**Assembly of Immunoglobulin Light Chains as a Prerequisite for Secretion**

A MODEL FOR OLIGOMERIZATION-DEPENDENT SUBUNIT FOLDING*

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Oligomeric proteins usually have to assemble into their final quartenary structure to be secreted. However, most immunoglobulin (Ig) light (L) chains can be exported as free chains, whereas only a few Ig L chains, here referred to as export-incompetent, have to assemble with Ig heavy (H) chains into antibody molecules to be secreted. In the absence of Ig H chain expression, these export-incompetent Ig L chains remain bound to BiP as partially folded monomers with only one of the two internal disulfide bonds being formed. To understand the apparent discrepancy in Ig L chain export, we performed assembly studies with chimeric Ig chains and found that the variable (V) domain of the export-incompetent NS1 x chain cannot mediate homodimer formation. Conversely, the V domain of the export-competent J558L λ chain supports homodimer formation and, accordingly, these Ig L chains are secreted as noncovalently or covalently linked homodimers. We show that the export-incompetent mutant λ1 FS62 chain forms disulfide bonds in both domains only upon pairing with Ig H chain and is secreted as part of an antibody. Therefore, Ig L chain assembly seems to be a prerequisite for complete folding, indicating that Ig L chain secretion generally depends on either homo- or heterodimer formation. We discuss a mechanism that controls oligomerization by monitoring the conformation of individual subunits that cannot proceed in folding prior to successful assembly.

In eukaryotic cells, various soluble and membrane-bound proteins travel along the secretory pathway to reach their final destination. These molecules first enter the endoplasmic reticulum (ER) as unfolded polypeptides, which are modified there to reach their final three-dimensional structure. Attributes such as conformation, structure of attached carbohydrates, and oligomeric state not only control the functional properties of a protein but are also critical for intracellular transport. Newly synthesized polypeptides are hampered in exiting the ER as long as they have not acquired a so-called export-competent conformation (for reviews, see Refs. 1–3). Oligomeric proteins such as influenza hemagglutinin or vesicular stomatitis virus glycoprotein are homo-oligomers that must be primed prior to exit from the ER (4–6). In other cases, export is not dependent on complete assembly. While the heptameric T-cell receptor complex only reaches the cell surface when completely assembled, in the absence of ζ chain homodimer association, pentamers leave the ER and are subsequently transported to lysosomes instead. Other assembly intermediates or isolated subunits are retained in the ER (7). The export of major histocompatibility complex class I antigens usually depends on the noncovalent assembly of a transmembrane heavy chain, the soluble β2-microglobulin, and peptide, but soluble β2-microglobulin can be secreted as free chains (9). Similarly, free immunoglobulin (Ig) heavy (H) chains are not exported (10, 11) unless assembled with Ig light (L) chains into antibody molecules (12), while most Ig L chains can also be secreted as free molecules (13, 14). Astonishingly, some Ig L chains (here referred to as export-incompetent) do not follow this rule but depend on Ig H chain association to be secreted (14–16). To date, there is no valid model to explain this apparent discrepancy.

Using immunoglobulins as a model to study the requirements of ER export, Sitia and colleagues have uncovered a quality control mechanism that acts on polypeptides to expose unpaired cysteine residues (17–20). Immunoglobulin L chains possess five cysteine residues, four of which are involved in two disulfide bonds that stabilize the variable (V) and constant (C) domain, respectively, and a carboxyl-terminal cysteine that is responsible for the intermolecular disulfide bond to Ig H chain. The thiol group of the carboxyl-terminal cysteine of unassembled Ig L chains causes a delay in secretion because it forms reversible disulfide bonds with the protein matrix of the ER (21). However, secreted Ig L chains no longer possess unpaired cysteines since the two internal disulfide bonds have already formed (22) and the carboxyl-terminal cysteine is either covalently linked to a second Ig L chain (or to Ig H chain) or paired with a free cysteine (21, 23).

Export-incompetent Ig L chains, which are not secreted in the absence of Ig H chains may also covalently interact with ER matrix proteins. However, the major cause of retention is probably the noncovalent binding to BiP, an ER-resident molecular chaperone (24, 25). It seems that only one of the domains can fold in vivo because such Ig L chains occur as partially oxidized BiP-bound monomers until they are degraded (22, 26). Complete oxidation of the Ig L chain reflecting disulfide bond formation in the second domain is only observed upon in vitro dissociation from BiP (22). As protein export is thought to be restricted to completely folded molecules, we reasoned that oligomerization might be a prerequisite for Ig L chain folding because Ig H chain association is sufficient to restore export

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1 The abbreviations used are: ER, endoplasmic reticulum; Ig, immunoglobulin; H, heavy; L, light; V, variable; C, constant; NEM, N-ethylmaleimide; PAGE, polyacrylamide gel electrophoresis; NP, X: (4-hydroxy-3-nitrophenyl)acetyl.
Complete Folding Depends on Assembly

EXPERIMENTAL PROCEDURES

Expression Vectors—Expression vector pY3 (named B1–8.VHC/S in Ref. 27) is derived from pECY3 (28) and contains the gene segments encoding the mouse y3 Ig H chain constant (C) region and the rearranged Ig H chain B1.8 variable (V) region. pSVE2neoA8-6 (identical to myeloma A8-8; Ref. 30) was taken as a 2.6-kilobase chain. J558L.V encoded Ig H chain. The transfectant NS1.VJ558L

Cell Lines and Transfections—Mouse myeloma X63Ag8.653 and hybridoma H62 express no Ig chains (31, 32). J558L is a Ig H chain loss variant of the mouse plasmacytoma J558a (Ref. 16). NS1 is a mouse plasmacytoma synthesizing but not secreting Ig chains (15). NSFS62 is a stable transfectant of NS1 that lost endogenous Ig chains and expresses the export-incompetent Ig L chain l, FS62 (22). Cell line E7, a mouse cell line obtained by transfecting E7 (a stable transfectant of H62 that expresses y3 Ig H chains) with the B1.8 Vg domain (34) with pSVE2neoA1. J558L–y3 co-expresses endogenous l chain and the y3 chain as E7 (a generous gift of T. Simon). Transfection of NSFS62 with pY3 gave rise to NSF62–y3 (l, FS62 Ig L and y3 Ig H chains). NS1–y3 is a stable transfectant of NS1 and expresses endogenous l Ig and the y3–encoded Ig H chain. The transfectant NS1.VC3 co-expresses the endogenous k Ig L chain and the vector-encoded chimeric VC3, Ig H chain. J558L–VC3 derives from J558L and co-expresses endogenous l Ig L chain and the chimeric VC3 Ig H chain. II4c.MEV (co-expressing chimeric VC3, Ig L and VC3 Ig H chains) was made by co-transfection of H62 with pEVHC.neo and pEVCy3. All cells were maintained as described (24). Transfection of cells by electroporation and selection of stable transfectants were performed in principle as described by Allen et al. (28). A list of cell lines, corresponding transfectants, and respective Ig chains expressed is given in Table I.

Bio synthetic Labeling, Immunoprecipitations, Size Fractionation, and Western Blotting—As detailed elsewhere (24, 33), starved cells were labeled with [35S]methionine (37 TBq/mmol; Amersham Corp.) and washed in phosphate-buffered saline prior to solubilization in NET lytic buffer (100 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 50 mM Tris/HCl, pH 7.4). When indicated, wash and lysis buffer contained 20 mM NEM to prevent oxidation of free sulfhydryl groups. Immunoprecipitation of Ig chains was performed with subunit-specific antibodies and/or protein A-Sepharose. Size fractionation of proteins was achieved by using a column (diameter, 1 cm; length, 40 cm) containing Sephacryl S-200HR (Pharmacia LKB, Freiburg, Germany). Proteins were separated by native or SDS-PAGE, transferred onto nitrocellulose filters by semidy blotting for Western blotting or visualized by autoradiography (24).

Native Gel Electrophoresis—Post-nuclear supernatant (10 μl; 4 x 106 cells/ml) of NET lysate buffer) was mixed with sample buffer (30 μl; 0.1 M Tris/HCl, pH 6.8, 8.7% glycerol, 10% β-mercaptoethanol) and applied onto a 6% nondenaturing slab gel. The separating gel was prepared by diluting 6 ml of a mixture containing 30% acrylamide and 0.44% bisacrylamide in 24 ml of 0.1 M glycine/NaOH, pH 9.8. The stacking gel contained 1 ml of the same acrylamide mixture, 2.5 ml of 0.5 M Tris/HCl, pH 6.8, and 6.3 ml of H2O. Electrophoresis in 0.1 M glycine/NaOH, pH 9.8, at 4°C was done at 10 mA for 30 min until the blue dye reached the separating gel and thereafter at 20 mA for 7 h. Proteins were transferred onto nitrocellulose sheets and the Western blots developed as described below.

Immunological Reagents—All antisera were purchased from Southern Biotechnology Associates (Birmingham, AL). Affinity-purified goat anti-mouse antibody subunit-specific antisera bound to protein A-Sepharose or protein A-Sepharose alone were used for immunoprecipitations. Antibodies bearing the B1.8 idiotype react with the hapten (4-hydroxy-3-nitro-phenyl)acetetyl (34) and could be isolated with NP-caproate coupled to Sepharose (NP-Sepharose). Western blots were developed with biotinylated affinity-purified goat anti-mouse l, y3 chain antisera, followed by a staining reaction catalyzed by streptavidin-alkaline phosphatase. Alternatively, streptavidin-horseradish conjugate was used for detection with the BM chemiluminescence blotting substrate (Boehringer, Mannheim, Germany).

RESULTS

The k chain expressed by NS1 cells is a well known example of an export-incompetent Ig L chain. Free NS1 k chain is disulfide-bonded in one domain only and bound to BiP as a monomer (22), whereas the same IgL chain is secreted when associated with Ig H chain (15). Therefore, we reasoned that oligomerization might be the prerequisite for secretion of Ig L chains. This model would predict that, in the absence of Ig H chain expression, export-competent Ig L chains differ from export-incompetent Ig L chains in their capability to form homodimers.

No Antibodies Are Formed From Subunits That Both Carry the V Domain of the Export-incompetent NS1 k Chain—As both export-competent and -incompetent Ig L chains may bear the same C domain (24, 35), the export competence of a free Ig L chain must be determined by its V region. That is, the V domain of the Ig L chain should determine whether or not homodimer formation is possible. To investigate this prediction, we used chimeric Ig chains in which the V regions of Ig H and L chains have been swapped. Such chimeric chains are assembled into antibodies as well as wild type chains (27). As the mode of Ig chain pairing does not seem to depend on the intramolecular V-C conjunction of the individual subunits, we can study the impact of specific V domains on chain pairing by investigating antibody formation in cells that expressed chimeric Ig C chains.

Using this system, we assayed the assembly of Ig H chain and L chains bearing identical V domains derived from either an export-competent Ig L chain (the l chain of J558L) or an export-incompetent Ig L chain (the k chain of NS1). Hybrid genes (VC3 and VC3) were constructed and stable transfectants of J558L (expressing l chains) or NS1 (expressing NS1 k chains) cells established (Fig. 1; J558L.VC3 and NS1.VC3, respectively). In order to verify that chimeric chains can, in principle, form antibodies, we made other transfectants ex-
pressing Ig H and L chains with complementary V domains. As summarized Fig. 1 (and Table I), these include NS1.\(\gamma\)3 (endogenous \(\kappa\) and wild type \(\gamma\)3 chains), II4c.MEV (V\(\kappa\)C\(\kappa\) and V\(\gamma\)3 chains with mutually exchanged V domains), and J558L.\(\gamma\)3 (endogenous \(\lambda\)1 and wild type \(\gamma\)3 chains).

To analyze the assembly state of the Ig chains, cellular proteins were biosynthetically labeled with \[^{35}\text{S}\]methionine and material containing Ig H chains was isolated with protein A-Sepharose and analyzed by SDS-PAGE under non-reducing conditions (Fig. 2). In the control cell line, NS1.\(\gamma\)3 (NS1 \(\kappa\) chains and wild type \(\gamma\)3 chains), most of the Ig H chains migrated as H\(\kappa\)L\(\kappa\) molecules. In addition, all covalent assembly intermediates as well as some free Ig H chains (H\(\kappa\) and H) were detected (Fig. 2a, lane 1). Only 40–50% of the labeled Ig L chains were associated with Ig H chains, as quantified by densitometry of the fluorograph (data not shown) seen in Fig. 2b (lanes 1 and 2), i.e. Ig L chain seems to be in a molar excess over Ig H chain. Ig chains expressed by II4c.MEV cells (V\(\kappa\)C\(\kappa\) produced (Fig. 2 with Ig H chains bearing either the VH domain or the V\(\lambda\)1 (endogenous (Fig. 2 lanes 7 compare c), able to assemble into antibody molecules because they capable of forming the homodomain dimers required to support Ig chain assembly. However, the individual chains are, in principle, able to assemble into antibody molecules because they

| Cell line | Endogenous Ig | Transfectant | Endogenous Ig | Transfected Ig |
|-----------|---------------|--------------|---------------|---------------|
| J558L     | \(\lambda\)1  | J558L.\(\gamma\)3 | \(\lambda\)1  | \(\gamma\)3 |
|           |               | J558L.VAc\(\gamma\)3 | \(\lambda\)1  | V\(\gamma\)3 |
| NS1       | \(\kappa\)    | NS1.\(\gamma\)3 | \(\kappa\)    | \(\gamma\)3 |
|           |               | NS1.VC\(\gamma\)3 | \(\kappa\)    | V\(\gamma\)3 |
|           |               | NSF862\(\gamma\)3 | \(\kappa\)    | V\(\gamma\)3 |
| H62       | II4c.MEV      | V\(\kappa\)C\(\kappa\) | \(\lambda\)1  | V\(\gamma\)3 |
|           |               | ET.\(\lambda\)1 | \(\lambda\)1  | \(\gamma\)3 |

Note that these transfectants lost endogenous NS1 \(\kappa\) Ig L chain expression.

To verify this assumption, we analyzed total cellular proteins from the culture supernatant of J558L cells by gel filtration (Fig. 4). Most of the intracellular covalently linked Ig L chain dimers eluted at a position corresponding to the expected molecular mass of 55 kDa (maximum at fraction 18 in Fig. 4, left panel). Most of the non-disulfide linked Ig L assembly with partner chains that carry the V domain of the Ig H chain. Covalent assembly of Ig H and L chains also takes place when both Ig chains bear the V domain of the export-competent J558L \(\lambda\)1 chain, a result indicating that these V domains are capable of homodomain pairing.

Free Export-competent J558L \(\lambda\)1 Chains Are Secreted as Covalently and Non-covalently Linked Homodimers—Our results indicated that the V domain of the export-competent J558L \(\lambda\)1 chains could support Ig L chain homodimer formation. Indeed, J558L \(\lambda\)1 chains migrate as a single species when total cellular proteins were separated under native conditions that ought to have preserved all native complexes (Fig. 3). Together with the fact that a fraction of the J558L \(\lambda\)1 chains migrate as covalently linked dimers in non-reducing SDS-gels (24), the finding of a single band in the native gel suggests that most intracellular Ig L chains are in the same assembly state, i.e. dimers.

To verify this assumption, we analyzed total cellular proteins as well as proteins from the culture supernatant of J558L cells by gel filtration (Fig. 4). Most of the intracellular covalently linked Ig L chain dimers eluted at a position corresponding to the expected molecular mass of 55 kDa (maximum at fraction 18 in Fig. 4, left panel). Most of the non-disulfide linked Ig L...
It was reported that the Phe to Ser mutation at position 62 in a conserved sequence exposed on the surface of the $\lambda_1$ chain V domain blocked the secretion of the Ig L chain whether free or associated with $\mu$ Ig H chain (37). We previously analyzed this particular Ig L chain, $\lambda_1$ FS62, expressed in the absence of Ig H chains and showed that they are indeed not transported but are degraded instead (22). Here, we report an analysis of the export of the same Ig L chain in stably transfected cells, NSFS62,$\gamma_3$, that co-express $\gamma_3$ Ig H chain. In contrast to the model used by Dul and Argon (37), our experiments involve (i) stable rather than transient expression and (ii) expression of $\gamma$ rather than $\mu$ chain. We observed that $\lambda_1$ FS62 was secreted as part of assembled IgG. In fact, IgG secretion was as efficient as in the control cell line, E7,$\lambda_1$, which expresses $\gamma_3$ chain in combination with a $\lambda_1$ chain that does not carry the Phe to Ser substitution (Fig. 5). Since the mutation in the V domain of the $\lambda_1$ chain does not lead to a general block in Ig secretion, these results demonstrate that it does not destroy a putative transport signal postulated by Dul and Argon (37).

Both Internal Disulfide Bonds Are Formed in Export-incompetent $\lambda_1$, FS62 Chains When Paired with an Ig H Chain—Previous studies had shown that the export-incompetent $\lambda_1$, FS62 chain, like NS1 $\kappa$ chain, is bound to BiP as a partially oxidized molecule, whereas the export-competent J558L $\lambda_1$ chain exhibits a fully oxidized conformation (22). In order to investigate the oxidation state of the $\lambda_1$ chain FS62 in cells co-expressing Ig H chains in stable transfected cell lines, NSFS62,$\gamma_3$, cells were prepared in the presence of N-ethylmaleimide (to prevent oxygen-mediated oxidation of thiol groups) and immunoprecipitated Ig was analyzed by Western blotting under non-reducing conditions (Fig. 6). Analysis of total Ig chains revealed two Ig L chain bands (Fig. 6, lane 4) corresponding to a partially and a completely oxidized form; both forms migrated more rapidly than completely reduced Ig L chain (compare lane 4 with lane 1). When Ig H chain-associated material was analyzed (lane 5), the co-precipitated Ig L chains migrated as fully oxidized molecules as did the J558L $\lambda_1$ chains (lane 2). In contrast, Ig L chains remaining in the Ig H chain-depleted fraction were partially oxidized (lane 6) as they were in the absence of Ig H chain expression (lane 3). Thus, in the same cell, free $\lambda_1$, FS62 chain is partially oxidized, while Ig L chain co-precipitated with Ig H chain is completely oxidized.

$\lambda_1$, FS62 Chains Can Assemble with Ig H Chains as Partially Oxidized Molecules—The finding that $\lambda_1$, FS62 chains have formed the second internal disulfide bond upon Ig H chain...
Complete Folding Depends on Assembly

Fig. 6. Oxidation state of Ig H chain-bound and free λ1 FS62 chains analyzed by Western blotting. From the equivalent of 1.5 × 10^6 J558L cells lysed in the presence of 20 mM NEM, proteins were immunoprecipitated and separated by SDS-PAGE on a 15% acrylamide gel under reducing (R; 50 mM 2-mercaptoethanol) or non-reducing (NR) conditions. The Western blot was stained for Ig H and L chains and developed using the chemiluminescence procedure. Total Ig chains (lanes 1–4), Ig H chains (lane 5), or total Ig chains remaining after Ig H chain precipitation (lane 6) were isolated with an anti-L chain antiserum bound to protein A-Sepharose (for total Ig chains) or anti-H chain antibodies bound to protein A-Sepharose (for Ig H chain). The completely reduced Ig L chains (Red) migrate at a position different from the partially (Ox1) and the completely oxidized (Ox2) forms. The Ig H chain (H) and various assembly intermediates (H₂L₂, H₂L₃, and H₂L₄) are seen in the upper part of the blot, which was developed to a lower intensity as the lower part. Note that two bands are visible for Ig H chains that migrate as monomers.

Fig. 7. Oxidation state of Ig H chain-bound and free λ1 FS62 chains analyzed by biosynthetic labeling. NSFS62-γ3 were pulse-labeled for 20 min with [35S]methionine (200 μCi/2 × 10⁶ cells/ml) and chased with an excess of unlabeled methionine for 0 or 4 h, as indicated. Cells were lysed in the presence of 20 mM NEM, and Ig chains contained in equivalent number of cells were immunoprecipitated and separated by SDS-PAGE on a 15% acrylamide gel under reducing (R; 50 mM 2-mercaptoethanol) or non-reducing (NR) conditions. Total Ig chains (lane 1), Ig H chains (lanes 2 and 3), or total Ig chains remaining after Ig H chain precipitation (lanes 4 and 5) were isolated with an anti-L chain antiserum bound to protein A-Sepharose (for total Ig chains) or anti-H chain antibodies bound to protein A-Sepharose (for Ig H chain). Migration positions of reduced (Red), partially oxidized (Ox1), and completely oxidized (Ox2) Ig L chains as well as the Ig H chains migrating as monomers (H) or in covalently linked assembly intermediates (H₂L₂, H₂L₃, and H₂L₄) are indicated. It is to be noted that at least four distinct bands are resolved at the position of monomeric Ig H chains under non-reducing conditions.

Association raises the question whether assembly precedes oxidation of the second Ig L chain domain or is required for stabilization of completely folded λ1 FS62 chain. To determine the sequence of events, we performed pulse-chase experiments with NSFS62-γ3 cells and analyzed the oxidation state of Ig H chain-bound L chains (Fig. 7). Both fully and partially oxidized labeled Ig L chains were co-precipitated with Ig H chain isolated directly after the pulse (lane 2), indicating that Ig L chains can assemble with Ig H chains as partially oxidized molecules. The majority of labeled Ig H chains migrated as monomers (lane 2) and resolved into at least four bands (clearly visible in shorter exposed autoradiographs; data not shown), which might not only reflect Ig H chain with different glycomoieties but could also be due to Ig H chain with various numbers of internal disulfide bonds, as the fully reduced Ig H chain exhibited a higher apparent molecular weight and resolved into only two bands (lane 1).

After a chase period of 4 h, a different pattern was obtained. As compared to the situation present directly after the pulse, more noncovalently linked labeled Ig L chains were co-precipitated with Ig H chain after 4 h of chase indicating that unlabeled Ig H chains synthesized during the chase associated with pre-existing labeled Ig L chains (Fig. 7, lane 3). The majority of these Ig L chains migrated as fully oxidized molecules. More label is also found with covalently linked Ig H-L chain assembly intermediates as well as with complete H₂L₂ molecules. Conversely, a decreased amount of labeled molecules was present in the monomeric Ig H chain pool (lane 3). Interestingly, the amount of lower migrating Ig H chain monomers was more reduced compared to the amount of the more rapidly migrating species. This could reflect that assembly with Ig L chain allowed Ig H chain to form internal disulfide bonds that increase Ig H chain motility (33).

Consecutive precipitation of total Ig chains remaining in the Ig H chain-depleted fraction revealed that the majority of labeled intracellular Ig L chains were unassembled and partially oxidized (Fig. 7, lane 4). The presence of Ig L chains migrating as fully oxidized molecules in this fraction could be due to disassembly of the molecules from Ig H chains during the first immunoprecipitation procedure because of weak binding. Decrease in the total amount of free partially oxidized Ig L chain during the chase period (compare lanes 4 and 5) might reflect assembly with Ig H chain, on the one hand, or the degradation of free Ig L chains as is the case with these Ig L chains when expressed in the absence of Ig H chains, on the other hand (22). BiP co-immunoprecipitated with Ig H chains as well as with Ig L chains present in the Ig H chain-depleted samples (lanes 2–5).

In conclusion, these experiments demonstrate that partially oxidized λ1 FS62 chain is able to bind Ig H chain and could oxidize in the second domain only as the result of Ig H chain co-expression. However, these experiments did not allow us to unambiguously determine the fate of partially oxidized Ig L chains in Ig H chain-expressing cells because the conversion of noncovalent Ig L-H chain complexes into covalently linked complexes interfered with the analysis of the Ig L chain oxidation state, which could only be followed in Ig L chains noncovalently associated with Ig H chain. Therefore, these data could not prove that assembly is a prerequisite for Ig L chain folding. Nevertheless, the fact that partially oxidized Ig L chains coprecipitated with Ig H chain together with the time-dependent increase in the amount of fully oxidized Ig H chain-associated L chains (that could have been recruited from the partially oxidized labeled Ig L chain pool) are compatible with the interpretation that newly synthesized partially oxidized Ig L chains can pair with Ig H chains and may fully oxidize as Ig H chain-bound molecules.

Discussion
A major aim of this study was to understand the molecular basis underlying Ig chain secretion. In particular, we were
puzzled by the phenomenon that some Ig L chains depend on Ig H chain association for export, whereas others are also secreted as free chains even in the absence of Ig H chain expression. The latter fact was interpreted as a possible example of polypeptides that may escape ER quality control (1). The model that led us to design the experiments was based on the information that, on the one hand, export-incompetent Ig L chains are monomeric, oxidized in one domain only, and turn into fully oxidized molecules when experimentally released from BiP interaction (22, 26). On the other hand, export-competent Ig L chains interact only transiently with BiP and are secreted as completely oxidized molecules (22, 24). The data presented here provide evidence for Ig L chain monomers having to assemble with another Ig chain prior to export. This means that export competence of an Ig L chain is determined by its capability to form homodimers in case Ig H chains are not available for assembly. Furthermore, the requirement of assembly for export could reflect that Ig L chain proceed in folding only upon chain association as demonstrated for the export-incompetent λ1 FS62 that forms both internal disulfide bonds only when associated with an Ig H chain.

When λ1 FS62 chains are co-expressed with Ig H chains, Ig H chain-bound L chains are completely oxidized whereas, in the same cell, free Ig L chains are still partially oxidized (Fig. 6). This could reflect that the partially oxidized Ig L chain is a folding intermediate used for Ig chain assembly, which implies that assembly precedes disulfide bond formation in the second Ig L chain domain. Alternatively, Ig H-L assembly could be required for stabilization of a completely folded λ1 FS62 chain. In favor of the first hypothesis is the fact that we never detected completely oxidized Ig L chains in the absence of Ig H chain expression. Furthermore, the results of our pulse-chase experiments showed that Ig L chains are principally able to bind to Ig H chains as partially oxidized molecules. Our attempt to determine the folding pathway of Ig L chains was impeded by the formation of covalently linked HL, H2L, and H2L2 molecules. However, the existence of assembly-competent Ig L chain folding intermediates is independently supported by the finding that Ig chain assembly is also a prerequisite for prolyl isomerization (38). Furthermore, export-competent Ig L chains accumulate in a partially oxidized conformation in COS cells that transiently overexpress a mutant BiP defective in its ATPase activity (39). From these results it seems that disulfide bond formation in one of the two Ig L chain domains is independent of BiP-ATPase function, whereas oxidation of the second domain is not. Interestingly, export-incompetent Ig L chains are in the partially oxidized form irrespective of whether the ATPase of BiP is functional or not. In the light of our results, it is tempting to speculate that oligomerization of Ig L chains is the initial step required for APD-dependent dissociation of the BiP-L chain complex.

It seems clear that structural features intrinsic to the V domain must determine whether Ig L chains are export-competent or not as both types of Ig L chains with identical constant domains are found (24, 35). Indeed, mutations in the V domain of Ig L chains can alter their secretory phenotype (37, 40). Our experiments with the chimeric chains demonstrate that the competence of an Ig chain to assemble in vivo with another chain is strongly influenced by the nature of its V domain. For example, antibodies are formed when both chains carry the V domain of the export-competent J558L λ chain, while the chains do not associate when both subunits bear the V domain of the export-incompetent NS1 κ chain. It was shown that secretion of NS1 κ chain is restored when the histidine in position 87 is replaced by tyrosine or phenylalanine (35), suggesting that histidine 87 located in the contact surface of paired V domains (41) prevented Ig L chain homodimer formation. However, in the light of our results, the final three-dimensional structure known from crystallographic data is only achieved upon Ig chain pairing. As a consequence, amino acid residues that are not embedded in the final contact area may also be involved in the process of V domain pairing. For instance, the λ1 FS62 chain, which is found exclusively as unpaired monomer in NFS62 cells, carries the mutation on the solvent accessible outer surface of the folded structure.2

If complete Ig L chain folding is required for secretion and depends on the capability of free Ig L chains to self-assemble, one should expect export-competent Ig L chains to be secreted as dimers. We found that the J558L λ1 chains are fully oxidized (22) and are secreted as covalently and noncovalently linked homodimers (Figs. 3 and 4). The monomeric Ig L chains reported in some cases (42) may correspond to molecules with low affinity interaction that fall apart easily when exported as noncovalently linked dimers. In fact, the in vitro analysis of 17 human κ Bence Jones proteins showed that the ratio between Ig L chain monomers and noncovalently linked dimers depended on the dimerization constant, which ranged from <103 to >106 M⁻¹ (36). In this context, it is interesting to note that the dimerization constant does not necessarily correlate with the ratio between covalently and noncovalently linked Ig L chain dimers (36).

It had been suggested that V domains of Ig L chains carry a transport signal conferring export competence to the isolated Ig L chain as well as to assembled antibody molecules (37). This notion was based on experiments performed with the λ1 chain mutant, λ1 FS62, in which this putative signal was destroyed. The λ1 FS62 mutation not only blocked secretion of the Ig L chain itself but also led to the retention of assembled IgM in a transient transfection model. Here, we have analyzed transport of the same Ig L chain in stably transfected cells that co-express a γ chain and found that assembled IgG molecules were secreted. Moreover, about the same amount of IgG was secreted when the antibodies contained J558L λ1 or λ1 FS62 chains, i.e., the mutation did not alter the efficiency of antibody export. The conflicting results might be explained by the different experimental systems used to study Ig secretion; for instance, transient transfections might not ensure that sufficient amounts of both chains are expressed in the same cell. Be that as it may, however, our results exclude the possibility that a putative transport signal located on the Ig L chain had been destroyed by the FS62 mutation.

Our studies on Ig L chain assembly and secretion provide an example to illustrate how the cellular machinery could control the oligomerization state of multi-subunit proteins by monitoring the folding state of the individual subunits. In our model, oligomeric proteins would become export-competent only when the subunits proceed in folding as the result of successful assembly. Retention in the ER of unassembled subunits or of partial complexes would be due to ER chaperones binding to incompletely folded structures as does BiP with unassembled, partially oxidized Ig L chains (22). This view clearly differs from a model in which the role of BiP is to cover the hydrophobic interface of a non-assembled folded subunit (1). The observation that BiP binding prevents complete oxidation of unassembled Ig L chains (22, 39) strongly indicates that BiP interferes with the in vivo folding pathway of Ig chains. A quality control mechanism that monitors oligomerization at the level of subunit folding could not exclude the formation of different oligomers when subunits fulfill their folding requirements by assembling with various partner chains. For exam-

2 M. R. Knittler and I. G. Haas, unpublished results.
ple, Ig L chains pass the export checkpoint by forming either heterodimeric antibody molecules or Ig L homodimers. The thiol-mediated retention described by Sitia and co-workers (18) monitors the oligomerization state of normally covalently linked subunits and may act as a second mechanism to retain folded molecules that still exhibit free thiol groups (17, 21). As β2-microglobulin also exhibits an Ig fold structure and molecules can be exported without major histocompatibility complex class I H chain association, our model predicts that free β2-microglobulin might form homodimers prior to exit from the ER.

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