Assembly and Secretion of Heavy Chains that Do Not Associate Posttranslationally with Immunoglobulin Heavy Chain-binding Protein

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Abstract. Heavy chain-binding protein (BiP) associates posttranslationally with nascent Ig heavy chains in the endoplasmic reticulum (ER) and remains associated with these heavy chains until they assemble with light chains. The heavy chain-BiP complex can be precipitated by antibody reagents against either component. To identify sites on heavy chain molecules that are important for association with BiP, we have examined 30 mouse myelomas and hybridomas that synthesize Ig heavy chains with well characterized deletions. Mutant Ig heavy chains that lack the C_H1 domain could not be demonstrated to associate with BiP, whereas mutant Ig heavy chains with deletions of the C_H2 or C_H3 domain were still able to associate with BiP. In two light chain negative cell lines that produced heavy chains with deletions of the C_H1 domain, free heavy chains were secreted. When Ig assembly and secretion were examined in mutants that did not associate with BiP, and were compared with normal parental lines, it was found that the rate of Ig secretion was increased in the mutant lines and that the Ig molecules were secreted in various stages of assembly. In one mutant line (C_H1^-) approximately one-third of the secreted Ig molecules were incompletely assembled, whereas the Ig molecules secreted by the parental line were completely assembled. Our data show the C_H1 domain to be important for association with BiP and that when this association does not occur, incompletely assembled heavy chains can be secreted. This implies a role for BiP in preventing the transport of unassembled Ig molecules from the ER.

IMMUNOGLOBULIN biosynthesis, assembly, and transport have been well characterized using both normal and malignant lymphoid cells (7, 27). The Ig molecule is composed of two identical heavy chain proteins and two identical light chain (LC)[1] proteins which are joined by interchain disulfide bonds. Nascent heavy and light chains contain a hydrophobic amino-terminal signal sequence which allows them to be cotranslationally translocated into the lumen of the endoplasmic reticulum (ER) (5). The addition of mannose core sugars to heavy chains occurs during this translocation (4). Very soon after translocation, heavy and light chains begin to assemble in the ER and interchain disulfide bonds are formed (24, 27). The sequence of subunit assembly appears to be determined by the heavy chain isotype. IgM and some IgG_{2b} assemble as heavy and light chain (HL) molecules and then H_2L_2 molecules, whereas IgG_1 and IgG_{2a} first form H_2 molecules, then H_2L, and finally intact H_2L_2 molecules (3). Once assembly is complete, the Ig molecule is transported to the Golgi complex for further processing of its carbohydrate side chains (14). The mechanism for directing the Ig molecule to Golgi is not well understood. Data gathered from studying the transport of other proteins to the Golgi complex suggest the existence of inherent transport signals on protein molecules which direct their transport to the Golgi complex (10, 20). It is not clear if transport sequences are present on the heavy chain, the light chain, or both. After processing in the Golgi complex, the Ig molecule is packaged into vesicles that are targeted to the plasma membrane for secretion.

Usually, only completely assembled Ig molecules are secreted. When assembly can not occur due to the absence of light chain synthesis or is inhibited due to the underglycosylation of heavy chains in the case of IgM, the free heavy chains are not transported to the Golgi complex and are degraded internally (13, 17). A notable exception to this occurs in lymphoproliferative heavy chain disease (HCD). This disorder is characterized by the production and secretion of heavy chains in the absence of LC production (11). The secreted heavy chains are abnormal in that they contain large protein deletions that usually involve the first constant region domain (C_H1) and occasionally the variable region

1. *Abbreviations used in this paper*: BiP, heavy chain-binding protein; ER, endoplasmic reticulum; LC, light chain; HCD, lymphoproliferative heavy chain disease; HL, heavy and light chain.

(25). There are also several myeloma and hybridoma cell lines that synthesize and secrete heavy chains without LC. Like the proteins produced in HCD, these heavy chains also have deletions of the C_H1 domain (8, 21).

Heavy chain-binding protein (BiP) has been demonstrated to coprecipitate with heavy chains synthesized in myeloma lines, that have lost the ability to sythesize LC (6, 23), and in Abelson virus transformed pre-B cell lines (μ^+, LC^-) (12). We have found BiP to be part of the normal posttranslational processing of Ig heavy chains (6). BiP associates with nascent heavy chains in the lumen of the ER and appears to be dissociated only when LC are added to heavy chains. When LC are not synthesized, BiP remains associated with heavy chains and these heavy chains are not transported to the Golgi or secreted (6). When IgM-producing cells are treated with tunicamycin, the unglycosylated μ-chains do not combine with LC efficiently, are not secreted (13), and remain associated with BiP (Hendershot, L., D. Bole, and J. F. Kearney, manuscript submitted for publication). We have proposed that BiP acts to hold heavy chains in the ER until assembly is complete by interfering with or blocking transport sequences on heavy chains. Only after BiP has been dissociated would the transport sequences on heavy chains be exposed.

To determine which portions of the heavy chains are involved in the association with BiP, we examined deletion mutants lacking different C_H domains. If mutant Ig heavy chains could be found that did not associate with BiP we could more directly determine how BiP affects Ig assembly and secretion. Thirty mouse lymphoid cell lines that produce heavy chains with well characterized deletions were examined for the ability of their heavy chains to associate with BiP. The C_H1 domain was found to be necessary for the association of α, γ_1, γ_{2a}, and γ_{2b} heavy chains with BiP. Heavy chains, which did not associate with BiP, could be secreted in the absence of LC synthesis. Cell lines that synthesized LC together with heavy chains with C_H1 domain deletions secreted completely assembled Ig molecules as well as all the assembling intermediates.

Materials and Methods

Cell Lines

The cell lines used in this study were obtained from a variety of sources. Most have been described previously and are summarized in Table I. Cell lines 45.6 (21), 10.1 (21), G403 (21), R15 (8), Δ15 (8), and K25.1 (22) were a gift from Dr. S. Morrison (Columbia University, New York). Cell lines Ar13.4 (29), ArM16 (29), ArM1 (29), S107 (30), S_1U_9 (30), RP3, RP4, th3, 24F3, 26C2, 6C4, 16D2, 20G12, and 3G3 were gifts from Drs. R. Pollack and M. D. Scharff (Albert Einstein College of Medicine, New York). Cell lines PC 700 (2), 562 (2), and 574 were a gift from Dr. M. Shulman (University of Toronto). Cell lines 208 (2), 482 (18), 662 (18), and RD3-2 (6) have been described previously. LBW-26 is a human Epstein-Barr Virus-transformed cell line which synthesizes IgG and was produced in this lab. All lines were maintained in RPMI 1640 with 10% FBS, 2 mM L-glutamine, and 100 U/ml penicillin-streptomycin.

Biosynthetic Labeling

For short pulse and pulse-chase experiments, the cells were pre-incubated in RPMI 1640 lacking methionine for 1 h and then labeled with 25 μCi of [^{35}S]methionine (Amersham Corp., Arlington Heights, IL) for 10 min. When chase experiments were performed, cells were resuspended in complete RPMI 1640 devoid of radioactive methionine and incubated for indicated times. For continuous labeling experiments, cells were placed directly in methionine-free RPMI 1640 containing 25 μCi [^{35}S]methionine and labeled for 90 min.

Immunoprecipitation

Labeled cells were harvested and lysed in half-strength PBS containing 0.5% deoxycholic acid and 0.5% NP-40. Nuclei were sedimented at 10,000 *g* for 20 min and the resulting supernatant was used for immunoprecipitation. Anti-mouse isotype specific antibodies were obtained from Southern Biotechnology Associates (Birmingham, AL). The rat anti-BiP monoclonal (IgGκ) was produced in this laboratory and has been described previously (6). A mouse anti-rat κ monoclonal antibody (26) was conjugated directly to Sepharose 4B beads and was used to precipitate the anti-BiP monoclonal. Anti-mouse isotype antibodies were precipitated by binding to Protein A conjugated Sepharose 4B beads. Immunoprecipitated proteins were washed three times with 0.05 M Tris buffer, pH 7.4, containing 0.4 M NaCl, 0.5% DOC, and 0.5% NP40, and analyzed by SDS PAGE (19). The SDS gels were then fluorographed using En^{3}Hance (New England Nuclear, Boston, MA), dried, and exposed on Kodak XAR-5 film to visualize bands.

Results

Reactivity of IgG_{2b} Mutant Proteins with BiP

Five IgG_{2b}-producing cell lines were examined for the ability of their heavy chains to associate with BiP posttranslationally. Ar13.4 cells produce antibody with specificity for arsonate and contain complete γ_{2b} heavy chains (29). Two mutant sublines of Ar13.4 with domain-spanning deletions were obtained: ArM16, which is missing the C_H2 domain, and ArM1, which has deleted the C_H3 domain of its heavy chain (29). 45.6 cells, which are the tissue culture line equivalent of the myeloma MPC 11, and its subline 10.1, which synthesizes and secretes IgG_{2b} antibodies lacking the C_H1 domain (21), were also examined. The cells were pulse labeled with [^{35}S]methionine and then lysed. The cell lysate was divided and one half was precipitated with anti-IgG_{2b} and the other half with anti-BiP antibodies. A major band representing heavy chains can be observed from each cell line when precipitating with anti-Ig (Fig. 1). The molecular weight of the heavy chains from each cell line vary according to whether they contain a deletion and which domain is deleted. A fainter band comigrating with BiP can be observed in the anti-Ig precipitated material from the Ar13.4, ArM16, and ArM1 clones. We were unable to detect any BiP coprecipitating with Ig in the 45.6 cell line. This could be due

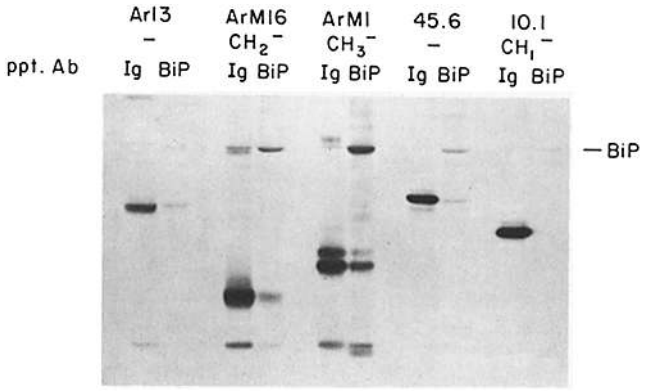

Figure 1. Ar13.4, ArM16, ArM1, 45.6, and 10.1 cells were pulse labeled with [^{35}S]methionine for 10 min. Cell lysates were divided and one half was precipitated with anti-IgG_{2b} and the other half with anti-BiP. The immunoprecipitates were analyzed on 10% SDS PAGE under reducing conditions.

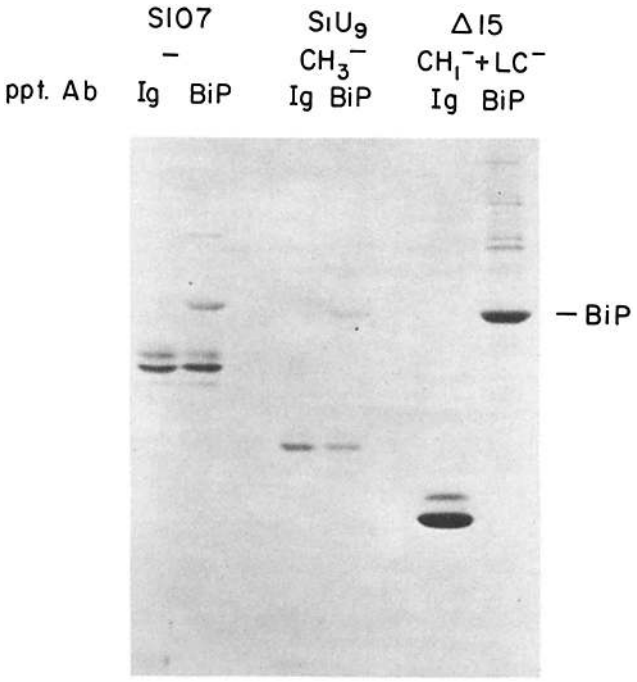

Figure 2. S107, S_1U_9, and Δ15 cells were pulse labeled for 10 min with [^{35}S]methionine. Cell lysates were divided and one half was precipitated with anti-IgA and the other half with anti-BiP. The precipitated proteins were analyzed on 10% SDS PAGE under reducing conditions.

to the fact that Ig in this cell line also assembles along the H → HL → H_2L_2 pathway, thus leaving fewer free heavy chains to associate with BiP. It is also possible that this line has a larger unlabeled intracellular pool of BiP which is associating with the γ_{2b} heavy chains. In the anti-IgG_{2b} precipitated material from both ArM16 and ArM1, a faint band is also observed which migrates slightly slower than BiP in the case of ArM1 and slightly faster than BiP in the case of ArM16. These do not appear to be alternate forms of BiP as they are not precipitated with anti-BiP. It is possible that they represent some incompletely reduced IgG_{2b}. When each of the lysates were immunoprecipitated with anti-BiP, a band migrating at 78 kD was obtained. In both of the parent lines (Ar13.4 and 45.6), normal γ heavy chains coprecipitated with BiP (Fig. 1). Examination of the mutant sublines showed that the heavy chains missing the C_H2 and C_H3 domains (ArM16 and ArM1) were also coprecipitated with BiP. However, heavy chains lacking the C_H1 domain from the 10.1 subline were not coprecipitated with BiP. Therefore, deletions of the C_H2 or C_H3 domain did not appear to affect significantly the association of γ_{2b} heavy chains with BiP whereas the C_H1 domain seemed to be necessary for this association.

Association of Mutant IgA Heavy Chains with BiP

Three IgA-producing lines were examined for the ability of their heavy chains to associate with BiP. S107 cells produce IgA immunoglobulin with specificity for phosphorylcholine (30). S_1U_9 is a subline of S107 and produces α heavy chains which lack the C_H3 domain. The Δ15 line, which produces α heavy chains missing the C_H1 domain, is a subline of W3129 which recognizes α1 → 6 dextran (8). The cells were pulse labeled and immunoprecipitated with anti-IgA or anti-BiP antibodies. The α heavy chains were precipitated from each of the cell lines with anti-IgA (Fig. 2). When lysates were immunoprecipitated with anti-BiP, BiP could be precipitated from each of the cell lines. In the case of S107 and S_1U_9 (C_H3^-) cells, α heavy chains were coprecipitated with BiP. However, no α heavy chains from the Δ15 cells (C_H1^-) could be demonstrated to coprecipitate with BiP (Fig. 2). Therefore, as in the γ_{2b} heavy chains, the C_H1 domain appeared to be necessary for the association of α heavy

Table I. Summary of Mutant Cell Lines and Their Reactivity with BiP

Cell lines	Isotype	Parent line	Deletion	Assembly	Reactivity with BiP
Ar13.5 (29)	IgG_{2b}	–	–	H_2L_2	+
ArM16 (29)	IgG_{2b}	Ar13.5	CH_2	H_2L_2	+
ArM1 (29)	IgG_{2b}	Ar13.5	CH_3	HL	+
45.6 (21)	IgG_{2b}	MPC11	–	H_2L_2	+
10.1 (21)	IgG_{2b}	45.6	CH_1	H_2L_2(noncov L)	–
G403 (21)	IgG_{2b}	10.1	CH_1 + LC^-	H	–
S107 (30)	IgA	–	–	H_2L_2(noncov L)	+
S_1U_g (30)	IgA	S107	CH_3	HL	+
W3129 (8)	IgA	–	–	H_2L_2(noncov L)	+
R15 (8)	IgA	W3129	LC^-	H_2	+
Δ15 (8)	IgA	R15	CH_1 + LC^-	H	–
RP3	IgG_1	3665	–	H_2L_2	+
RP4	IgG_1	RP3	CH_2	H_2L_2	+
24F3	IgG_1	3665	CH_3	H_2L_2	+
26C2	IgG_1	3665	CH_2	H_2L_2	+
th3	IgG_1	3665	CH_1	H_2L_2(noncov L)	–
Ag8(8) (6)	IgG_1	P3X63Ag8	LC^-	H_2	+
K25.1 (22)	IgG_{2b}	–	–	H_2L_2(noncov L)	–
PC700 (2)	IgM	–	–	$(H_2L_2)_5$	+
208 (2)	IgM	PC 700	CH_{1-3}	HL	–
562 (2)	IgM	PC 700	Carboxyterm	H_2L_2	+
574 (31)	IgM	PC 700	LC	H_2	+
482 (18)	IgM	Sp6	CH_{3-4}	H_2L_2	+
662 (18)	IgM	Sp6	CH_{3-4}	H_2L_2	+

chains with BiP. Deletion of the C_H3 domain of the α heavy chain did not appear to affect this association.

Summary of Mutant Cell Lines and Their Reactivity with BiP

Altogether 30 mouse myeloma and hybridoma cell lines, including 6 cell lines producing IgG_{2b}, 4 producing IgA, 1 producing IgG_{2a}, 10 producing IgG_1, and 9 synthesizing IgM, were examined for association of heavy chains with BiP (Table I). The Ig subclass, degree of Ig assembly, and the extent of the heavy chain deletion for each line has been characterized previously and are summarized in Table I. For most of the isotypes, lines were obtained that produced heavy chains with deletions of each of the C_H domains. Six cell lines were found that produced heavy chains that did not associate with BiP as judged by the inability of anti-Ig to coprecipitate BiP or anti-BiP to coprecipitate heavy chains. These included two cell lines that synthesized γ_{2b} heavy chains, one cell line making γ_1 heavy chains, one producing γ_{2a} heavy chains, one synthesizing α heavy chains, and one producing μ heavy chains. In each case these heavy chains lacked the C_H1 domain. The deletion of other C_H domains did not appear to affect the posttranslational association of BiP with heavy chains.

Association of BiP with Heavy Chains in LC⁻ Lines

Four cell lines that produce heavy chains but do not synthesize LC (H^+, LC^-) were examined for posttranslational association of heavy chains with BiP and heavy chain secretion. Ag8(8) ($\gamma1^+$, LC^-) and 574 (μ^+, LC^-) cells produce complete heavy chains that are not secreted (6, 28a). The Δ15 (α^+, LC^-) and G403 (γ_{2b}^+, LC^-) cells produce heavy chains that lack the C_H1 domain and are secreted in the absence of LC synthesis (8, 21). The cell lines were labeled for 90 min and after harvesting, both cell lysates and culture supernatants were analyzed. Cell lysates were immunoprecipitated with either anti-heavy chain isotype specific or anti-BiP antibodies, and the culture supernatants were precipitated with anti-isotype specific antibodies. Anti-heavy chain precipitation of Ag8(8) and 574 cell lysates resulted in the precipitation of γ_1 and μ heavy chains, respectively, as well as coprecipitation of BiP (Fig. 3). Anti-BiP precipitation of these cell lysates demonstrated two bands: one representing BiP and the other representing γ_1 heavy chains in the case of Ag8(8) cells and μ in the case of 574 cells. The heavy chains were not secreted from these cells as determined by our inability to precipitate them from the culture supernatants (Fig. 3). When Δ15 and G403 cells were analyzed in the same manner, the anti-heavy chain antibodies were shown to precipitate only α heavy chains (Δ15) or γ_{2b}-heavy chains (G403). We were unable to detect any coprecipitating BiP in either of these lines. Likewise, anti-BiP precipitated only BiP from Δ15 and G403 cells with no coprecipitating bands representing α or γ_{2b} heavy chains. When culture supernatants from these two lines were examined for the secretion of heavy chains, C_H1-deleted α chains, in the case of Δ15 cells, and C_H1-deleted γ_{2b} chains, in the case of G403 cells, could be found (Fig. 3). These experiments suggest that the secretion of free heavy chains occurs when the heavy chains fail to associate with BiP.

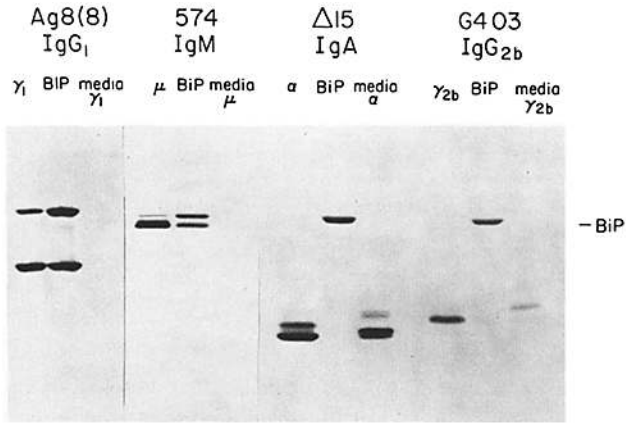

Figure 3. Ag8(8) d(γ_1), 574 (μ), Δ15 (α), and G403 (γ_{2b}) cells were labeled for 90 min with 25 μCi of [^{35}S]methionine. Cell lysates were split and immunoprecipitated with either anti-heavy chain specific antibodies or anti-BiP. Culture supernatants were immunoprecipitated with anti-heavy chain-specific antibodies to detect any secreted heavy chains. Precipitated proteins were analyzed on 10% SDS PAGE under reducing conditions.

Assembly of Secreted Ig Molecules in C_H1^- Mutant

Secreted Ig from a cell line producing γ heavy chains with a C_H1 domain deletion was compared with normal secreted IgG to determine if the inability of heavy chains to associate with BiP affected their assembly and secretion. Four cell lines that produce normal γ heavy chains as well as 10.1 cells, which produce γ heavy chains with a C_H1 domain deletion, were labeled for 2 h. The culture supernatant was immunoprecipitated with anti-Ig and analyzed under nonreducing conditions by SDS PAGE. The four cell lines that synthesize normal γ heavy chains secreted them as completely polymerized H_2L_2 molecules (Fig. 4 *a*, Ig). When secreted Ig from the 10.1 cell line was examined in the same manner, three major bands were precipitated with anti-Ig (Fig. 4 *a*, Ig). They represent H_2, H, and LC molecules. This agrees with previously reported data on this cell line (21).

When the C_H1 domain is missing, LC are attached to the C_H2 or C_H3 domain of the heavy chain by noncovalent bonds (21). After SDS denaturation of these noncovalent bonds, it was impossible to tell if the H_2 band was derived from H_2L_2, H_2L, H_2 molecules, or all three and whether the H band came from H or HL molecules (Fig. 4 *b*). To clarify this, the labeled culture supernatant from 10.1 cells was first immuno-absorbed with monoclonal anti-κ conjugated sepharose beads to precipitate all LC-containing molecules. The remaining Ig molecules (H and H_2) were then precipitated with anti-Ig. When the precipitated material was examined by SDS PAGE, we found that the anti-κ antibody precipitated all of the LC band, all of the H band (HL), and about half of the H_2 band (H_2L_2, H_2L) (Fig. 4 *a*, k′). The remaining H_2 molecules were precipitated with anti-Ig (Fig. 4 *a*, Ig′). Therefore, the three bands shown in the secreted material from 10.1 cells represent H_2L_2, H_2L, H_2, HL, and LC molecules. When th3 (γ_1, C_H1^-) and K25 cells (γ_{2a}, C_H1^-) were examined in the same way, they were also found to secrete unassembled Ig molecules (data not shown).

This demonstrates that in the absence of posttranslational association with BiP, heavy chains and all of the assembly

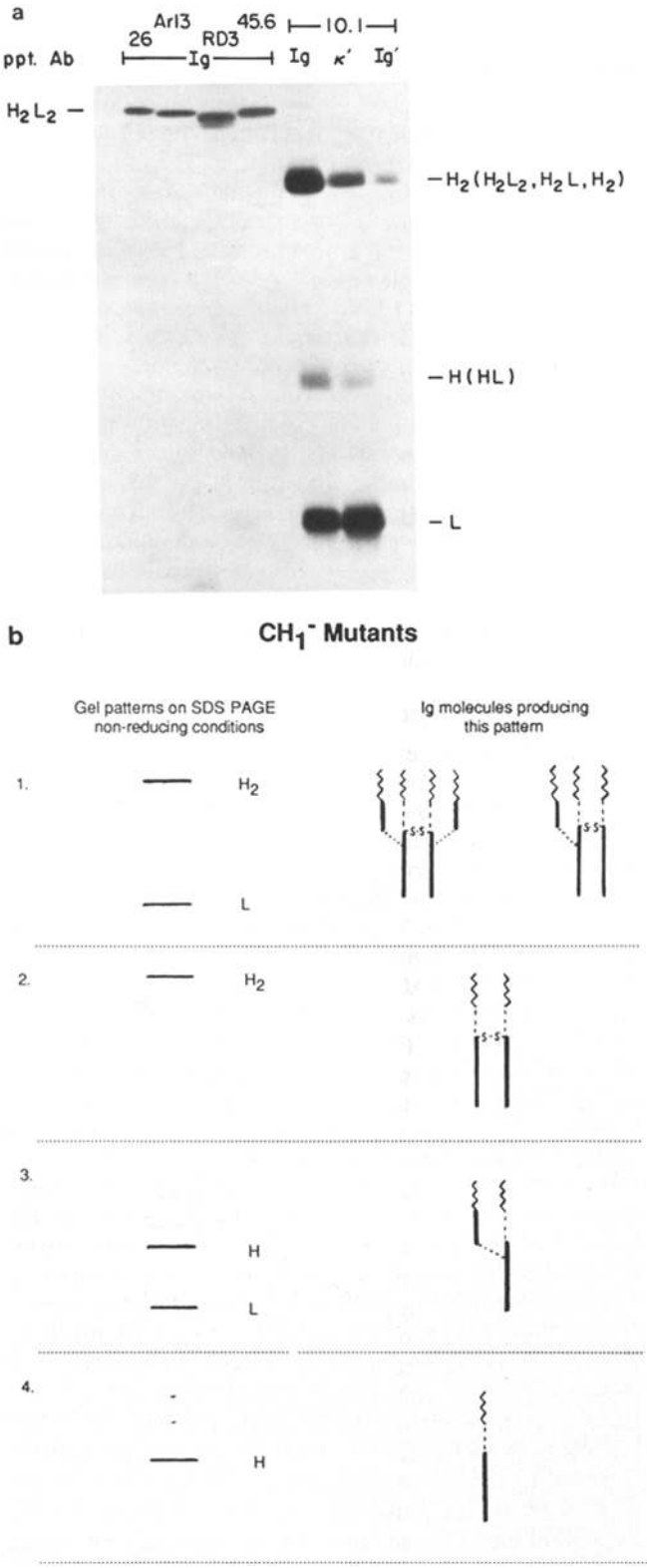

Figure 4. (*a*) Four cell lines producing normal γ heavy chains (26, Arl3.4, RD3, and 45.6) and one cell line producing γ heavy chains with a C_H1 domain deletion (10.1) were labeled for 2 h with [^{35}S]-methionine. Culture supernatants were precipitated with anti-Ig and separated on 8% SDS PAGE under nonreducing conditions (*Ig*). To determine the identity of the three bands precipitated from the 10.1 cells, the supernatant was reacted first with anti-κ (lane *k'*) to precipitate LC-containing molecules, followed by precipitation with anti-Ig (lane *Ig'*) to isolate remaining heavy chains. (*b*) A schematic drawing to show the assembly of Ig molecules when the C_H1 domain is missing, and the gel patterns observed when this molecule is analyzed on SDS PAGE under nonreducing conditions.

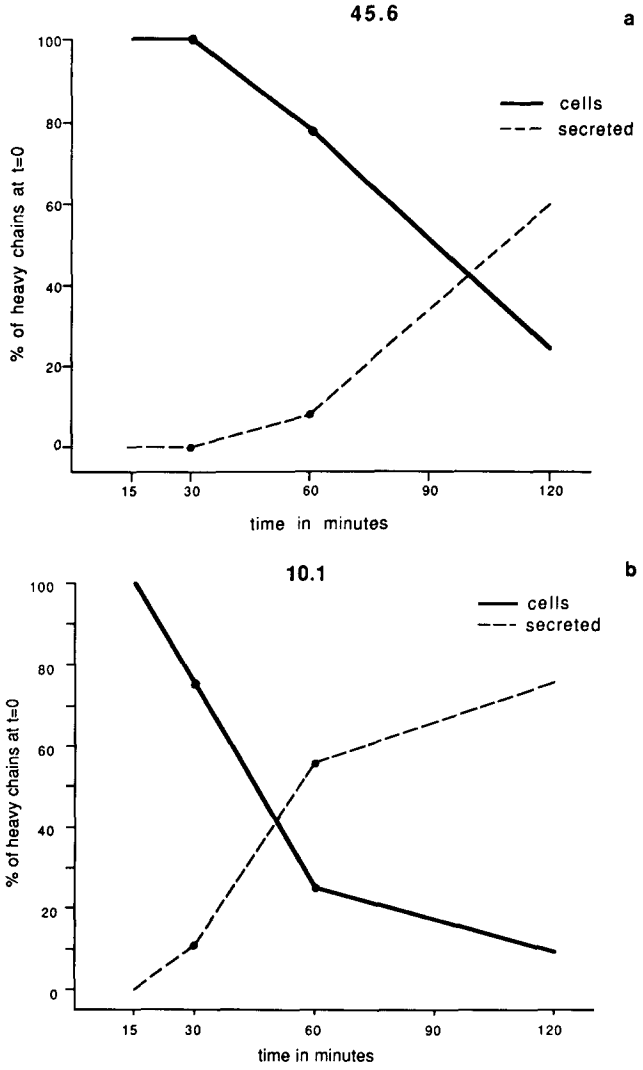

Figure 5. 45.6 cells (*a*) and 10.1 cells (*b*) were pulse labeled for 10 min with [^{35}S]methionine, resuspended in complete RPMI 1640, and chased for 0, 15, 30, 60, and 120 min. Cell lysates and culture supernatants were immunoprecipitated with anti-Ig and analyzed on 10% SDS PAGE under reducing conditions. The γ heavy chains from each time point on the autoradiograph were quantitated by densitometer tracing and are represented as percent of cell-associated heavy chains at $t = 0$.

intermediates of Ig are secreted. This is in direct contrast to heavy chains containing the C_H1 domain that associate with BiP and are not secreted except as completely assembled molecules

Rate of Secretion of Ig in the Absence of Association with BiP

To determine whether the association of BiP with heavy chains affected the rate of Ig transport, the kinetics of Ig secretion were examined in 10.1 cells (C_H1^-) and the parent line 45.6. Cells were pulse labeled with [^{35}S]methionine for 15 min and chased for 0, 15, 30, 60, and 120 min. Ig was

precipitated from both the cell lysates and culture supernatants. Immunoprecipitated proteins were analyzed by SDS PAGE, and cellular and secreted Ig was quantitated by densitometer tracings of the autoradiograph. Densitometer tracings of each time point were calculated as percent of cell-associated heavy chain at $t = 0$ (Fig. 5, *a* and *b*). The $t_{1/2}$ for transport of heavy chains from the 45.6 cells was calculated to be 100 min, whereas the $t_{1/2}$ for transport of heavy chains from 10.1 cells was only 50 min. The C_H1^- heavy chains from the 10.1 cells that did not associate with BiP were secreted at twice the rate of their normal counterparts syntheized by 45.6 cells, which do associate with BiP.

Discussion

In this study we have compared the posttranslational association of mutant and wild type heavy chains with BiP. Six cell lines were found which produced heavy chains that did not appear to associate with BiP as judged by the inability of anti-Ig to coprecipitate BiP or anti-BiP to coprecipitate heavy chains. These cell lines produced heavy chains representing five different isotypes. In each case these heavy chains lacked the C_H1 domain. The deletion of other C_H domains did not appear to prevent the posttranslational association of BiP with heavy chains. This implies that the C_H1 domain contains or contributes to a site necessary for BiP association. It is of interest that LC are covalently attached to heavy chains at the C_H1 domain. It is possible that BiP interacts with a portion of the heavy chain that is involved in the association with LC.

When LC^- cell lines were examined, a direct correlation was found between the lack of association of BiP with heavy chains and the ability of these free heavy chains to be secreted. Two LC^- cell lines (Δ15 and G403) were examined here that produced heavy chains that did not associate with BiP and were secreted. In both cases, the heavy chains contained deletions of the C_H1 domain. This situation closely parallels that seen in human HCD where free mutant heavy chains are secreted. These mutant heavy chains contain large deletions that usually include the C_H1 domain (25). We have shown that human lymphoid cell lines also produce BiP which associates with heavy chains in much the same fashion as in mouse cell lines (Hendershot, L., D. Bole, and J. F. Kearney, manuscript submitted for publication). It is, therefore, very tempting to suggest that the inability of HCD proteins to associate with BiP is responsible for the secretion of free heavy chains in this disease.

Biosynthetic studies of pre-B hybridomas and LC^- myelomas have demonstrated that free heavy chains are not normally secreted from lymphoid cells (17, 23). It has been suggested that heavy chains do not contain transport signals necessary for targeting them to the Golgi and that the transport signals are on the LC (28). Only after combination with LC would heavy chains be transported. However, the secretion of mutant heavy chains in HCD and in certain LC^- cell lines suggests that heavy chains do contain transport signals (21, 25). The two LC^- cell lines examined here (Δ15 and G403), which secrete free heavy chains, produce heavy chains that do not associate with BiP. This strengthens our hypothesis that BiP either obscures the transport signals inherent to heavy chains or prevents the formation of cryptic transport signals (6). Only after BiP is displaced by LC or when it is unable to bind (in the case of C_H1 domain mutants) would heavy chain transport signals be exposed, resulting in the transport of heavy chains to the Golgi.

The examination of Ig assembly and secretion in 10.1 cells (C_H1^-, LC^+) contributes to our understanding of the role of BiP in preventing heavy chain secretion. The fact that some complete assembly of Ig occurred demonstrates that BiP is not necessary for assembly of heavy and light chains. But the finding that unassembled and assembling heavy chains can be secreted, even in the presence of LC synthesis and assembly, is perhaps the most compelling evidence that BiP acts to prevent the secretion of unassembled heavy chains. In most cases, heavy chains are only secreted as intact H_2L_2 molecules. It is noteworthy that a trace amount of HL molecules are secreted from 45.6 cells. A portion of Ig assembly in this cell line follows the $H \rightarrow HL \rightarrow H_2L_2$ pathway. We have found that the HL molecule in this cell line is not associated with BiP (unpublished data). Previous studies have shown that mutant IgA molecules that cannot form interchain H–H bond are secreted as HL molecules (30). This might be interpreted to suggest that any Ig intermediate not associated with BiP is capable of being secreted. It is also noteworthy that the heavy chains from the 10.1 cell line, which do not associate with BiP, are secreted at a faster rate than their normal counterparts. This suggests that association with BiP might delay heavy chain transport until assembly with LC is complete.

When examining IgM mutants, we found that the absence of the C_H1 domain did not entirely prevent the association of μ heavy chains with BiP. A very weak association of μ heavy chains with BiP remained in 427 (18) and 128 (2) cells, which are missing the C_H1 and C_H2 domains, and 43 (18) cells, which are lacking the C_H1 domain. This suggests a second domain with the ability to associate weakly with BiP. This association could only be observed after overexposure of gels even in LC^- mutants (unpublished data). It is interesting that only the μ heavy chains appear to have a second (albeit weak) site for BiP association. When the C_H1 and C_H2 domain of μ chains were absent, LC were able to form disulfide bonds with the C_H3 or C_H4 domains (18). However, when the C_H1 domain is missing from α or γ heavy chains, the LC can only form noncovalent bonds with the C_H2 or C_H3 domain. It is possible that BiP interacts with either the free sulfhydryl involved in H–L bond formation or a hydrophobic region of the C_H1 domain, which is eventually covered by the constant domain of the LC (9, 16). Both of these must be duplicated in the μ heavy chains since there is a second site for LC attachment. However, the fact that BALB/c α heavy chains (1) and human α_2 heavy chains (15) both have mutations, which change the heavy chain cysteine residue involved in H–L formation but are still able to associate with BiP, suggests that this sulfhydryl is not involved in BiP association.

The data presented here demonstrate that the C_H1 domain of heavy chains is necessary for their association with BiP. In the absence of association with BiP, free heavy chains and assembling intermediates of Ig can be secreted, whereas heavy chains that associate with BiP are secreted only as completely assembled Ig molecules.

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