording was turned on, followed by the pressure machine. Protein dissociation was followed by monitoring scattering at 400 nm (excitation and emission slits were 2 mm each). To reach the desired pressure, the rate of pressurization was controlled through the Advanced Pressure Products software. In typical experiments, to reach pressures of 1 kbar required 1 min, 2 kbar required 3 min, and 3 kbar required 4 min at a pump speed of 2.0. This introduces severe limitations on following kinetics that would occur in less than the time taken to achieve the target pressure. In some instances, we were able to pressure and depressurize much faster than the indicated times, but in most cases, rapid pressure change caused damage by shattering the quartz windows or sample bottles. To ensure that the intensity changes were not contributed by dimension changes of the cell due to high pressure, controls were run with latex beads in the sample bottle. The scattering intensity increased slightly under the pressure gradient and then reversed back to the original intensity upon depressurization (data not shown).

### Nondenaturing Gel Electrophoresis—
The method for nondenaturing gel electrophoresis for the analysis of GroEL monomer and resolution of GroEL$_{14}$ from GroES$_{7}$-GroEL$_{14}$ complexes on native gels has been described in an earlier publication (46).
Mg$_2^+$, which represent the pressure at the midpoint of dissociation
KCl. In all of these gels, the monomers, given an equivalent number of subunits, always stain more intensely than the 14-mers. Upon comparing the intensities of the bands for the 14-mer and monomers with the standard 6.5% PAGE. It may be noted that the gel analysis provided a reasonable measure of the formation of monomers because their reassociation rate is extremely slow, with a $t_{1/2}$ of 150 h (40). The results are presented in Fig. 3. In these gels, the monomers, given an equivalent number of subunits, always stain more intensely than the 14-mers. Upon comparing the intensities of the bands for the 14-mer and monomers with the standard (native 6.5% PAGE), it is evident that the GroEL showed maximum stability when either Mg$_2^+$ or AMP-PNP + Mg$_2^+$ were present in the sample. This is a clear indication that Mg$_2^+$ induces subunit interactions that lead to a tight oligomeric structure, which can be destabilized by adding ADP (lane 8) or ATP. The dissociated sample when ATP alone (not shown) was present resembles that of ATP + Mg$_2^+$ (lane 6). The gel pattern indicates that, although the 14-mer did not dissociate completely at this pressure, significant dissociation occurred, depending upon the nature of nucleotide (see lanes 6 and 8), even in Tris buffer alone without Mg$_2^+$ and nucleotides (lane 3).

Kinetics of dissociation of the GroEL 14-mer at different fixed pressures were studied in the absence of Mg$_2^+$ or any nucleotide. Two of the kinetics plots are shown in Fig. 4, one showing monoexponential (A) and the other biexponential (B) behavior at pressures above and below 1.75 kbar, respectively. We observed that the amplitudes of both the first and second phases decreased with the decrease in pressure. Although the kinetics at 1.75 kbar could be fitted nicely to a biexponential equation to yield a rate for the slow phase (Fig. 4B), the two phases could not be resolved at lower pressures because of small amplitudes. With the intention of obtaining information on the process as a function of pressure, the major kinetic phase was analyzed. The plots of $k_{obs}$ and amplitude of this phase as a function of pressure are shown in Fig. 5. The $k$ versus pressure plot (Fig. 5A) shows that the observed rates increase slowly with pressure until 2 kbar (slope = 0.53 ± 0.06 s$^{-1}$ kbar$^{-1}$) and then rapidly as the pressure was increased (slope = 3.52 ± 0.15 s$^{-1}$ kbar$^{-1}$). The amplitude versus pressure plot shows a sigmoid (Fig. 5B), and the amplitudes beyond 2 kbar reach a plateau. These two results indicate that the dissociation of GroEL is governed by both pressure-dependent kinetics and pressure-dependent equilibrium. A transient kinetic intermediate is indicated because of the observance of a biphasic behavior at lower pressures. An attempt was made to detect a stable intermediate using native PAGE gel of the laboratory and has been shown to have a $t_{1/2}$ of 150 h at 25 °C (40). The observation that ATP + Mg$_2^+$ destabilized the 14-mer in the pressure dissociation experiments reported in this investigation is in agreement with the earlier results from urea dissociation (40). The stability of GroEL in the presence of Mg$_2^+$ has also been seen in the case of its dissociation by urea (21), where the $U_g$ values (urea concentration at the midpoint of transition) were in the following order: Mg$_2^+$ > AMP-PNP + Mg$_2^+$ > buffer only > ADP + Mg$_2^+$.

Kinetics of dissociation of GroEL samples at 2.5 kbar in the presence of Mg$_2^+$ and different nucleotides were monitored by light scattering. The observed rates are summarized in Table II. The dissociation rates are in the following order: Mg$_2^+$ = AMP-PNP + Mg$_2^+$ > ATP-$\gamma$S = ATP = ADP > ATP-$\gamma$S + Mg$_2^+$ + KCl > ATP + Mg$_2^+$ + KCl > buffer only > ADP + Mg$_2^+$ + KCl. The general trend is similar to the order of dissociation rates presented in Table I, with the stabilization of the oligomeric structure attributed to the binding of Mg$_2^+$ and its destabilization when both Mg$_2^+$ and an adenine nucleotide were present. The kinetics in the presence of ATP-$\gamma$S + Mg$_2^+$ + KCl was biphasic, and both a slow and a fast rate could be evaluated (see Table II). The depressurized samples were analyzed by native 6.5% PAGE. It may be noted that the gel analysis provided a reasonable measure of the formation of monomers because their reassociation rate is extremely slow, with a $t_{1/2}$ of 150 h (40). The results are presented in Fig. 3. In these gels, the monomers, given an equivalent number of subunits, always stain more intensely than the 14-mers. Upon comparing the intensities of the bands for the 14-mer and monomers with the standard (native 6.5% PAGE), it is evident that the GroEL showed maximum stability when either Mg$_2^+$ or AMP-PNP + Mg$_2^+$ were present in the sample. This is a clear indication that Mg$_2^+$ induces subunit interactions that lead to a tight oligomeric structure, which can be destabilized by adding ADP (lane 8) or ATP. The dissociated sample when ATP alone (not shown) was present resembles that of ATP + Mg$_2^+$ (lane 6). The gel pattern indicates that, although the 14-mer did not dissociate completely at this pressure, significant dissociation occurred, depending upon the nature of nucleotide (see lanes 6 and 8), even in Tris buffer alone without Mg$_2^+$ and nucleotides (lane 3).
samples that had been pressurized. The gel is shown in Fig. 6. The results confirm the observations made in the kinetics experiments at different pressures (see Fig. 5) that the dissociation produced increasing amounts of monomers until 2 kbar, after which it proceeded completely to monomers.

Dissociation of Isolated GroES-GroEL-(ADP)$_7$ Complex—We studied the dissociation of the isolated GroES-GroEL-(ADP)$_7$ complex (see "Materials and Methods") in the absence and presence of additional nucleotides. A typical kinetics plot followed by scattering, upon pressurization and depressurization, is shown in Fig. 7. It may be noted that there is approximately 25% reassociation upon depressurization, as seen from the increase in intensity (Fig. 7C) relative to the total intensity after completion of the kinetics. In all cases, the kinetics were biphasic and showed reasonable amplitudes for the evaluation of two rate processes. The results are presented in Table III. The faster rates ($k_1$, s$^{-1}$) were in the following order: buffer only > ADP > AMP-PNP > ATP. The slower rates ($k_2$, s$^{-1}$) were as follows: ADP > ATP > buffer ≥ AMP-PNP. The results suggest that a destabilized intermediate is formed in all cases, which subsequently dissociates into monomers in a slower process.

DISCUSSION

We investigated the dissociation of GroEL, GroES, and GroEL-GroES-(ADP)$_7$ complex by the action of high hydrostatic pressure in the range of 1–3 kbar. The dissociation of GroEL
was investigated both in the absence and presence of Mg$^{2+}$ and adenine nucleotides. The results from this investigation are important in understanding the effect of functional ligands on the quaternary structure of GroEL in the absence of chemical denaturants. Pressure is a thermodynamic variable that does not require use of chemical denaturants, which can have complex effects in addition to dissociation of subunits.

The binding sites on GroEL for functional ligands, polypeptides, and Mg$^{2+}$ have been shown from its x-ray crystal structure and mutation studies. The equatorial domain residues 6–133 and 409–523 contribute to the intra- and interring contacts across the equatorial plane of the tetradecamer and provide the nucleotide-binding site (8–10, 12). The apical domain residues 197–376 contain the binding sites for GroES and other peptides (48, 49). The intermediate domain (residues 134–190 and 377–408) connecting the apical and equatorial domains acts as a hinge for intraring allosteric communication (12, 20). The x-ray structure shows that the carboxylate oxygen of residue Asp$^{389}$ is directly involved in Mg$^{2+}$ coordination (10).

GroES heptamer dissociation is fully reversible both in the absence and presence of Mg$^{2+}$ and nucleotides (Fig. 1). These results from the pressure dissociation presented in this investigation are consistent with the observations made by dissociating GroES in urea and reassociating it upon dilution (50).

The GroEL 14-mer is stabilized in the presence of Mg$^{2+}$ alone and when, additionally, a nonhydrolyzable analog of ATP is present. It is destabilized in the presence of ADP, ATP, ADP-Mg$^{2+}$, and ATP-Mg$^{2+}$ (See Table II). There is only a slight increase in scattering intensity upon the release of pressure in all other cases except when Mg$^{2+}$ alone is present. In the presence of 10 mM Mg$^{2+}$, there is only a 20% decrease in light scattering, and upon release of pressure there is complete reversal to the nonpressurized sample (see Table I; Fig. 2 data...
represented by triangles). The complete, rapid reversal of light scattering upon depressurization makes it impossible in the present investigation to determine the nature of the species formed under pressure. It cannot be due to an expansion in the size of the molecule under pressure, because the light scattering would have increased instead of the observed decrease. It is clear, however, that the behavior is not that of a mixture of monomers and 14-mers, since control experiments in which partially dissociated species of GroEL dissociated completely do not show any reversal (data not shown). It is possible that scattering represents dissociation to 7-mers or species that still retain some degree of quaternary structure. The rate of GroEL dissociation is dependent upon the pressure applied. The equilibrium shifts toward monomers as the applied pressure is increased and GroEL completely dissociates near 2 kbar.

From sedimentation velocity studies on the dissociation of the tetradecamer \( (s_{20,w} = 20 \text{ S}) \) (51), it has been shown that the monomers formed by urea-induced dissociation (51) did not differ significantly in their average sizes \( (s_{20,w} < 3 \text{ S}) \) from those formed by pressure dissociation (40). However, the monomers formed in these two different processes are not the same, as seen from their proteolytic susceptibility and reassociation properties. The pressure-induced monomers show a proteolytic susceptibility to chymotrypsin that is intermediate between native GroEL and GroEL in 2.5 mM urea (40). It is known from the labeling of the monomers by 6-iodoacetamidofluorescein that only one sulfhydryl of the three present on the GroEL monomer was readily available in the urea-induced monomers (52), whereas all three were available in the case of pressure-dissociated monomers (40). The pressure-dissociated monomers have been shown to reassociate very slowly to oligomers \( (t_{1/2} = 150 \text{ h at } 25 \text{ °C}) \) (53). However, the monomers incubated on ice did not reassociate even after 7 days of incubation, regardless of whether \( \text{Mg}^{2+} \) and adenine nucleotides were present or absent during pressurization. The urea-dissociated monomers, on the other hand, are known to form GroEL 14-mer in the presence of \( \text{Mg}^{2+} \), ATP or ADP, and ammonium sulfate (54). One of the interesting results of the present investigation is that we did not see any reassembly of the pressurized monomers, whether \( \text{Mg}^{2+} \) and adenine nucleotides were absent or present during pressurization. The combination and optimum amounts of \( \text{Mg}^{2+} \), ATP or ADP, and ammonium sulfate necessary for reassociating urea-dissociated monomers (54) were also ineffective in reassociating the monomers from all of the pressure dissociation experiments presented in this investigation.

The GroES-GroEL-(ADP)$_7$ complex is reversible in the early stages, and the profile under the pressure gradient (not shown) resembles that of GroEL in the presence of \( \text{Mg}^{2+} \) (Fig. 2). However, it is not able to reassociate completely after a 5-h incubation at 2.5 kbar (Fig. 7). The kinetics of GroES-GroEL-(ADP)$_7$ complex dissociation at 2.5 kbar are biphasic. The regain in scattering intensity after depressurization is fast, and it is approximately the same as the amplitude of the second phase of the kinetics (Fig. 7, A and C). Although it is not possible to propose a detailed mechanism from the data presented in this investigation, we speculate that the dissociation of the complex proceeds through an intermediate whose stability could be altered by the addition of adenine nucleotides during pressurization. There is a complex dependence of the observed rates and the amplitudes of the fast and slow phases of the biphasic kinetics on the nature of externally added nucleotides (Table III). At present, we are employing other methods to characterize this intermediate that are beyond the scope of the present investigation.

The following conclusions can be made based on the results from this investigation using high hydrostatic pressure as a probe to study the effect of functional ligands on GroES, GroEL, and the GroES-GroEL-(ADP)$_7$ complex. GroES heptamer dissociation is reversible both in the absence and presence of \( \text{Mg}^{2+} \). The GroEL tetradecamer is stabilized in the

![Image](http://www.jbc.org/)

**Fig. 7.** Typical dissociation kinetics of the isolated GroES-GroEL-(ADP)$_7$ complex monitored by light scattering (see "Materials and Methods"). Pressure was maintained at 2.5 kbar. Reaction conditions were as follows: 50 mM Tris-HCl, pH 7.8; \([\text{complex}] = 0.1 \mu \text{M; } T = 20 \text{ °C.} \ A, \text{open circles; data; solid line through circles, the bieponential fit obtained using the equation described under "Materials and Methods."} \ B, \text{the continued acquisition of data until the dissociation was nearly complete.} \ C, \text{the resulting increase in scattering intensity after depressurization to 1 bar.}

**Table III**

| Condition$^a$ | \( k_1 \) | Percentage of total amplitude$^b$ | \( k_2 \) | Percentage of total amplitude$^b$ |
|---------------|-----------|-----------------|-----------|-----------------|
| Buffer only   | \( 4.6 \pm 0.1 \times 10^{-3} \) | 55               | \( 1.4 \pm 0.1 \times 10^{-4} \) | 45               |
| With ADP      | \( 3.9 \pm 0.2 \times 10^{-3} \) | 87               | \( 2.5 \pm 0.2 \times 10^{-4} \) | 13               |
| With ATP      | \( 1.2 \pm 0.4 \times 10^{-3} \) | 84               | \( 2.9 \pm 0.4 \times 10^{-4} \) | 16               |
| With AMP-PNP  | \( 2.6 \pm 0.7 \times 10^{-3} \) | 89               | \( 1.3 \pm 0.3 \times 10^{-4} \) | 61               |

$^a$ [Complex] = 0.1 \mu \text{M; } [\text{Tris-HCl}] = 50 \text{ mM; } \text{pH 7.8; } [\text{Mg}^{2+}] = 10 \text{ mM; } [\text{KCl}] = 10 \text{ mM; } T = 20 \text{ °C.} \ [\text{ATP}] = [\text{ADP}] = [\text{AMP-PNP}] = 1.0 \text{ mM.}$

$^b$ The dissociation kinetics reach about the same plateau (infinity) value at the completion of the reaction, as seen from the biphasic fits.
presence of Mg$^{2+}$ alone. It is also stabilized when a nonhydrolyzable analog of ATP along with Mg$^{2+}$ is used. This effect is probably due to intraring cooperativity as seen from the results of dissociation under a pressure gradient and kinetics at 2.5 kbar (see Tables I and II). In all other cases, the tightness of oligomeric structure was lost due to hydrolysis of ATP, leading to interring negative cooperativity.

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