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BiP Binding Sequences in Antibodies*

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During the process of folding and assembly of antibody molecules in the endoplasmic reticulum, immunoglobulin heavy and light chains associate transiently with BiP, a resident endoplasmic reticulum protein that is a member of the Hsp70 family of molecular chaperones. BiP is thought to recognize unfolded or unassembled polypeptides by binding extended sequences of approximately seven amino acids that include bulky hydrophobic residues not normally exposed on the surface of native proteins. We used a computer algorithm developed to predict BiP binding sites within protein primary sequences to identify sites within immunoglobulin chains that might mediate their association with BiP. Very few of the sequential heptapeptides in the heavy or light chain sequences were potential BiP binding sites. Analysis of the ability of synthetic heptapeptides corresponding to 24 potential sites in heavy chains to stimulate the ATPase activity of BiP indicated that at least half of them were authentic BiP binding sequences. These sequences were not confined to a single domain of the heavy chain but were distributed within both the V_H and C_H domains. Interestingly, when the BiP binding sequences were mapped onto the three-dimensional structure of the Fd antibody fragment, the majority involve residues that participate in contact sites between the heavy and light chains. Therefore, we suggest that *in vivo* BiP chaperones the folding and assembly of antibody molecules by binding to hydrophobic surface regions on the isolated immunoglobulin chains that subsequently participate in interchain contacts.

BiP,[1] the sole ER-located member of the Hsp70 family of molecular chaperones, was originally described as the immunoglobulin heavy chain binding protein, found in non-covalent association with heavy chains in myeloma cells that do not synthesize immunoglobulin light chains (Haas and Wabl, 1983). Interestingly, BiP was independently identified as a protein (GRP78) expressed in response to glucose starvation (Pouyssegur *et al.*, 1977). BiP is now known to associate transiently not only with immunoglobulin heavy and light chains during their folding and assembly (Bole *et al.*, 1986; Hendershot *et al.*, 1987; Nakaki *et al.*, 1989; Dul and Argon, 1990, Ma *et al.*, 1990; Knittler and Haas, 1992) but also with a wide variety of other newly synthesized wild-type exocytotic proteins (for reviews, see Gething and Sambrook (1992); Gething *et al.* (1994); Haas (1994)). BiP interacts more permanently with malfolded or unassembled proteins whose transport from the ER is blocked but does not bind to native proteins. These observations indicate that BiP plays a role in the folding and assembly of newly synthesized proteins in the ER lumen. Like other Hsp70 proteins, BiP is thought to function by recognizing hydrophobic sequences exposed on unfolded or unassembled polypeptides and, by inhibiting intra- or intermolecular aggregation, maintaining them in a state competent for subsequent folding and oligomerization (Gething and Sambrook, 1992; Craig *et al.*, 1993; Becker and Craig, 1994; Hightower *et al.*, 1994).

Although the transient interaction between BiP and immunoglobulin molecules has been well documented, little is known about the location or nature of the sequences on the heavy and light chains that are recognized by the chaperone. Indirect evidence points to the C_H1 domain of the heavy chain being required for association with BiP (Hendershot *et al.*, 1987). Thus, whereas mutant heavy chains lacking C_H2 or C_H3 domains are complexed with BiP and remain in the ER, chains lacking C_H1 do not appear to interact with BiP and are transported along the secretory pathway. Other studies indicate that sequences within the V_H domain influence the association of heavy chains with BiP (Haas, 1991). To identify potential BiP binding sequences in Ig heavy chains, we took advantage of a computer scoring system designed to predict BiP binding sites in synthetic peptides and naturally occurring polypeptides (Blond-Elguindi *et al.*, 1993a). We scored sequences from two different but related antibodies, MAK 33 and 3D6. Monoclonal antibody MAK 33 has been used in a number of studies on antibody folding *in vitro* (Buchner and Rudolph, 1991; Buchner *et al.*, 1991; Lilie *et al.*, 1995a, 1995b) and on the influence on this process of folding catalysts and chaperones (Wiech *et al.*, 1992; Schmidt and Buchner, 1992; Schmidt *et al.*, 1994; Lilie *et al.*, 1993, 1994; Lilie and Buchner, 1995). Since the three-dimensional structure of this antibody is not yet known, we also scored the Fd portion of the monoclonal antibody 3D6, which three-dimensional structure had been determined to a resolution of 2.7 Å (He *et al.*, 1992).

We report that it is possible to predict potential BiP binding sites in the two different immunoglobulin sequences using the

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[1] The abbreviations used are: BiP, immunoglobulin heavy chain binding protein; ER, endoplasmic reticulum; Fab, proteolytically derived antibody fragment consisting of the entire immunoglobulin light chain and the two amino-terminal domains of the heavy chain; Fd, part of the immunoglobulin heavy chain consisting of the two amino-terminal domains (V_H and C_H1); Fc, part of the immunoglobulin heavy chain consisting of the two carboxyl-terminal domains (C_H2 and C_H3); MAK33, murine antibody of the subclass κIgG1, directed against human creatine kinase; 3D6, human monoclonal antibody of the subclass κIgG1 from the cell line 3D6 directed against the gp41 protein of the human immunodeficiency virus-1; Fmoc, *N*-(9-fluorenyl)methyloxycarbonyl.

scoring procedure. Only a small number of potential BiP binding sites were identified, with the majority of the antibody sequences having a very low probability of binding to BiP. The potential binding sites in the Ig heavy chains are distributed within both the V_H and C_H domains, and the majority involve residues that participate in contact sites between the heavy and light chains. We suggest that BiP chaperones the folding and assembly of antibody molecules by binding to hydrophobic surface regions on the isolated immunoglobulin chains that subsequently participate in interchain contacts.

MATERIALS AND METHODS

Purification of BiP from Bovine Pancreas—BiP used in ATPase stimulation assays was isolated from bovine pancreas based on a procedure described previously (Wiech *et al.*, 1993). All steps of the isolation were performed at 4 °C. Bovine pancreas was homogenized, MgATP was added, and the homogenate was centrifuged twice to remove cell fragments, nuclei, and mitochondria. Subsequently, the microsomes were pelleted by ultracentrifugation. The post-microsomal supernatant contained a considerable amount of BiP, which could be separated from Hsc70 and Hsp90 by DE52 anion exchange chromatography with a linear NaCl gradient (100–200 mM). BiP-containing fractions were pooled, concentrated, and dialyzed against 40 mM HEPES, 100 mM KCl, 20 mM KOH, 10 mM $(NH_4)_2SO4$, 4 mM $MgCl_2$, 2 mM potassium acetate, 1 mM dithiothreitol, 5% (v/v) glycerol, 0.005% (v/v) Triton X-100, pH 7.8 (buffer L). The resuspended rough microsomes were supplemented with NaCl and Triton X-100 to a final concentration of 100 mM and 0.1% (v/v), respectively. After homogenization, the membranes were removed by centrifugation at 140,000 × *g* for 120 min. Subsequent DE52 anion exchange chromatography was performed as described previously (Wiech *et al.*, 1993). The dialyzed pools of BiP were then applied to a C8 ATP-agarose column (Sigma). After washing the column with buffer L containing 10 mM EDTA, but no $MgCl_2$, BiP was eluted with buffer L supplemented with 0.5 M KCl and 4 mM MgATP. The eluate was dialyzed against 40 mM potassium phosphate, pH 7.0 (buffer B), $(NH_4)_2SO_4$ was added to a final concentration of 2 M, and the solution was then applied to a butyl-Sepharose 4 Fast Flow column (Pharmacia Biotech Inc.). BiP was released from the column by a linear gradient from 2 to 0 M $(NH_4)_2SO_4$ (in buffer B). The fractions of pure BiP were concentrated and stored in buffer L containing 25% (v/v) glycerol at −70 °C.

ATPase Measurements—ATPase assays were performed according to Kornberg *et al.* (1978). Approximately 4 μg of purified BiP were incubated at 37 °C with 500 μM (final concentration) unlabeled ATP and 10 μCi of [α-^{32}P]ATP in a total volume of 20 μl containing 40 mM HEPES, pH 7.0, and 2 mM $MgCl_2$. The stimulation of the ATPase activity by peptide was determined in the presence of concentrations of 10 μM to 1 mM of the respective peptide. Following different times of incubation, 3-μl aliquots of the reaction mixture were added to 2 μl of a mixture of ATP, ADP, AMP, and EDTA (each 12 mM) to terminate the reaction. Thin layer chromatography was performed in 0.5 M LiCl and 2 N formic acid. The plates were dried, and the spots corresponding to ATP and ADP were located with UV light and excised; the radioactivity was then determined by liquid scintillation. The radioactivity in each spot was expressed as a fraction of the total recovered in each lane.

Peptide Synthesis and Purification—Peptides were synthesized using a 9050 PepSynthesizer (Milligen) and Fmoc-protected amino acids (Atherton *et al.*, 1978). The first residue was coupled to polystyrene beads via an acid-labile linker (Tenta-Gel, Rapp-Polymere). Double coupling cycles were used for residues following proline or glycine and when identical or similar residues were clustered, in order to avoid incomplete coupling reactions. *t*-Butyl (Ser, Thr, Tyr), *t*-butylester (Asp, Glu), trityl (Cys, His, Gln, Asn), *t*-butyloycarbonyl (Lys), and 4-methoxy-2,3,6-trimethylbenzene-sulfonyl (Arg) groups were used for side chain protection. Fmoc-protected amino acids were converted to hydroxy-benzotriazol-activated esters with 1.5 mmol of hydroxybenzotriazol and 1.2 mmol of diisopropycarbodiimide per mmol of amino acid, prior to the coupling procedure. The subsequent coupling reaction was performed in *N*,*N*-1-dimethylformamide, and coupling times of 10 min were used. Fmoc groups were removed by 20% piperidine, and the completeness of this reaction was monitored by fluorometric measurements (all amino acids were purchased from Bachem AG, solvents were from Merck AG, and chemicals were from Aldrich). After synthesis, side chain protecting groups were removed by treatment for 4 h in 50% trifluoroacetic acid in dichloromethane or in 100% trifluoroacetic acid overnight when Arg residues were present in the peptide sequence. 3% anisol and 3% phenol was routinely added as scavengers including 5% 2-mercaptoethanol in cases where Arg and Trp residues were combined in the peptide sequence. Subsequently, solvents were evaporated, the peptide suspended in 1–2 ml acetic acid and precipitated in an excess of ice-cold *t*-butylethylether, washed several times, and suspended in water. The peptide was lyophilized and purified by reversed phase high performance liquid chromatography using a C2/C18 copolymer column (PepS, Pharmacia) and a gradient of 0–70% acetonitrile in 0.1% trifluoroacetic acid. Finally, the peptide-containing fractions were lyophilized.

RESULTS

Prediction of Potential BiP Binding Sites in the Sequences of Monoclonal Antibodies 3D6 and MAK33—The sequences of the MAK33 and the 3D6 antibodies were screened for potential BiP binding sites using the BiP Score computer program (Blond-Elguindi *et al.*, 1993a). The algorithm used in this program scores amino acid sequences with a moving window of seven residues, the apparent size of the (poly)peptide binding cleft on BiP (Flynn *et al.*, 1991; Blond-Elguindi *et al.*, 1993a). An individual score is assigned to each amino acid depending on its position within the heptapeptide, and the sum of the seven scores provides a measure of the probability that a given heptapeptide would bind to BiP. Heptapeptides with scores greater than 10 are very likely to bind to BiP, while peptides with scores between +6 and +10 have odds of 3 to 1 of displaying binding activity. Scores from 0 to +5 have little predictive value, but peptides with negative scores almost invariably lack the capacity to bind to BiP.

Fig. 1 shows the results of the scoring procedure for heavy chain sequences of the two antibodies. The scores range from −28 to +12 for heptapeptides contained in the 3D6 Fd fragment and from −31 to +20 for those from the MAK33 heavy chain (Fd + Fc). Only a small fraction of the heptapeptides (<8% for 3D6 sequences, <5% for MAK33 sequences) had scores greater than +6, indicating that they were potential sites for binding to BiP. The majority of these had scores in the range between +6 and +10 (14 out of 223 peptides in the case of 3D6, 18 out of 448 peptides in the case of MAK33) and should therefore bind to BiP with a probability of 3 to 1. Only a very few (3 in the case of 3D6 and 4 in the case of MAK33) had scores greater than +10, indicating a very high probability of binding. Peptides with positive scores were frequently clustered in small groups of 2–5 peptides but were distributed throughout the 3D6 Fd fragment and the MAK33 heavy chain, indicating no special preference for a distinct antibody domain. Very similar results were obtained when the light chains of the two antibodies were scored (Fig. 2).

Many Synthetic Peptides Corresponding to Predicted BiP Binding Sites in Ig Heavy Chains Stimulate the ATPase Activity of BiP—To test the predictive capacity of the BiP Score program, we checked the ability of synthetic peptides corresponding to predicted BiP binding sites in the Ig heavy chains to stimulate the ATPase activity of BiP (Flynn *et al.*, 1989). 34 peptides were synthesized. 26 of these corresponded to sequences from the heavy chain of antibody 3D6 (named HD14–183), including 15 potential binding sites with scores ranging from +5 to +12, and 11 sites with scores ranging from +1 to −28, which are unlikely to be BiP binding sequences. A further 8 peptides corresponding to potential sites in the heavy chain of MAK33 (named HM27 to 430) had scores of +7 to +20. The peptide HD111R does not correspond to a natural 3D6 peptide but is a randomized version of peptide HD111 having the same amino acid composition but a different sequence and a significantly lower BiP Score (−6 *versus* +10). Most of the synthetic peptides were heptamers, but when the carboxyl-terminal residue of the chosen heptapeptide was proline or tryptophan, thus causing problems with synthesis, octapeptides that in-

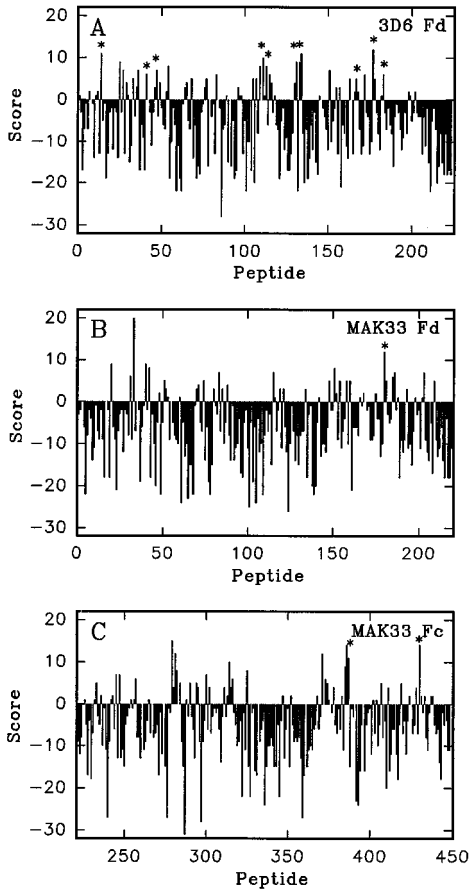

FIG. 1. **Prediction of BiP binding sequences in the primary structure of the heavy chains of antibodies 3D6 and MAK33.** Overall scores for each of the overlapping heptapeptides in these sequences were calculated using the BiP Score program described by Blond-Elguindi *et al.* (1993a) within the Fd fragment of antibody 3D6 (*A*) and the Fd (*B*) and the Fc (*C*) fragments of antibody MAK33, respectively. *Asterisks* indicate the positively scoring sequences in the 3D6 Fd fragment that, when tested as synthetic peptides, stimulated the ATPase activity of BiP (see Table I).

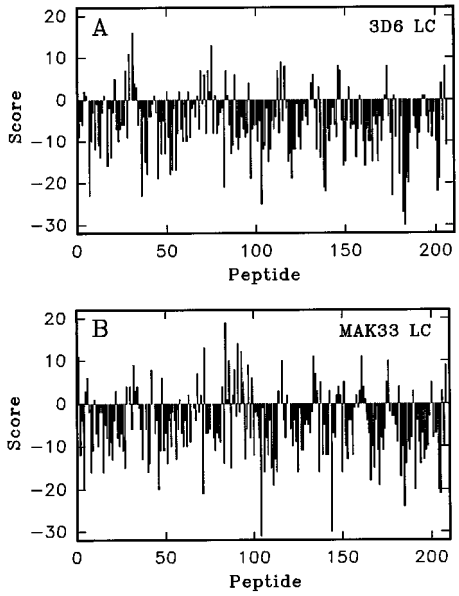

FIG. 2. **Prediction of BiP binding sequences in the primary structure of the light chains of antibodies 3D6 (*A*) and MAK33 (*B*).** Overall scores for each of the overlapping heptapeptides in these sequences were calculated using the BiP Score program described by Blond-Elguindi *et al.* (1993a).

cluded the next residue in the sequence were synthesized (see peptides HD47/48, HD133/134, and HM387/388). In each case, these peptides contain two potential seven-residue binding sites, and the overall BiP score for the second heptapeptide is also provided in Table I.

Table I presents the sequences and scores of the synthetic peptides together with the results of assays of their ability to stimulate the ATPase activity of BiP when added at a concentration of 500 μM. Of the 23 peptides having BiP scores of at least +5, 12 stimulated BiP ATPase activity by factors ranging from 1.9 to 3.8. These stimulation factors correspond well to those obtained in the same experiment for two peptides, pp28 and pp37, selected as BiP binding peptides by bacteriophage display (Blond-Elguindi *et al.*, 1993a) and to those reported previously for other peptides tested at this concentration (Flynn *et al.*, 1989; Blond-Elguindi *et al.*, 1993b; Fourie *et al.*, 1994). An additional two peptides displayed consistent but lower stimulatory capacity (1.4- and 1.6-fold, respectively). As "negative controls" we tested a set of 10 heptapeptides that had negative BiP scores and one peptide with a score of +1. All of these peptides failed to stimulate the ATPase activity of BiP (Table I). The fact that peptide HD111 (score +10) displayed one of the highest stimulation factors (3.0) while its randomized version peptide 111R (score, −6) failed to stimulate BiP ATPase activity confirms that it is not the composition of amino acids that determines the ability of a peptide to bind to BiP but rather their specific sequence in the peptide.

The concentration dependence of ATPase stimulation was measured for four peptides corresponding to sequences from the 3D6 heavy chain and for the previously characterized pp28 peptide. The data shown in Fig. 3 yielded a K_m value of 60 ± 10 μM for peptide HD111, defined as the concentration of peptide required for half-maximal stimulation of the ATPase activity of BiP. As shown in Table II, peptides HD131, HD133, and HD177 caused half-maximal stimulation at even lower concentrations (6–17 μM), while the K_m obtained for peptide pp28 was 16 ± 3 μM, which is in good agreement with the value of 10 ± 2 μM obtained previously (Blond-Elguindi *et al.*, 1993a).

Finally, the capacity of peptide HD111 to bind to BiP was tested using the ability of the peptide to compete the binding of BiP to reduced and carboxymethylated lactalbumin as described previously (Fourie *et al.*, 1994). Competition of reduced and carboxymethylated lactalbumin binding was approximately 30–40% at a peptide concentration of 50 μM, while 100 μM peptide caused approximately 70–80% competition. Extrapolation of this data suggests that half-maximal competition of binding would be achieved at 60–70 μM, a value in excellent agreement with the K_m value measured for ATPase stimulation (data not shown).

Localization of BiP Binding Sequences in the Three-dimensional Structure of Antibody 3D6—Finally we investigated the position in the three-dimensional structure of 3D6 of the BiP binding sequences that we had identified as causing significant stimulation of the ATPase activity of BiP. Fig. 4 shows the V_H and C_H1 sequences of antibody 3D6, with the 10 peptides that were predicted and shown to be positive with respect to BiP binding outlined by *frames*. The residues of 3D6 involved in the interface between the H-chain and the L-chain (shown by *shaded boxes*) were determined by comparing the surface exposure of side chains in the crystal structure of the dimer and the artifically separated monomers. With only two exceptions (HD14 and HD167), the BiP binding sequences contain multiple residues that participate in the contact sites. In total, of 48 contact residues 25 are located within BiP binding sequences. These 25 residues include the majority of the contact site residues that are hydrophobic in character. These observations

TABLE I
BiP scores and ATPase stimulation of antibody peptides

Peptide	Sequence	Score for position							BiP score	Stimulation factor
		1	2	3	4	5	6	7		
V_H										
HD14	PGRSLRL	−1	2	2	2	3	−2	5	+11	3.3 ± 0.2
HD25	SGFTFND	−3	2	8	−2	2	0	2	+9	1.3 ± 0.5
HD27	FTFNDYA	7	2	8	2	0	0	−12	+7	1.0 ± 0.1
HD36	WVRQAPG	5	1	2	0	−1	0	0	+7	1.1 ± 0.1
HD41	PGKGLEW	−1	2	0	−2	3	0	4	+6	3.1 ± 0.2
HD44	GLEWVSG	−2	−1	−9	4	0	−3	0	−11	1.0 ± 0.2
HD47	WVSGISW(D)	5	1	0	−2	2	−3	4	+7 (−4)	2.7 ± 0.3
HD86	LRAEDMA	2	−6	−4	−6	0	−2	−12	−28	1.1 ± 0.1
HD97	VKGRDYY	0	0	0	0	0	0	1	+1	1.3 ± 0.1
HD109	FTVAFDI	7	2	−2	0	2	0	−1	+8	1.0 ± 0.2
HD111	VAFDIWG	0	−2	8	0	2	2	0	+10	3.0 ± 0.6
HD111R	DVAWIFG	1	−10	1	−4	2	1	0	−6	1.1 ± 0.2
HD113	FDIWGQG	7	−1	0	4	−2	0	0	+8	1.9 ± 0.5
C_H1										
HD125	SSASTKG	−3	−2	−4	2	0	−2	0	−9	0.9 ± 0.1
HD131	GPSVFPL	−2	−6	0	−2	2	12	5	+9	2.5 ± 0.2
HD132	PSVFPLA	−1	−2	−2	−1	−6	2	−12	−22	1.1 ± 0.1
HD133	SVFPLAP(S)	−3	1	8	1	3	0	−1	+9 (+1)	2.5 ± 0.4
HD138	APSSKST	−5	−6	0	2	−8	−3	1	−19	1.1 ± 0.1
HD151	LGCLVKD	2	2	2	1	0	−2	2	+7	1.3 ± 0.2
HD167	WNSGALT	5	0	0	−2	−1	2	1	+5	2.4 ± 0.3
HD169	SGALTSG	−3	2	−4	1	0	−3	0	−7	1.1 ± 0.2
HD173	TSGVHTF	−2	−2	0	−2	−1	2	2	−3	1.0 ± 0.2
HD175	GVHTFPA	−2	1	0	−2	2	0	−12	−13	1.0 ± 0.2
HD177	HTFPAVL	0	2	8	1	−1	−3	5	+12	3.0 ± 0.4
HD181	AVLQSSG	−5	1	1	0	−6	−3	0	−12	1.1 ± 0.2
HD183	LQSSGLY	2	1	0	2	−2	2	1	+6	2.4 ± 0.4
V_H										
HM27	FTFSDYY	7	2	8	2	0	0	1	+20	1.4 ± 0.1
HM36	WVRQTPE	5	1	2	0	0	0	0	+8	1.1 ± 0.1
HM110	AMDYWGQ	−5	3	−2	3	4	−1	5	+7	1.2 ± 0.1
C_H3										
HM371	LTCMITD	2	2	2	0	2	2	2	+12	1.6 ± 0.2
HM385	VEWQWNG	0	−1	6	0	4	0	0	+9	1.0 ± 0.1
HM386	EWQWNGQ	−2	6	2	4	0	−1	5	+14	1.0 ± 0.1
HM387	WQWNGQP(A)	5	1	6	2	−2	0	−1	+11 (−15)	2.0 ± 0.4
HM430	FTCSVLH	7	2	2	2	0	2	−1	+14	1.9 ± 0.2
pp28	(H)WDFAWPW	5	−1	8	0	4	12	4	(+3) +32	2.1 ± 0.3
pp37	(F)TYGSRWL	−2	−1	0	2	1	2	5	(−1) +7	2.8 ± 0.1

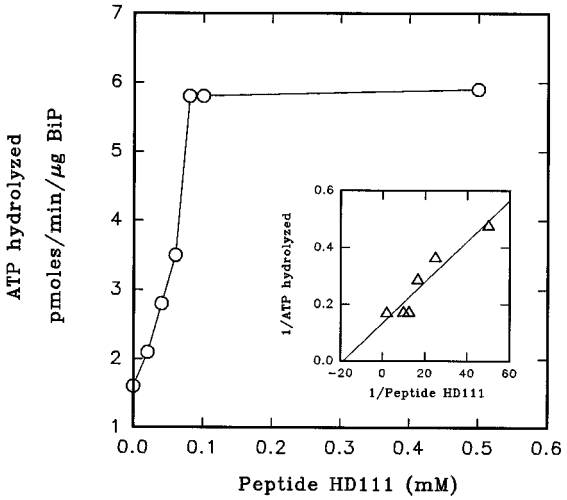

FIG. 3. **Stimulation of the ATPase activity of BiP by peptide HD111.** The concentration dependence of the stimulation of ATP hydrolysis by peptide HD111 was determined as described under "Materials and Methods." The concentration of peptide necessary for half-maximal stimulation was calculated to be 60 μM.

suggest that binding of BiP to one or more of several potential binding sites exposed within the contact interface on folded but unassembled heavy chains would shield the hydrophobic surfaces and inhibit unproductive aggregation reactions.

TABLE II
Affinity of antibody peptides for BiP

Peptide	Score	Stimulation factor	K_M ATPase stimulation
			μM
HD111	+10	3.0	60 ± 10
HD131	+9	2.3	9 ± 4
HD133	+9	2.5	6 ± 4
HD177	+12	3.4	17 ± 6
pp28	+32	2.8	16 ± 3

DISCUSSION

Using a computer program that predicts recognition sites within protein sequences for binding of the ER-located chaperone BiP (Blond-Elguindi *et al.*, 1993a), we identified a number of potential BiP binding sites in the primary sequences of antibodies 3D6 and MAK33. Only a small proportion of all the sequential heptapeptides within the immunoglobulin sequences are potential BiP binders (*i.e.* have BiP Scores ≥+5). Measurement of the ability of synthetic peptides corresponding to 23 potential sites in the immunoglobulin heavy chains to stimulate the ATPase activity of purified BiP demonstrated that 12 of them were indeed BiP binding sequences. Our results confirm that the predictive power of the scoring program is greater than 50% for positive peptides, rising to ~80% when peptides with BiP scores ≥10 are considered, and it is close to 100% for negative peptides. Analysis of the concentration dependence of stimulation of BiP by a subset of the peptides

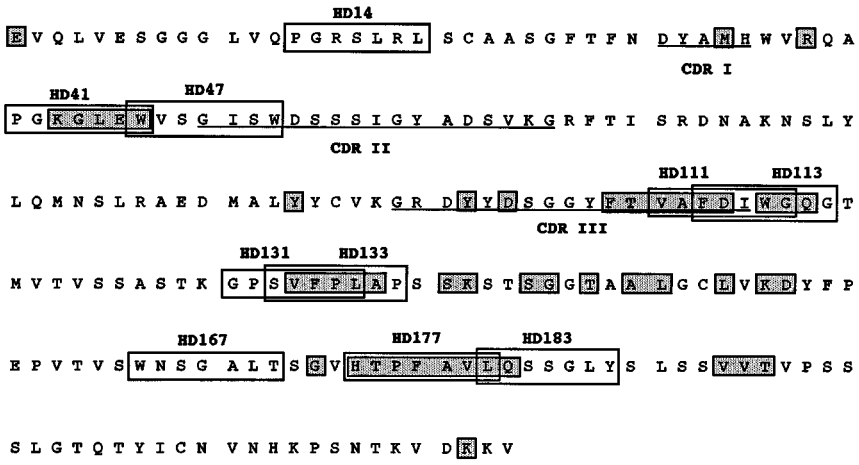

FIG. 4. **Localization of BiP binding sequences in the primary structure of the Fd fragment from antibody 3D6.** *Underlined* amino acids correspond to the complementarity-determining regions (CDR's), while *shaded segments* in the 3D6 sequence represent amino acids that are involved in interchain contacts. These residues were determined by comparing the surface exposure of side chains in the crystal structure of the dimer and the respective monomers. Peptides that stimulate the ATPase activity of BiP are *framed*.

yielded apparent K_m values of 6–60 μM that lie within the range previously defined for high affinity sites (Blond-Elguindi *et al.*, 1993a; Fourie *et al.*, 1994).

Confirmed BiP binding sequences are located within both the V_H and C_H1 domains of the 3D6 heavy chain and within the C_H3 domain of the MAK33 heavy chain. In addition, a number of untested sequences having BiP scores >10 (and therefore having a high probability of being BiP binding sites) are present in the MAK33 C_H2 domain. Thus, potential BiP binding sites are not confined to the C_H1 domain of the heavy chain, as was previously suggested on the basis of experiments that showed that mutant heavy chains lacking C_H1 do not interact stably with BiP (Hendershot *et al.*, 1987). Rather our data are consistent with the recent results of Kaloff and Haas (1995), who showed that the C_H1 domain is not the only portion of the heavy chain that interacts with BiP during the biosynthesis and initial folding of the molecule. What apparently sets the C_H1 domain apart from the other three domains of the heavy chain is its propensity to continue to expose at least one BiP binding site after the heavy chain dimer is formed, and this is probably the result of the C_H1 domain being uniquely unable to homodimerize.

Fig. 5 presents a schematic model of the role of BiP during the folding and assembly of immunoglobulin molecules. During the translocation of heavy and light chains as extended polypeptides into the lumen of the endoplasmic reticulum, BiP binds to one or more of multiple potential sites present within all four domains of the heavy chains and within both domains of the light chains. Some potential binding sites become occluded as the domains begin to fold, but sites displayed on surfaces destined to become subunit interfaces remain exposed until assembly occurs, first of heavy or light chain homodimers and then of the H_2L_2 heterotetramers.

Our data suggest that BiP binding sites in immunoglobulins may in fact be concentrated in sequences that participate in subunit interfaces. The localization within the three-dimensional structure of antibody 3D6 of confirmed potential binding sites in the Fd portion of the heavy chain showed that almost every site includes amino acids that are part of the contact surface between the heavy and light chains. Furthermore, the hydrophobic residues that are destined to become buried in the interface are disproportionately present within the potential BiP binding sites. By protecting hydrophobic patches on the surface of Ig folding intermediates from forming inappropriate interactions with other partially folded polypeptides, nonspecific aggregation and unproductive side reactions would be suppressed, and the generation of correctly assembled antibodies would be favored. It will be of great interest to determine if BiP binding sites in other multisubunit proteins are also preferentially localized in sequences that form subunit interfaces, thus indicating a general and important role for BiP during subunit assembly.

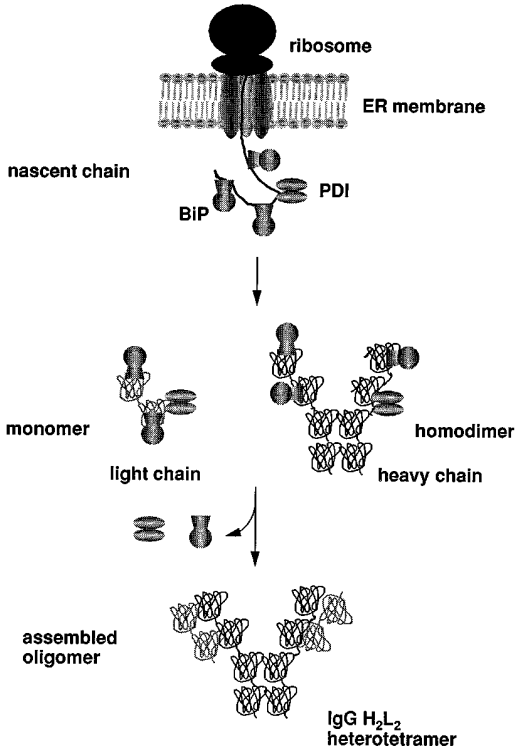

FIG. 5. **A schematic model of the role of BiP during the folding and assembly of immunoglobulin molecules.** This model concentrates on the role of BiP in binding to linear epitopes exposed in the nascent chain and (partially) folded but unassembled structures. The simultaneous binding of BiP and protein disulfide isomerase (*PDI*) to antibody chains is still hypothetical. Furthermore, the interaction of BiP with antibodies may occur via ATP-dependent cycles of binding, release, and rebinding. The undoubtedly important roles of other chaperones and co-chaperones, for example Grp94 (Melnick *et al.* 1994), have not been addressed.

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