THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 268, No. 17, Issue of June 15, pp. 12730-12735, 1993
Printed in U.S.A.

Peptide-dependent Stimulation of the ATPase Activity of the Molecular Chaperone BiP Is the Result of Conversion of Oligomers to Active Monomers*

(Received for publication, February 24, 1993)

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The molecular chaperone BiP purified from bovine liver (bBiP) exhibits a low basal level of ATPase activity that can be stimulated 3–6-fold by synthetic peptides (Flynn, G. C., Chappell, T. G., and Rothman, J. E. (1989) *Science* 245, 385–390). By contrast, recombinant murine BiP (rBiP) purified to homogeneity following expression in *Escherichia coli* exhibits a higher basal level of ATPase activity and is much less stimulated by synthetic peptides. Nondenaturing gel electrophoresis showed that rBiP is predominantly monomeric, while bBiP exists in multiple forms probably corresponding to differentially modified monomeric, dimeric, and higher oligomeric species. Some, but not all, synthetic peptides cause conversion of the oligomeric and modified species of bBiP to a monomeric form. We propose that the peptide-dependent ATPase stimulation observed for BiP reflects the conversion of inactive oligomeric and/or modified species into active monomers.

The endoplasmic reticulum (ER)[1] of eukaryotic cells contains a single member of the 70-kDa heat shock (HSP70) protein family (reviewed in Ref. 2). This protein, now named BiP, was originally described independently as the immunoglobulin heavy chain binding protein (3, 4) and as the 78-kDa glucose-regulated protein, grp78 (5). BiP is a major constituent of the lumen of the ER, and its rate of synthesis can be further increased by the accumulation of secretory precursors (6, 7) or mutant proteins (8, 9) in the ER, or by a variety of stress conditions (reviewed in Ref. 10) that also lead to the accumulation in the ER of unfolded polypeptides (6, 8). In the ER of mammalian cells, BiP associates transiently with a variety of nascent, wild-type polypeptides (11–14) and more permanently with misfolded, underglycosylated or unassembled polypeptides whose transport from the ER is blocked (15–18). In *Saccharomyces cerevisiae*, BiP is involved in protein folding and assembly in the ER (19) and in addition is required for translocation of newly synthesized polypeptides across the ER membrane (20, 21).

Like other members of the HSP70 family, BiP binds ATP with high affinity (22) and exhibits a low basal level of ATPase activity that can be stimulated by the binding of synthetic peptides (1). HSP70 proteins are composed of a ~44-kDa N-terminal domain that includes the ATP binding/hydrolysis site and a C-terminal domain of ~30 kDa that contains the (poly)peptide binding site (22–24). The structure of the N-terminal ATPase domain of bovine cytosolic hsc70 has been defined to 2-Å resolution (24, 25); the structure of the C-terminal domain has not yet been characterized although it has been predicted to resemble that of the $\alpha_1\alpha_2$ domain (*i.e.* the peptide binding platform) of class I major histocompatibility antigens (26, 27). There must be significant interaction between these two domains in the BiP molecule, since ATP hydrolysis catalyzed by the N-terminal domain causes the dissociation of polypeptides bound to the C-terminal domain (28, 29).

In mammalian cells, BiP is post-translationally modified by both phosphorylation and ADP ribosylation (30, 31) and exists in interconvertible oligomeric and monomeric forms (32, 33). Because the post-translational modifications appear to be restricted to oligomeric forms of BiP that are not bound to nascent polypeptides, Freiden *et al.* (34) proposed that the various forms of the protein are functionally distinct with the monomeric, unmodified form being the biologically active species able to bind to unfolded or unassembled proteins. However, the experiments described in this paper demonstrate that synthetic peptides that stimulate the ATPase activity of BiP bind to both oligomeric and monomeric forms of BiP and thereby induce their conversion to enzymatically active, monomeric species.

EXPERIMENTAL PROCEDURES

Recombinant DNA Techniques—Buffers and reaction conditions for restriction enzymes, T4 DNA ligase, and the Klenow fragment of DNA polymerase I were those listed by the commercial sources, New England Biolabs Inc. and Boehringer-Mannheim. Isolation of DNA fragments, oligonucleotide-directed mutagenesis, preparation of plasmid DNAs, and other standard recombinant DNA techniques were carried out as described (35).

Construction of pSecB115—A plasmid (pT7-mBiP) containing the cDNA sequence encoding the mature mouse BiP polypeptide (lacking the signal sequence) under the control of the T7 RNA polymerase promoter was constructed by a three-way ligation of the following components: a 1.8-kb *ClaI*/*Bam*HI fragment encoding amino acids 34–636 of mouse BiP (6, 29), a 2.4-kb *NcoI*/*Bam*HI fragment from the NpT7 vector (36) that contains plasmid maintenance sequences and the T7 RNA polymerase promoter, and two annealed bridging oligonucleotides, 5′-C ATG GAG GAG GAG GAC AAG AAG GAG GAT GTG GGC ACG GTG GTC GGC AT-3′ and 5′-CG ATG CCG ACC ACC GTG CCC ACA TCC TCC TTC TTG TCC TCC TCC TC-3′, that form a *NcoI*/*ClaI* restriction fragment encoding an initi-

* This work was supported in part by National Institutes of Health grants (to M. H. G. and J. F. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Recipient of a postdoctoral fellowship from the European Molecular Biology Organization.

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[1] The abbreviations used are: ER, endoplasmic reticulum; kb, kilobase; PAGE, polyacrylamide gel electrophoresis.

ation codon and the N terminus of the mature BiP polypeptide (amino acids 19–33; the signal sequence comprises residues 1–18 (29)). Site-directed mutagenesis using the oligonucleotide 5′-CTC CAT GGA TCC TCT CCT T-3′ was then employed to introduce a *Bam*HI restriction site immediately before the *Nco*I site that includes the BiP initiation codon. The resulting plasmid (pT7-mBiP-B) was cut with *Bam*HI, and the 2-kb fragment encoding mature BiP was inserted into the *Bam*HI site of plasmid pET-12a (Novagen, Madison, WI), in frame with the sequences encoding the OmpT signal peptide, yielding the expression vector SecB115.

Purification of BiP Expressed in Escherichia coli—BL21(DE3) cells (37) were transformed with SecB115 DNA. One ampicillin-resistant bacterial clone was used to inoculate M9ZB medium. The culture was grown, and the expression of T7 RNA polymerase was induced with isopropyl-1-thio-β-D-galactopyranoside as described previously (37). After 2 h of induction the cells were harvested, and the periplasmic extract was prepared as described by Neu and Heppel (38). A 60–80% ammonium sulfate fraction containing the expressed BiP protein was prepared and exhaustively dialyzed against 20 mM Tris, pH 7.0, containing 40 mM NaCl, 2 mM $MgSO_4$, and 0.25 mM phenylmethylsulfonyl fluoride (purification buffer). The dialysate was then applied at a flow rate of 20 ml/h to a DEAE-Sephacel column (100 ml) equilibrated in the same buffer. The column was washed until no further protein could be detected by Bradford protein assay (Bio-Rad), and then bound proteins were eluted with a linear gradient of purification buffer containing 40–275 mM NaCl. Aliquots of the fractions were analyzed by SDS-PAGE (Fig. 1), and the BiP-containing fractions were pooled, dialyzed against 20 mM Hepes pH 7.0, containing 20 mM KCl, 2 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 0.5 mM dithiothreitol, and 0.25 mM phenylmethylsulfonyl fluoride (Buffer A), and applied to a column (5 ml) of ATP-agarose (linked through C8, Sigma) at a flow rate of 10 ml/h. The column was washed with 5 volumes of Buffer A, and then BiP was eluted with 3 mM ATP dissolved in the same buffer (Fig. 1). Free ATP as well as ATP bound to BiP was removed by precipitation of the protein with 80% $(NH_4)_2SO_4$. The BiP pellet was washed once with a 90% saturated solution of $(NH_4)_2SO_4$ dissolved in 20 mM Hepes pH 7.0, containing 20 mM NaCl, 3 mM $MgSO_4$ (storage buffer) and stored in that solution at a concentration equal to 2–10 mg/ml. For use, the protein was dialyzed extensively against buffer A. In a typical experiment, 20 liters of an induced culture yielded 8–10 mg of BiP that was estimated to be 99% pure by densitometry following analysis by SDS-PAGE and staining with Coomassie Brilliant Blue, and by N-terminal amino acid sequence analysis performed by the automated Edman degradation method using an Applied Biosystems Inc. (Foster City, CA) model 470A amino acid sequenator according to the manufacturer's standard protocols.

ATPase Assays—Approximately 1 μg of BiP was incubated at 37 °C in 25 μl of Buffer A supplemented with ATP (0.1–8 μM final concentration containing 20 μCi of [γ-^{32}P]ATP). Following different times of incubation, 1-μl aliquots of the reaction mixtures were spotted on TLC plates that had been prespotted with 1 μl of a mixture of nonradioactive AMP, ADP, and ATP (each 10 mM). The plates were chromatographed for 15 min in 0.5 M LiCl, 0.5 M formic acid and then dried and exposed for autoradiography. Spots corresponding to the released [^{32}P]phosphate were located, excised, and put in scintillation fluid for determination of radioactivity. The amount of phosphate released was also estimated by densitometric scanning of the autoradiograph by comparison with a calibration set of standard ^{32}P-labeled solutions spotted and run under the same conditions. Assays of stimulation of ATPase activity were performed in the presence of peptide A (30 μM to 1 mM). The peptide A concentration was determined by absorbance, using a molar extinction coefficient estimated to be equal to $\epsilon_{280}^{GdnHCl} = 1280\ M^{-1}\ cm^{-1}$ according to Gill and Von Hippel (39).

Peptide Synthesis and Characterization—Peptides were prepared by continuous flow solid-phase synthesis performed on a 430A peptide synthesizer from Applied Biosystems Inc. (Foster City, CA) using fluorenylmethoxycarbonyl- or *tert*-butyloxycarbonyl-protected amino acids with *N*-methylpyrrolidone as a solvent system. Amino acids were activated with 1-hydroxybenzotriazole/2-(1-benzotriazol)-1,1,3,3-tetramethylluronium hexafluorophosphate. The peptides were analyzed by high pressure liquid chromatography on a Brownlee RP-300 column (Applied Biosystems Inc.) and by mass spectrometry carried out on a VG 30–250 quadrapole instrument equipped with an Ion-tech fast atom bombardment gun. The molar extinction coefficients at A_{280} of peptides ROS8 and PP52 were determined by amino acid analysis to be 5354 $M^{-1}\ cm^{-1}$ and 6127 $M^{-1}\ cm^{-1}$, respectively. N-terminal biotinylation of peptides PP52 and P17G was performed by incubation of resin-bound peptide with a 20-fold molar excess of a 1-hydroxybenzotriazole ester of biotin for 1 h at room temperature.

RESULTS

To examine the mechanisms involved in the regulation of the ATPase activity of BiP, we expressed murine BiP in *E. coli* with the aim of obtaining a homogeneous, unmodified species whose properties could be compared with those of BiP purified from bovine microsomes. The cDNA sequence encoding mature (signal-cleaved) mouse BiP (29) was subcloned into the pET12a expression vector (26) and expressed under the control of the T7 RNA polymerase promoter as described (37). Upon addition of isopropyl-1-thio-β-D-galactopyranoside to induce the synthesis of the polymerase, BiP was efficiently expressed and translocated into the bacterial periplasm whence it could be purified to homogeneity by chromatography on DEAE-Sephacel and ATP-agarose (Fig. 1). The BiP polypeptides bound to ATP-agarose and could be specifically eluted with ATP, suggesting that they were properly folded. The identity of the purified recombinant protein as rBiP was confirmed by immunoreactivity with an anti-mouse BiP antibody (results not shown) and by N-terminal sequence analysis, which also demonstrated that the protein was processed and did not contain the OmpT signal peptide. It, however, retained 5 extra residues, Ser-Thr-Gly-Ser-Met, at the N terminus that are encoded by OmpT and linker sequences. Periplasmic expression of rBiP has the following advantages. (i) We observed that expression of BiP in the cytosol of *E. coli* caused cell lysis after 2 h of induction. Secretion of BiP into the periplasmic space avoids this toxic effect and results in much higher expression levels. (ii) BiP purified from cytosolic extracts was significantly contaminated[2] with *E. coli* DnaK, the endogenous member of the HSP70 family (40). No DnaK protein is detectable when BiP is purified from periplasmic extracts (Fig. 1).

The ability of purified rBiP to hydrolyze [γ-^{32}P]ATP was assayed at 37 °C. The K_m for ATP was determined to be 0.4 μM, while the k_{cat} was 1.35 pmol of ATP hydrolyzed per min per μg (Fig. 2). The K_m value is similar to that described previously for canine BiP (22), but the k_{cat} is significantly different from the value of 0.35 pmol/min/μg reported for bBiP by Flynn *et al.* (1) and confirmed by ourselves (Fig. 2) using bBiP kindly provided by G. Flynn (University of Oregon). These results suggest that the basal activity of bBiP is approximately 4-fold lower than that of rBiP. bBiP and rBiP also differ in the extent to which their ATPase activities can be stimulated by the addition of a synthetic peptide (Fig. 2). As previously described (1) a 15-residue peptide, peptide A, whose sequence KRQIYTDLEMNRLGK corresponds to the C terminus of the G protein of vesicular stomatitis virus, stimulated the basal ATPase activity of bBiP in a concentration-dependent manner. Analysis of the data shown in Fig. 2 yielded values of 2.1 pmol of ATP hydrolyzed per min per μg of bBiP for k_{cat} and 670 μM for the concentration of peptide causing half-maximal stimulation, in agreement with the published data (1). The ATPase activity of rBiP was much less stimulated by peptide, although the maximum activity (about 2 pmol of ATP hydrolyzed per min per μg) was the same as that of bBiP. These results suggest that binding of peptide A causes bBiP to be converted from an inactive to an active form; however, the majority of rBiP molecules is already in an activated form in the absence of peptide.

Nondenaturing polyacrylamide gel electrophoresis revealed very different migration patterns for rBiP and bBiP (Fig. 3).

[2] S. Blond-Elguindi, unpublished data.

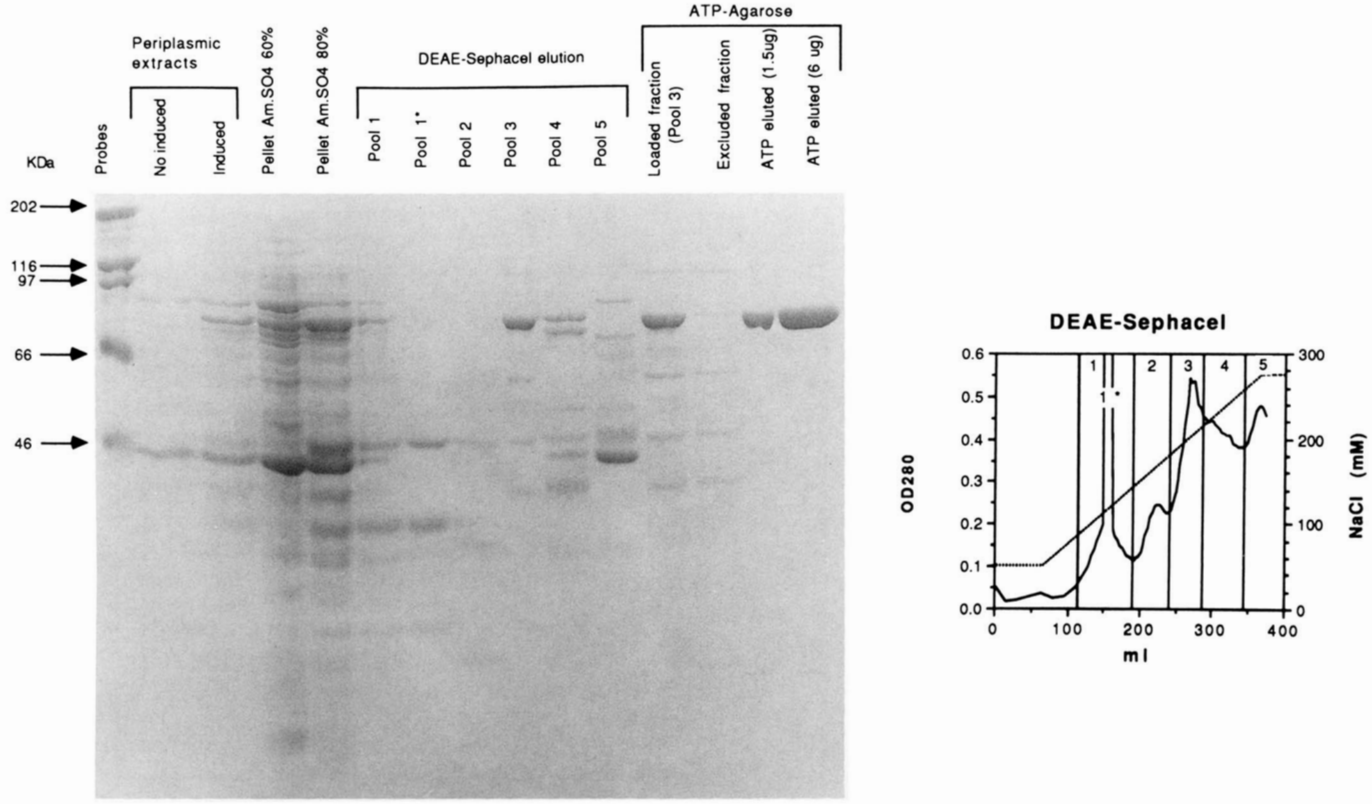

FIG. 1. **Purification of murine BiP expressed in *E. coli*.** A 10% SDS-polyacrylamide gel stained with Coomassie Brilliant Blue shows the protein species present in aliquots of samples from different stages of the procedure for purification of BiP expressed in *E. coli* BL21(DE3) cells carrying the SecB115 vector. The elution profile of the DEAE-Sephacel column is shown below the gel. ——, OD_{280}; · · · ·, NaCl.

bBiP consists of monomers, dimers, trimers, and higher molecular weight oligomers whose ratios vary somewhat under different experimental conditions (compare *lane 1* in Fig. 3, *a*, *b*, and *c*). In addition to each major species, minor, more slowly migrating species were also observed (best seen in Fig. 3*a*, *lane 1*). Although we have not attempted to identify the cause of this heterogeneity in the electrophoretic mobilities of the monomeric and oligomeric forms of bBiP, it is likely that the various species differ in their extent of post-translational modification by phosphorylation and/or ADP-ribosylation (30–32, 34). rBiP was predominantly present as monomers, with a small proportion of dimeric species (Fig. 3*a*, *lane 2*, and Fig. 3*d*, *lanes 1* and *3*). The rBiP monomers comigrate with a minor species of monomeric bBiP, whose mobility is significantly slower than that of the major monomeric bBiP species. Since bBiP and rBiP migrate identically as single 78-kDa species on SDS-PAGE (data not shown), the differences in migration between the major bBiP and rBiP species are probably the result of post-translational modification of the majority of the bBiP molecules. Lack of extensive modification of rBiP produced in *E. coli* was previously demonstrated by two-dimensional PAGE of recombinant hamster BiP (grp78) (33). However, rBiP monomers show some microheterogeneity, perhaps due to a limited degree of phosphorylation, which has been reported for the *E. coli* HSP70 protein, DnaK (41).

We investigated the effect of peptides on the state of oligomerization of bBiP and rBiP. Addition of peptide A induced the dissociation of all oligomeric forms of bBiP and the conversion of the various monomeric forms into a single species (Fig. 3*b*, *lanes 1–3*). This effect was dependent on the concentration of the peptide, being incomplete at a concentration (0.12 mM) below the K_m (0.67 mM) for stimulation of

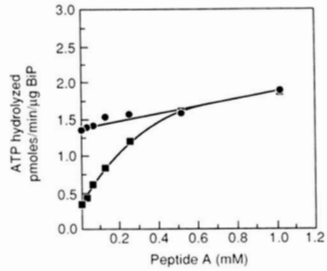

FIG. 2. **Peptide A-dependent stimulation of the ATPase activity of rBiP and bBiP.** ATP hydrolysis as a function of peptide A concentration for rBiP (●) and for bBiP (■). Assays were performed using an ATP concentration of 8 µM.

the ATPase activity of bBiP (see above) but complete at a concentration (2.5 mM) above that required for maximal stimulation (Fig. 2). Peptide A also induced a decrease in the mobility of rBiP monomers which, at decreased loading and in the absence of peptide, migrated as a triplet in which the three closely spaced bands are present in about equal amounts (Fig. 3*b*, *lane 4*). In the presence of peptide A, the slower band of the triplet was significantly enriched (Fig. 3*b*, *lane 6*). The slight mobility change may reflect the small increment in mass of the complex and/or a conformational change in the monomer induced by the binding of the peptide. These effects are not unique to peptide A since they were also observed with peptide PP52 (YVDRFIGW), identified as a BiP binding sequence by panning of a library of peptides displayed on bacteriophage fd particles[3] (Fig. 3*c*, *lane 3*; Fig. 3*d*, *lanes 2* and *4*; and Fig. 4*a*, *lane 2*), and with peptide P17G

[3] S. Blond-Elguindi, S. E. Cwirla, W. J. Dower, R. J. Lipshutz, J. F. Sambrook, and M. J. Gething, manuscript in preparation.

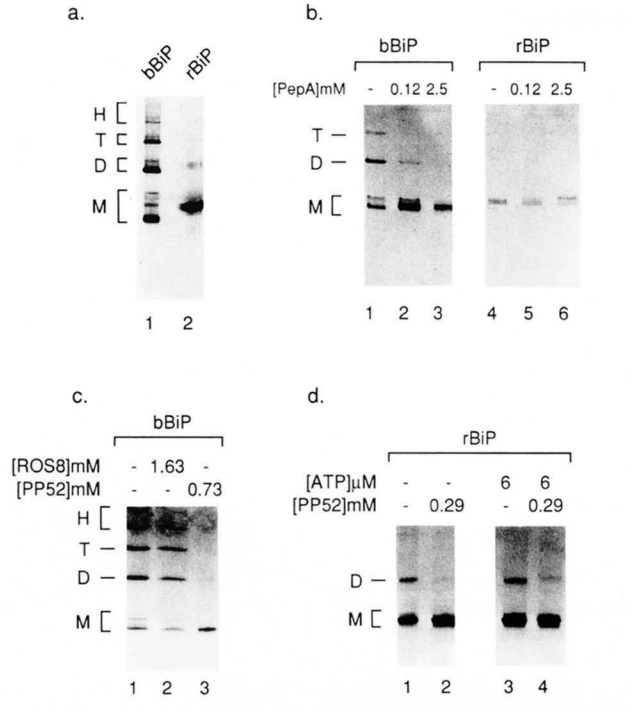

FIG. 3. **Effect of peptides on the oligomerization states of bBiP and rBiP.** BiP samples (0.2–1 μg in Buffer A) were incubated in the absence or presence of peptides for 30 min on ice (*panels a* and *b*) or at 37 °C (*panels c* and *d*) before analysis by electrophoresis on 8–25% polyacrylamide gels run under nondenaturing conditions and visualization by silver staining. In the experiment shown in *panel b*, 8 μM ATP was included in the incubation mixture. *M*, monomer; *D*, dimer; *T*, trimer; *H*, higher oligomers.

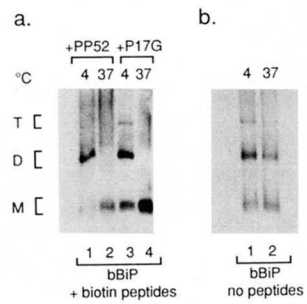

FIG. 4. **Peptide binding to oligomeric and monomeric forms of bBiP.** *a*, bBiP (0.5 μg in 40 mM Hepes pH 7.0 containing 75 mM KCl, 4.5 mM MgOAc) was incubated with 1 mM biotinylated peptides PP52 or P17G for 45 min at 4 °C. The incubation was then continued at 4 °C (*lanes 1* and *3*) or at 37 °C (*lanes 2* and *4*) for a further 45 min. The different oligomeric forms of BiP were then separated from free peptide by electrophoresis on 7% polyacrylamide gels run under nondenaturing conditions. The proteins were then transferred to nitrocellulose, and complexes between BiP and biotinylated peptides were detected by incubation with streptavidin-peroxidase and the ECL detection system (Amersham Corp.). *b*, control BiP samples were treated as in *a* but in the absence of peptides and were visualized by silver staining. *T*, trimer; *D*, dimer; *M*, monomer.

(PLSQETFSGLWKLLPPEDG), which corresponds to a hsc70 binding site in p53 (42) (Fig. 4*a*, *lane 4*). These peptides contain the motif (underlined) of three or four hydrophobic residues in alternating positions shared by the majority of BiP-binding peptides identified in the bacteriophage panning experiments.[3] However, not all synthetic peptides are able to induce either the dissociation of BiP oligomers or conformational changes within the monomer since a fourth peptide, ROS8 (DIYKNDYY), which lacks the binding motif and does not stimulate the ATPase activity of bBiP or rBiP (data not shown), did not induce any change in the mobility of bBiP conformers (Fig. 3*c*, *lane 2*).

The bBiP and rBiP proteins used in this study were both purified by protocols that included ATP-agarose chromatography, but the ATP was removed by ammonium sulfate precipitation and exhaustive dialysis. The addition of ATP at concentrations (6–8 μM) above the K_m for the ATPase reaction did not cause significant dissociation of bBiP oligomers (Fig. 3, *a* and *b*). Furthermore, the addition of ATP was not required for the peptide-induced dissociation of oligomeric forms of bBiP (Fig. 3*c*) or rBiP (Fig. 3*d*). Other workers have reported that the addition of ATP at a significantly higher concentration (1 mM) promotes the dissociation of oligomeric forms of bBiP even at 0 °C (33). We found that the addition of millimolar range ATP did not induce the dissociation of rBiP dimers, although bBiP oligomers were partially dissociated under these conditions (data not shown).

Finally, we investigated whether peptides bind to bBiP oligomers and directly cause their dissociation or whether they bind solely to BiP monomers and cause a readjustment of the equilibrium between monomers and oligomers in favor of the monomeric species. bBiP was incubated with biotinylated peptides PP52 and P17G under conditions (4 °C) that retard peptide-induced dissociation of bBiP oligomers. Incubations were also performed at 37 °C to promote peptide-induced dissociation. BiP-peptide complexes were separated from free peptides by nondenaturing polyacrylamide gel electrophoresis and then transferred to nitrocellulose. Complexes of BiP and biotinylated peptides were visualized by incubation of the nitrocellulose sheet with peroxidase-conjugated streptavidin and an enhanced chemiluminescence detection system (Fig. 4*a*), while silver staining was used to visualize BiP species present in the absence of peptides (Fig. 4*b*). At 4 °C, biotinylated peptides bound to the trimeric, dimeric, and monomeric forms of BiP (Fig. 4*a*, *lanes 1* and *3*), preferentially associating with the predominant dimeric form of the protein (see Fig. 4*b*, *lane 1*). Following incubation at 37 °C in the presence of peptides the oligomeric forms were dissociated, and peptides were associated only with monomeric BiP species (Fig. 4*a*, *lanes 2* and *4*). Incubation at 37 °C of bBiP in the absence of peptides caused only partial dissociation of the oligomers (Fig. 4*b*). These results suggest that peptide-induced dissociation of BiP oligomers occurs as a consequence of binding of peptides to the oligomeric species.

DISCUSSION

We conclude that differences in the basal levels of ATPase activity of recombinant BiP and of BiP prepared from bovine liver reflect differences in the state of oligomerization and, possibly, modification of the two purified proteins. Monomeric species (which predominate in the recombinant protein purified from *E. coli*) display maximal activity, while the various oligomeric species (which form a significant proportion of bovine BiP) are less active or inactive. The ability of some peptides to stimulate the ATPase activity of BiP *in vitro* (1) is the result of their capacity to bind to the oligomeric species and promote their dissociation to active monomers. Dissociation of oligomers and stimulation of ATPase activity display the same dependence on peptide concentration, suggesting that the two processes are directly linked. The magnitude of the stimulatory effect depends on the relative proportions of monomers and oligomers in the bBiP and rBiP preparations because the maximum specific activity, achieved when all the protein is monomeric, is the same for both recombinant and bovine BiP. We have obtained similar results in studies with bovine and recombinant cytosolic hsc70

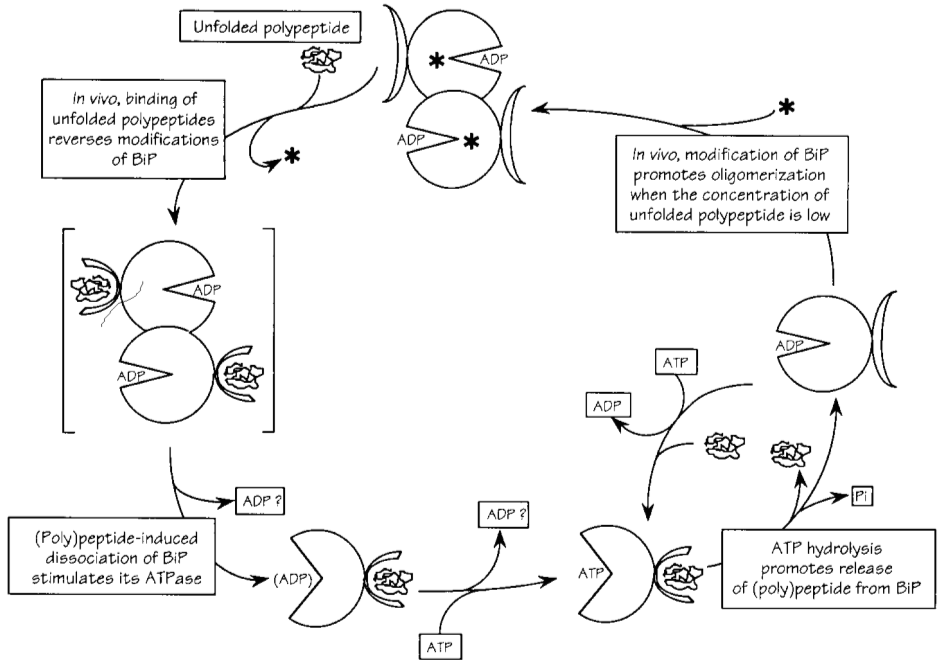

FIG. 5. **A model illustrating proposed pathways of interconversion of oligomeric and monomeric species of BiP.** Counterclockwise from *top*: in quiescent, unstressed cells the majority of BiP molecules is present as oligomeric species that are post-translationally modified by phosphorylation and/or ADP ribosylation (30–32, 34). Conditions that result in an increase in the concentration of unfolded polypeptides in the ER promote the reversal of modifications and the dissociation of BiP oligomers (30, 34). Our data demonstrate that both the oligomeric and monomeric species are capable of binding peptides and that peptide-induced stimulation of BiP's ATPase activity reflects the conversion of inactive or less active oligomers into active monomers. Hydrolysis of ATP by BiP then promotes release of the (poly)peptide from the complex (28, 29). In the continued presence of unfolded polypeptides and ATP, cycles of polypeptide binding, ATP hydrolysis, and polypeptide release continue. When the concentration of unfolded polypeptides in the ER decreases, free BiP molecules become modified and oligomer formation is favored.

proteins,[4] suggesting that peptide-induced dissociation of oligomeric forms of proteins of the HSP70 family is a general phenomenon. A model illustrating proposed pathways of interconversion of the various forms of BiP (or other HSP70 family members) that is compatible with our observations and with published data (28–34) is shown in Fig. 5.

An important feature of the model is that changes in the steady state levels of unfolded polypeptides in the ER affect the degree of post-translational modification of BiP, which in turn alters the propensity of BiP to oligomerize. Mammalian BiP exists *in vivo* as an apparently interconvertible mixture of post-translationally modified oligomers and unmodified monomers, whose proportions vary with the physiological state of the cell (34). Conditions that increase the levels of unfolded polypeptides in the ER cause a decrease in the extent of modification and an increase in the proportion of monomeric species (30, 34). Only unmodified, monomeric BiP molecules were found in complexes with unfolded or unassembled polypeptides (32, 34). Our *in vitro* results are consistent with these findings, since at 37 °C synthetic peptides that bind BiP cause the conversion of a complex mixture of oligomeric and monomeric BiP species to a single monomeric form. However, our results do not support the suggestion that only the monomeric species is active in complexing other proteins (34), since we have shown that biotinylated peptides bind to both monomeric and oligomeric forms of BiP. Taken together, the available data suggest that oligomeric and modified forms of BiP represent an inactive storage pool of the chaperone, which can be rapidly mobilized in the presence of an increased level of unfolded polypeptides in the lumen of the ER. Because the rate of *de novo* synthesis of BiP is also increased in response to the accumulation in the ER of unfolded polypeptides (6, 8, 9, 43), the size of the storage pool may be monitored via sensing of the degree of modification of BiP molecules (34). Thus the proximal signal for induction of transcription of BiP mRNA in the nucleus may be the disappearance of modified and/or oligomeric forms of BiP in the ER (44). Work is in progress to understand the relationship between post-translational modification of BiP, its state of oligomerization, and the regulation of its activity *in vivo* and *in vitro*.

[4] A. M. Fourie, unpublished results.

Acknowledgments—We are indebted to Dr. Greg Flynn for the gift of purified bovine BiP, and we thank Dr. Maurine Linder for the Np7T7 vector, Dr. Douglas Young for the idea of using biotinylated peptides, and Lynn DeOgny for help in their preparation. We thank Dr. Ibrahim Elguindi for critical comments on the manuscript.

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