Ligand-induced conformational changes of GroEL alone and with bound rhodanese, citrate synthase, or dihydrofolate reductase were studied by limited proteolysis. Similar digestion patterns of GroEL, with or without bound substrate polypeptide, were obtained in the absence and presence of the chaperonin ligands, K\(^+\), Mg\(^{2+}\), or ATP. The rates of formation and degradation of the six produced proteolytic fragments were significantly different, however. Strikingly, only with Mg\(^{2+}\)/ATP or K\(^+\)/Mg\(^{2+}\)/ATP an additional fragment of approximately 25 kDa was generated during digestion of GroEL alone or with bound rhodanese or dihydrofolate reductase, but not with bound citrate synthase. Most of the trypsin-sensitive sites in GroEL were localized in the flexible apical domain, which contains the putative polypeptide-binding region. Our data indicate that subtle structural changes in the trypsin-sensitive regions of GroEL occur as a result of the binding of the chaperonin ligands. However, these structural changes are influenced by the GroEL substrate polypeptides.

Chaperonin GroEL, a tetradecamer of 14 equal subunits of 57,259 Da each arranged in a double-stacked ring structure with a 7-fold symmetry and a central hole (1), has been shown to facilitate the folding of other polypeptides (2, 3). Although the mechanism of action of the chaperonins in assisting the folding of other proteins is not completely understood, important aspects have been identified (4, 5). A crucial step in chaperonin-mediated folding is the stabilization of incompletely folded intermediates through the formation of complexes between these species and GroEL. Substrate polypeptides remain bound to GroEL in inactive states that subsequently can be released into active forms, but only after addition of the GroEL ligands, K\(^+\), Mg\(^{2+}\), ATP, and sometimes GroES (6). It was proposed that conformational changes of GroEL, induced by the binding of those ligands, lead to the release of GroEL-bound polypeptides (7). This has been supported with recent reports of nucleotides and other ligand-induced conformational changes of GroEL (8–12).

Recently the x-ray crystal structure of GroEL was determined (13). Each of the monomers is organized into three distinct domains: apical, intermediate, and equatorial. The equatorial domain is composed of residues 6–133 and 409–523, and it accounts for most of the interactions between the monomers and contact regions between the two heptameric rings. It also contains the ATP binding site. The intermediate domain, which connects the equatorial domain with the apical domain, consists of residues 134–190 and 377–408. The apical domain, composed of residues 191–376, forms the opening of the central cylindrical channel of GroEL. This domain contains highly flexible segments, and mutations in this domain prevent polypeptide binding (14).

The enzymes rhodanese, citrate synthase and dihydrofolate reductase have been used to study the interaction of GroEL with other polypeptides (15–19). In this report, we have prepared and used functional complexes of GroEL with urea-unfolded rhodanese, citrate synthase (CS),\(^1\) or dihydrofolate reductase (DHFR) to detect conformational changes of the chaperonin upon ligand binding by limited proteolysis. We present data indicating the sources of significant conformational changes that occur in the trypsin-sensitive regions of GroEL. We found that a unique proteolytic fragment was generated from GroEL alone or with bound rhodanese or DHFR in the presence of Mg\(^{2+}\)/ATP or K\(^+\)/Mg\(^{2+}\)/ATP. This indicates that a specific conformational change of the chaperonin occurs upon interaction with these ligands. Further, we have localized this change to the putative polypeptide-binding region of the chaperonin. Since in these experiments, GroEL had a bound substrate polypeptide during proteolytic digestion, the detected conformational changes are likely to represent functional structural changes of GroEL induced by its ligands, which lead to the release of bound polypeptides.

**EXPERIMENTAL PROCEDURES**

Materials and Proteins—All of the reagents used were of analytical grade. CS from pig heart, DHFR from chicken liver, and trypsin (crystallized, bovine type XI) were purchased from Sigma. Electrophoresis quality urea was purchased from Bio-Rad. GroEL and GroES were purified, as described elsewhere (20, 21), from lysates of Escherichia coli cells bearing the multicopy plasmid pGroESL (22). After purification, the chaperonins were dialyzed against 50 mM Tris-HCl, pH 7.8, and kept at 4°C. The GroEL-polypeptide complexes were prepared by mixing GroEL with substrate polypeptide, thus forming a 1:1 mixture of GroEL monomers and contact regions between the two heptameric rings. It also contains the ATP binding site. The intermediate domain, which connects the equatorial domain with the apical domain, contains of residues 134–190 and 377–408. The apical domain, composed of residues 191–376, forms the opening of the central cylindrical channel of GroEL. This domain contains highly flexible segments, and mutations in this domain prevent polypeptide binding (14).

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\(^1\) The abbreviations used are: CS, citrate synthase; DHFR, dihydrofolate reductase.

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enzymes. After incubation of mixtures for different times at 23 °C, enzymatic activities of each enzyme were determined as described previously (18, 19, 28).

Limited Proteolysis—GroEL with or without bound substrate polypeptide was supplemented with one or more of the following ligands: K⁺, Mg²⁺, and ATP at final concentrations of 10, 10, and 2 mM, respectively. After incubation for 15 min at 23 °C, the mixture was transferred to a 37 °C water bath and incubated with trypsin at a ratio of GroEL:trypsin of 40/1 (w/w), and the reaction monitored at various times. The addition of ATP on the activity of trypsin itself has been shown to be negligible (29). The reaction was stopped by adding phenylmethylsulfonyl fluoride (in 20% ethanol) to a final concentration of 3.5 mM. The sample was then incubated for 10 min, at 23 °C, supplemented with SDS-gel loading buffer, and heated for 3 min, at 100 °C. Analysis of the digested material was carried out in SDS-containing 12% polyacrylamide gels (30). Gels were silver-stained (31), and gel bands were quantified by videodensitometric analysis using the NIH-Image program.

Amino Acid Sequence Analysis—For the amino acid sequence analysis, peptide fragments from tryptic digests were separated by SDS-polyacrylamide gel electrophoresis (on 12% polyacrylamide gels) and the proteins were electrophoretically blotted onto a polyvinylidene difluoride membrane (Gelman Sciences), utilizing a Bio-Rad electroblotting transfer apparatus. The membrane was stained with 0.1% (w/v) Amido Black-10B (in 10% methanol and 2% acetic acid) for 20 min. The peptides bands were excised, destained with a 7% acetic acid, 20% methanol solution, and allowed to air dry. The fragments were analyzed on a model 494 Procise Sequencer (Applied Biosystems). Peptides were subjected to five cycles of sequencing. Using this information, the sequence of each fragment was compared to the published sequence of the corresponding polypeptide.

RESULTS

Digestion of GroEL with Trypsin—Fig. 1A, panel a, shows the time course of trypsin digestion of GroEL. Digestion was virtually complete after 60 min, and six fragments were generated of 38, 32, 31.5, 22, 18, and 16 kDa, as estimated by SDS-polyacrylamide gel electrophoresis. The band with an apparent molecular mass of 28 kDa was found to correspond to trypsin (by amino acid sequencing). Proteolysis under similar conditions was used as a conformational probe to detect possible ligand-induced changes in the trypsin-sensitive regions of GroEL. Fig. 1A, panels b, c, and d, shows the time courses of trypsin digestion of GroEL in the presence of K⁺, Mg²⁺, and ATP, respectively. Digestion patterns of GroEL obtained in the presence of each ligand were similar to that of GroEL alone. Strikingly, it was only in the presence of K⁺/Mg²⁺/ATP (Fig. 1A, panel e) that an additional fragment of approximately 25 kDa was generated. The effect on GroEL digestion by trypsin with combinations of any two of the ligands also was examined. Generation of the 25-kDa fragment also was obtained during digestion of GroEL in the presence of Mg²⁺/ATP (not shown).

Fig. 1B shows that the rates of GroEL cleavage were significantly decreased in the presence of each of the three ligands. Fig. 1B also shows that the rates of formation of the six proteolytic fragments were significantly changed in the presence of each of the GroEL ligands, as well as of K⁺/Mg²⁺/ATP.

Digestion of the GroEL-Rhodanese and GroEL-CS Complexes with Trypsin—Complexes of GroEL with urea-unfolded rhodanese or CS were prepared, as described previously for the efficient formation of the complexes at low temperatures (16, 32). Addition of the components needed to cause the release of folded enzymes from GroEL led to recoveries of active enzymes similar to those previously reported (not shown). Fig. 2, panel a, shows the time courses of digestion of both complexes under the same conditions used for digestion of GroEL alone. GroEL digestion was almost complete after 60 and 45 min when complexed with rhodanese and CS, respectively. For both complexes, a GroEL digestion pattern similar to that of the chaperonin alone, was obtained. Time courses of digestion of GroEL with bound rhodanese or CS in the presence of K⁺, Mg²⁺, ATP

**Fig. 1. Digestion of GroEL with trypsin.** A, time course of the proteolysis of GroEL. GroEL (321 μM) was incubated in 50 mM Tris-HCl, pH 7.8, in the absence (panel a) or presence of K⁺ (panel b), Mg²⁺ (panel c), ATP (panel d) or K⁺/Mg²⁺/ATP (panel e), digested and treated as described under “Experimental Procedures.” The first lane (MW) corresponds to low molecular mass standard proteins (Bio-Rad). B, relative amounts of intact GroEL and the fragments in the absence (open circles) and presence of K⁺ (filled triangles), Mg²⁺ (filled inverted triangles), ATP (filled diamonds), or K⁺/Mg²⁺/ATP (filled circles). The relative amounts were calculated as described under “Experimental Procedures.”
or K\(^{1+}\)/Mg\(^{2+}\)/ATP are shown in Fig. 2, panels b, c, d, and e, respectively. Strikingly, in the presence of K\(^{1+}\)/Mg\(^{2+}\)/ATP (panel e) or Mg\(^{2+}\)/ATP (not shown) the 25-kDa fragment was generated from the GroEL-rhodanese complex, as obtained for GroEL alone. However, interestingly, binding of CS to GroEL completely suppressed the generation of the 25-kDa fragment. Further this fragment was not observed in the presence of CS in any ligand combination.

EffectsoftheGroELLigandsontheProductionofProteolyticFragmentsandtheDegradationofGroELDuringDigestionoftheGroEL-RhodaneseandGroEL-CSComplexes—Fig. 3 shows the effects of each of the GroEL ligands and K\(^{1+}\)/Mg\(^{2+}\)/ATP on the formation of each of the proteolytic fragments that were produced during digestion of the GroEL-rhodanese and the GroEL-CS complexes. Some of the effects that the ligands had on the formation of the proteolytic fragments were similar for both complexes. For example, Mg\(^{2+}\) or ATP had similar effects on inducing the formation of larger amounts of the 38-kDa fragment, but suppressed the formation of the 32 and 31.5 kDa fragments in both complexes. However, many of the effects of the ligands on the formation of the proteolytic fragments were shown to be dependent on the bound polypeptide. For example, in the GroEL-rhodanese complex, K\(^{1+}\) induced the formation of higher amounts of the 32 and 31.5 K fragments, while for the GroEL-CS complex, those fragments were generated in higher amounts in the absence of ligands or presence of K\(^{1+}\)/Mg\(^{2+}\)/ATP. In the presence of ATP, the 22-kDa fragment was not detected for the complex of GroEL with rhodanese, while a very significant amount of the fragment was observed for the GroEL-CS complex.

Fig. 4, panel A, shows that the rates of cleavage of GroEL were significantly decreased during digestion of the GroEL-rhodanese and GroEL-CS complexes in the presence of either K\(^{1+}\), Mg\(^{2+}\), ATP or K\(^{1+}\)/Mg\(^{2+}\)/ATP. Fig. 4, panel B, shows the formation of the 25 kDa fragment that was produced during digestion of GroEL alone and the GroEL-rhodanese complex in the presence of Mg\(^{2+}\)/ATP and K\(^{1+}\)/Mg\(^{2+}\)/ATP. Both ligand combinations had very similar effects on the rate of formation of that fragment.

Digestion of the GroEL-DHFR Complex with Trypsin in the Presence of GroEL Ligands—The observed effect that Mg\(^{2+}\)/ATP or K\(^{1+}\)/Mg\(^{2+}\)/ATP had on inducing cleavage of GroEL, generating the 25-kDa fragment only in the absence of a bound polypeptide or with the bound 33-kDa rhodanese, but not with the bound larger 50-kDa CS, raised the possibility of observing a polypeptide size-dependent effect. Binding of rhodanese has been suggested to occur within the central cavity of GroEL (33). Also, it was suggested, in the same study, that larger proteins could not be readily accommodated within the GroEL double ring. Thus, we reasoned that this was why the generation of the 25-kDa fragment was prevented during digestion of the chap-
Conformational Changes of GroEL Complexes

Erinon when CS was bound to GroEL. Therefore, we examined the trypsin digestion of another functional complex of GroEL. The 20-kDa DHFR has been used to study the interaction of GroEL with other polypeptides. Nonfolded forms of this protein were shown to interact with GroEL (19, 34). Thus, a functional complex of GroEL with urea-unfolded DHFR was prepared and incubated with trypsin in the absence or presence of the GroEL ligands. Fig. 5 shows the time-course of digestion of GroEL with bound DHFR in the absence (panel a) or presence (panel b) of Mg$^{2+}$/ATP. A GroEL digestion pattern similar to that of the chaperonin alone, was obtained for the GroEL-DHFR complex. However, in the presence of Mg$^{2+}$/ATP, the 25-kDa fragment also was generated, as with GroEL alone or with bound rhodanese. The 25-kDa fragment also was generated in the presence of K$^+$ or Mg$^{2+}$/ATP (not shown).

Identification of Sites of Tryptic Hydrolysis in GroEL—Sequencing of the proteolytic fragments gave the following results. The 38-kDa fragment started at Ala-2. The 22-kDa fragment started at Val-346 and continued to the C-terminal end of GroEL. Thus, cleavage between Arg-345 and Val-346, generates the 38- and 22-kDa proteolytic fragments, as suggested by their similar rates of production. Similarly, the 31.5- and 32-kDa fragments started at Ala-2 and Gly-269 and continued to the C-terminal end of GroEL, respectively. Thus, cleavage between Arg-268 and Gly-269 generated those two fragments, which appear to be produced at similar rates. The 16-kDa fragment begins at Ala-287 and the 18-kDa fragment begins at Ala-405 and continues to the C-terminal end of GroEL. Thus, cleavages between Lys-286 and Ala-287 and between Arg-404 and Ala-405 seem to generate those fragments which are formed at similar rates. Finally, the 25-kDa fragment begins at Ala-2 and ends in a region located in the apical domain of GroEL.

**DISCUSSION**

Ligand-induced conformational changes of GroEL are generally believed to lead to the release of the GroEL-bound polypeptides. Our results show that GroEL ligands induce different conformational changes of GroEL as evidenced by changes in digestion rates of the chaperonin and formation of its proteolytic fragments. The results presented here are consistent with a recent study showing that ligand-induced conformational changes occur in the apical domain of GroEL (12).

The increased resistance of GroEL to proteolysis in the presence of its ligands is remarkable. Mg$^{2+}$ or ATP were shown to have the largest protective effect. This is interesting in view of the opposing effects that those ligands have on the stability of GroEL; Mg$^{2+}$ was shown to significantly stabilize the oligomeric structure of the chaperonin (11), while ATP binding to GroEL has been shown to cause structural changes that tend to destabilize the oligomeric structure of GroEL and ease its dissociation into monomers by urea (36). Formation of monomers was accompanied by an increased exposure of hydrophobic surfaces (37) and by the incorporation of the fluorescent probe, 1,1'-bis(4-anilino-5-naphthalenesulfonic acid), in the apical domain of GroEL (38). Also, ATP was shown to facilitate the pressure-induced dissociation of GroEL (39). These experiments, however, were fundamentally monitoring the stability of the oligomeric structure of GroEL and they should not necessarily correlate with the sensitivity of tetradecameric-GroEL to protease digestion. Interestingly, the ATP binding site is located in the equatorial domain, which provides all of the contacts between the GroEL heptameric rings, as well as, many of the interactions between the monomers. Thus, it is likely that binding by the nucleotide could directly alter those interactions within the equatorial domain leading to destabilization of the oligomeric structure.

Three of the four identified sites are localized in the flexible apical domain of GroEL; residues 191 to 376. The identified peptides revealed that 268, 286, and 345 are the amino acid sites of tryptic hydrolysis in the apical domain (Fig. 6, upper left domain) and that 404 is the only amino acid site of tryptic hydrolysis in the intermediate domain (Fig. 6, upper right domain). No proteolytic sites were identified to occur in the large and well ordered equatorial domain (Fig. 6, lower domain). Possible explanations for the apparent resistance of the intermediate and equatorial domains to further tryptic hydrolysis are the maintenance of their structures or their continued contact with the cleaved apical domain after hydrolysis of this region (12); either of which could have prevented or retarded attack by trypsin.
Arg-268 is in the putative polypeptide-binding site on the inside surface of the apical domain, facing the central channel (14), and proteolysis at this site was previously detected in the presence of GroEL ligands (12) and when GroEL was dissociated into monomers with 2.5 M urea (37). In contrast, Arg-345 lies on the outer surface of the cylinder in an α-helix (340–354) of the apical domain (14). Proteolysis of the K⁺/Mg²⁺/ATP-complexed form of GroEL at Arg-404 was significantly decreased. Arg-404 is in an α-helix (388–408) near the base of the intermediate domain which flanks the ATP binding site (13). This suggests that binding of those ligands might directly block proteolysis at that site. Similarly, proteolysis of the K⁺/Mg²⁺/ATP-complexed form of GroEL at Lys-286 was significantly decreased. Significantly, Lys-286 lies on the top surface of the apical domain in an α-helix (283–296). This suggests that binding of those ligands induces interdomain conformational changes in the apical domain decreasing the exposure of that specific proteolytic site.

One of the most interesting results concerns the specific conformational change that exposes an additional trypsin cleavage site in the apical domain caused by Mg²⁺/ATP, which bind to GroEL in the top of the equatorial domain (14). It would, therefore, be reasonable to suggest that binding of Mg²⁺/ATP is involved in distant conformational changes between the two large domains; equatorial and apical, exerted through the small intermediate domain. It is possible that binding of Mg²⁺/ATP prevents the apical domain from exhibiting a form of flexibility suggested to be caused by hinge-like movements at its union with the intermediate domain (13). Alternatively, binding of Mg²⁺/ATP could directly induce conformational changes, since the top of the equatorial domain which bears the binding site of those ligands is close to the undersurface of the apical domain (13). These could be functional conformational changes that could be required for the discharge of bound substrate polypeptides from GroEL. A specific Mg²⁺/ATP-induced conformational change also was previously detected with fluorimetric techniques in a chemically modified form of GroEL (35).

Interestingly, the effect that Mg²⁺/ATP had on inducing cleavage of GroEL generating the 25 kDa fragment was observed during digestion of GroEL alone and in complexes of the chaperonin with unfolded DHFR (20 kDa) or rhodanese (33 kDa), but not with the larger CS (50 kDa). Therefore, although a larger number of complexes of GroEL with substrate proteins need to be examined for the production of the 25 kDa fragment during their digestion in the presence of Mg²⁺/ATP, the present data suggest that there might be a correlation between the size of the GroEL-bound substrate polypeptide and the Mg²⁺/ATP-induced generation of the 25 kDa fragment. Thus, the results presented here demonstrate a variety of subtle structural changes induced in the trypsin-sensitive regions of the apical domain of GroEL as a consequence of binding of the chaperonin ligands, which are dependent on the nature of the GroEL-bound substrate polypeptide.

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REFERENCES

1. Georgopoulos, C., and Ang, D. (1990) Semin. Cell Biol. 1, 19–26
2. Gething, M.-J., and Sambrook, J. (1992) Nature 355, 33–45
3. Hendrick, J. P., and Hartl, F.-U. (1993) Annu. Rev. Biochem. 62, 349–384
4. Martin, J., Mayhew, M., Langer, T., and Hartl, F. U. (1993) Nature 366, 228–233
5. Todd, M. J., Viitanen, P. V., and Lorimer, G. H. (1993) Nature 366, 228–233
6. Lorimer, G. H., Todd, M. J., and Viitanen, P. V. (1993) Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 339, 297–304
7. Creighton, T. E. (1991) Nature 352, 17–18
8. Baneyx, F., and Gatenby, A. A. (1992) J. Biol. Chem. 267, 11637–11644
9. Jackson, G. S., Staniforth, R. A., Halsall, D. J., Atkinson, T., Holbrook, J. J., Clarke, A. R., and Burston, A. R. (1993) Acta Chem. Scand. 7, 1129–1136
10. Hansen, J. E., and Gafni, A. (1993) J. Biol. Chem. 268, 21632–21636
11. Azem, A., Diamant, S., and Goloubinoff, P. (1994) Biochemistry 33, 6671–6675
12. Gibbons, D. L., and Horowitz, P. M. (1996) J. Biol. Chem. 271, 238–243
13. Braig, K., Otwinowski, Z., Hegde, R., Bösvert, D. C., Jachmiak, A., Horwich, A., and Sigler, P. B. (1994) Nature 371, 578–586
14. Fenton, W. A., Kashi, Y., Furtak, K., and Horwich, A. L. (1994) Nature 371, 614–619
15. Mendoza, J. A., Rogers, E., Lorimer, G. H., and Horowitz, P. M. (1991) J. Biol. Chem. 266, 16973–16976
16. Mendoza, J. A., Loremar, G. H., and Horowitz, P. M. (1991) J. Biol. Chem. 266, 16973–16976
17. Smith, K. E., and Fisher, M. T. (1995) J. Biol. Chem. 270, 21517–21523
18. Buchner, J., Schmidt, M., Fuchs, M., Jaenicke, R., Rudolph, R., Schröd, F. X., and Kiefhaber, T. (1991) Biochemistry 30, 1586–1591
19. Viitanen, P. V., Donaldson, G. K., Lorimer, G. H., Lubben, T. H., and Gatenby, A. A. (1991) Biochemistry 30, 9716–9723
20. Hendrix, R. W. (1979) J. Biol. Chem. 254, 375–392
21. Chandrasekhar, G. N., Tilly, K., Woolford, C., Hendrix, R., and Georgopoulos, C. (1986) J. Biol. Chem. 261, 12414–12419
22. Goloubinoff, P., Christeller, J. T., Gatenby, A. A., and Lorimer, G. H. (1989) Nature 342, 884–889
23. Viitanen, P. V., Lubben, T. H., Reed, J., Goloubinoff, P., O'Keefe, D. P., and Lorimer, G. (1990) Biochemistry 29, 5665–5671
24. Fischer, M. T. (1992) Biochemistry 31, 3955–3963
25. Hemmingsen, S. M., Woolford, C., van der Vies, S. M., Tilly, K., Dennis, D. T., Georgopoulos, C. P., Hendrix, R. W., and Ellis, R. J. (1988) Nature 333, 330–334
26. Horowitz, P. (1978) Anal. Biochem. 65, 751–753
27. Ploegman, J. H., Drent, G. H., Kalk, K. H., Hol, W. G. J., Heinrikson, R. L., Kelm, P., Weng, L., and Russell, J. (1978) Nature 273, 1245–1249
28. Sorbo, B. H. (1955) Acta Chem. Scand. 7, 1129–1136
29. Erlanger, B. F., Kokowsky, N., and Cohen, W. (1961) Arch. Biochem. Biophys. 95, 271–278
30. Laemmli, U. K. (1970) Nature 227, 680–685
31. Wadrychowski, A., Olimski, R., and Hnilica, L. S. (1986) Anal. Biochem. 159, 323–328
32. Schmidt, M., Buchner, J., Todd, M. J., Lorimer, G. H., and Viitanen, P. V. (1994) J. Biol. Chem. 269, 10304–10311
33. Langer, T., Pfeifer, G., Martin, J., Baumeister, W., and Hartl, F.-U. (1992) EMBO J. 11, 4757–4765
34. Mendoza, J. A., and Horowitz, P. M. (1994) J. Biol. Chem. 269, 25963–25965
35. Hansen, J. E., and Gafni, A. (1994) J. Biol. Chem. 269, 6286–6289
36. Horowitz, A., Bochkareva, E. S., Kovalenko, O., and Girsova, A. S. (1993) J. Mol. Biol. 231, 58–64
37. Horowitz, P. M., Hua, S., and Gibbons, D. L. (1995) J. Biol. Chem. 270, 1535–1542
38. Seale, J. W., Martinez, J. L., and Horowitz, P. M. (1995) Biochemistry 34, 7443–7449
39. Gorovitz, B., Raman, C. S., and Horowitz, P. M. (1995) J. Biol. Chem. 270, 2061–2066