Degradation of Distinct Assembly Forms of Immunoglobulin M Occurs in Multiple Sites in Permeabilized B Cells*

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Protein degradation is essential for quality control which retains and eliminates abnormal, unfolded, or partially assembled subunits of oligomeric proteins. The localization of this nonsylosomal pre-Golgi degradation to the endoplasmic reticulum (ER) has been mostly deduced from kinetic studies and carbohydrate analyses, while direct evidence for degradation within the ER has been provided by in vitro reconstitution of this process. In this article, we took advantage of the transport incompetence of permeabilized cells to directly demonstrate that the selective degradation of secretory IgM (sIgM) in B lymphocytes is transport-dependent. We show that, upon permeabilization of the plasma membrane with either streptolysin O or digitonin, sIgM is not degraded unless transport is allowed. Nevertheless, upon complete reduction of interchain disulfide bonds with thiols, the free μ heavy chains are degraded by a transport-independent quality control mechanism within the ER. This latter degradation is nonselective to the secretory heavy chain μs, and the membrane heavy chain μm, which is normally displayed on the surface of the B cell, is also eliminated. Moreover, the degradation of free μs is no longer restricted to B lymphocytes, and it takes place also in the ER of plasma cells which normally secrete polymers of sIgM. Conversely, when assembled with the light chain, the degradation is selective to sIgM, is restricted to B lymphocytes, and is a transport-dependent post-ER event.

Protein degradation plays a key role in a myriad of regulatory processes (1). A quality control mechanism is responsible for retention and elimination of abnormal, unfolded, or partially assembled subunits of oligomeric proteins (2–4). Metabolically controlled turnover is reported for various proteins, including the α and β subunits of the T cell receptor (14–16), the H2a subunit of the asialoglycoprotein receptor (17–19), a truncated form of ribophorin (20), the PZ variant of αs-antitrypsin (21), unassembled immunoglobulin light chain (22), and two mutated yeast vacuolar proteins (23), as well as HMG-CoA reductase (5, 6) and apoB (7–10). Localization of these processes to the ER has been directly demonstrated only for the yeast proteins (23). In most cases, however, it has been deduced from temperature and ATP requirements, kinetic studies, structure of carbohydrate moieties, and insensitivity to drugs that block export of proteins from the ER.

Secretory IgM (sIgM) is an oligomeric protein that is rapidly degraded in B lymphocytes but is efficiently secreted from plasma cells (24, 25). We have shown that, in the 38C B lymphocytes, sIgM degradation is also nonsylosomal and occurs prior to the trans-Golgi (26, 27). Moreover, this degradation is selective to sIgM, while the membrane form of IgM (mIgM) is transported to the surface of 38C cells (26–28). Nevertheless, sIgM degradation does not comply with the postulated role of the quality control mechanism, since it is not the consequence but rather the cause for the incomplete assembly of sIgM. As we have shown in the 38C-derived D2 hybridoma, complete assembly of sIgM to polymers occurs in or beyond the trans-Golgi, while in the 38C B lymphocytes monomers are degraded prior to this compartment (27).

The localization of sIgM degradation further indicates that in the 38C cells this process does not represent the ER quality control. Degradation of sIgM requires ATP (27) and displays a characteristic lag and a threshold temperature of 18°C (26). Moreover, unlike the pre-Golgi degradations described above, sIgM degradation is strongly inhibited by brefeldin A (BFA) (28). Thus, we have proposed a novel post-ER site for sIgM degradation, a mechanism in which export of sIgM from the ER is obligatory.

The most direct evidence for ER degradation has been provided by in vitro reconstitution of this process. Permeabilized cells were used to identify the ER as the site of the quality control degradation of subunits of T cell receptor (16) and asialoglycoprotein receptor (19) and the metabolically regulated degradation of HMG-CoA reductase (6, 29) and apoB (9). We took advantage of the transport incompetence of cytosol-depleted permeabilized cells (30, 31) to directly demonstrate the transport dependence and hence the post-ER localization of sIgM degradation. Moreover, this in vitro system sheds light on the biochemical characterization of the complex process of protein degradation.
tein degradation along the secretory pathway and on the interrelations between sIgM folding, assembly, and degradation.

In this article we show that sIgM is not degraded in 38C B lymphocytes permeabilized with either streptolysin O (SLO) or digitonin. However, the post-ER μ-specific degradation is reconstituted if transport is allowed, and ER quality control degradation of free μ heavy chains is observed upon light chain dissociation by disulfide reduction with either dithiothreitol (DTT) or bis-(2-mercaptoethanesulfone) (BMS). This latter degradation is nonselective to free μ and μm is also eliminated and is no longer restricted to B lymphocytes, and it takes place in the ER of D2 hybridoma as well.

MATERIALS AND METHODS

Cell Lines—The murine B lymphocyte cell line 38C and the IgM-secreting D2 hybridoma derived from it were previously described (26). Biosynthetic Labeling of Cells—Cells (1–2 × 10^6 cells/ml) were starved for 1 h in methionine-deficient RPMI medium, pulse-labeled for 5–30 min with either [35S]methionine or a mixture of [35S]methionine and [35S]cysteine (DuPont or Amersham Corp.) (50 Ci/ml; 1000 Ci/mmol) and chased by dilution in 4 volumes of RPMI containing 2 mM methionine. At the end of the pulse, or at various chase periods, cells and medium were separated, and cells were either lysed as described previously (26), or permeabilized (see below). The level of [35S]methionine incorporation into de novo synthesized proteins was estimated as material precipitated by hot trichloroacetic acid. Addition of DTT to lysosomal pre-Golgi degradation has been assigned to the μm is also eliminated and is no longer restricted to B lymphocytes, and it takes place in the ER of D2 hybridoma as well.

Cell Permeabilization and in Vitro Incubation—At the end of the pulse labeling or at various chase periods, cells were permeabilized with either SLO or digitonin. For SLO permeabilization, cells were washed and resuspended in phosphate-buffered saline (4°C) and resuspended in phosphate-buffered saline, further incubated for 10–15 min at 37°C and collected (3,000 × g). SLO (Welcome), at 1.5 IU/ml for 38C cells and 0.4–0.6 IU/ml for D2 cells, was added and cells were incubated for 20 min on ice. The cells were collected by centrifugation (3,000 × g, 5 min, 4°C), resuspended in the same volume of fresh phosphate-buffered saline, further incubated for 10–15 min at 37°C and collected (3,000 × g, 5 min, 4°C) (adapted from Millere and Moore (32)). For digitonin permeabilization, cells were washed and resuspended (2 × 10^5 cells/ml) in KHM buffer (110 mM KCl, 20 mM HEPES, 2 mM MgCl₂, pH 7.2). Digitonin (high purity, Calbiochem, 35 μg/ml) was added, and cells were incubated for 5 min on ice. Cells were diluted with 9 volumes, washed in ice-cold KHM, and collected (3,000 × g, 5 min, 4°C) (adapted from Plunter et al. (33)). Optimal SLO and digitonin concentrations were determined to yield permeabilization of more than 95% of the cells, as judged by uptake of trypan blue and release of lactate dehydrogenase. This 150-kDa cystolic enzyme was estimated by its activity measured spectrophotometrically as a decrease in A₅₅₀ due to NADH oxidation (reaction mixture: 50 mM sodium phosphate buffer (pH 7.4), 1 mM NADH, 1% Triton X-100, 5 mM sodium pyruvate (modified from Tan et al. (34)). Permeabilized cells were resuspended in RPMI medium and chased in vitro by incubation at 37°C in a humid CO₂-air incubator.

Immunoprecipitation, Immunoblotting, and Treatment with Endoglycosidase H—Total IgM was immunoprecipitated from comparable amounts of trichloroacetic acid-precipitable 35S counts from lysates of whole or permeabilized cells and from culture supernatants. An excess amount of goat anti-mouse IgM (GAM IgM) antibodies was followed by sufficient protein A- bacterial adsorbent (BioMakor) to immunoprecipitate all IgM. Immunoprecipitated IgM was eluted and incubated with or without endoglycosidase H (endo H; BioLabs) (26). IgM was resolved by reducing or nonreducing SDS-PAGE and quantified by either laser (26) or Imaging Plate (Fuji) densitometry. IgM was electroblotted onto nitrocellulose, probed with horseradish peroxidase-conjugated anti-mouse μ antibodies (Sigma) (27), and visualized by ECL (Amersham Corp.).

RESULTS

Degradation of sIgM Does Not Occur in Permeabilized B Cells Unless Transport Is Allowed.—The recently described nonlysosomal pre-Golgi degradation has been assigned to the ER by part of the quality control mechanism (4). Although sIgM degradation is also a nonlysosomal pre-trans-Golgi event, its localization prior to the polymerization and its inhibition by low temperatures, energy poisons, and BFA indicate that this process does not comply with quality control and occurs in a post-ER compartment (26–28). To characterize this degradation and directly demonstrate its novel localization, we have established an in vitro degradation assay in permeabilized cells. Since transport between the ER and the Golgi and between the Golgi cisternae requires a myriad of cytosolic factors and ATP, their depletion from permeabilized cells prevents any vesicular transport (30, 31). Consequently, proteins are trapped in the compartment they have reached prior to permeabilization.

As shown in Fig. 1, when 38C cells were permeabilized immediately after the pulse labeling and incubated in vitro for up to 5 h, the levels of both μ and μm remained relatively constant with an estimated half-life of 90 h. Similar stability of IgM was observed in cells permeabilized by either SLO or digitonin (Fig. 1). Conversely, if prior to cell permeabilization intracellular transport was allowed in vivo for 90 min, a gradual loss of μ was observed, and about 50% of μ was eliminated during the subsequent 4 h of the in vitro incubation, reflecting the net degradation of μ with a half-life of 4.6 h (Fig. 1). This in vitro degradation was specific, since the level and pattern of total labeled proteins remained relatively constant (data not shown). Thus, even when newly synthesized sIgM is trapped in the ER of permeabilized 38C cells it is not degraded, indicating that this specific degradation of sIgM does not occur within the ER and requires transport to a post-ER compartment. Moreover, unlike the in vivo degradation which is inhibited by energy poisons (27), after arrival to the degradation site, the degradation process per se requires neither cytosolic components nor externally added ATP.

Degradation of Both sIgM and μIgM Occurs in the Endoplasmic Reticulum of Permeabilized or Intact Cells in the Presence of Thiol-reducing Agents—Degradation of T cell receptor subunits in permeabilized cells was sensitive to the thioldox state and was detected only in the presence of DTT (16). This, together with studies with specific protease inhibitors (35), suggested the involvement of a cysteine protease in ER degradation. We have shown that a cysteine protease participates also in the in vivo degradation of sIgM in 38C cells (28). It is possible that oxidizing thioldox state unfavorable for the cysteine proteases activity was generated upon permeabilization. To assess the contribution of redox state, the thiold-reducing agents ME and DTT were added to the in vitro incubation. Interestingly, these two thiold-reducing agents exerted very different effects on the fate of newly synthesized μ and μm trapped in the ER. As shown in Fig. 1, in untreated as well as in ME-treated 38C cells permeabilized at the end of the pulse labeling, μ was essentially stable. Conversely, treatment with DTT caused a striking effect and within 5 h of in vitro incubation less than 10% of μ was recovered (Fig. 1). Regardless of whether cells were permeabilized by SLO or by digitonin, a rapid degradation of μ with a half-life shorter than 2 h was reconstituted in vitro in the presence of DTT (Fig. 1). Moreover, under these conditions, both μ and μm were rapidly degraded, as revealed after treatment with endo H (Fig. 1C).

These results demonstrate two locally distinct degradations of μ chains which are both reconstituted in permeabilized cells but exhibit different thioldox requirements. In the presence of DTT, μ and μm are degraded within the ER, as indicated by the persistence of this process in permeabilized cells. On the other hand, in the presence of ME or in the absence of any thiold-reducing agent, μ is degraded in a post-ER compartment, as concluded from the stability of μ in permeabilized cells (Fig. 1). Similar results were obtained with intact cells in which the ER-to-Golgi transport was blocked with BFA. As shown in Fig.
2A, in 38C cells incubated in the presence of DTT both µ and µm were rapidly degraded with a half-life of 1 h. The same short half-life was measured when DTT-treated cells were incubated in the presence of BFA, indicating degradation within the ER. Conversely, the rapid degradation (t½ = 1.5 h) of µ in untreated or ME-treated cells was inhibited by BFA (t½ = 4.4 h), thus this process took place in a post-ER compartment. Intrigued by the opposite effects exerted by DTT and ME, we examined a third thiol-reducing agent, BMS. As shown in Fig. 3A, the strong inhibition exerted by BFA (t½ was 3-fold prolonged, from 1.6 to 4.9 h) was moderated at a low (1 mM) BMS concentration (t½ was only 1.6-fold prolonged, from 2.3 h to 3.6 h), while at a higher (10 mM) BMS concentration, degradation was essentially insensitive to BFA (t½ was 1.1-fold prolonged, from 1.2 to 1.3 h). In this respect, BMS exerted both the effects of ME and DTT, in a dose-dependent fashion.

We could not rule out the possibility that only the stronger divergent reducing agents DTT and BMS, but not the milder monovalent ME, activated a relevant ER cysteine protease. Indeed, based on the criteria that indicated the involvement of a cysteine protease in the post-ER degradation of sIgM (28), participation of a cysteine protease was demonstrated also in the ER degradation. As shown in Fig. 4, a strong inhibition was exerted by ALLN on the post-ER degradations, as well as on the DTT-dependent degradation within the ER. However, if the in vitro post-ER proteolytic activity shown in Fig. 1 was carried out by the same cysteine protease that was involved in the in vivo degradation, this enzyme did not require any exogenous thiol to function in vitro. In fact, in 38C cells permeabilized after 90 min of chase, the µ that reached the post-ER site was degraded most efficiently in the absence of any reducing agents (Fig. 1D). Conversely, in cells permeabilized at the end of the pulse labeling, degradation within the ER was completely dependent upon DTT (Fig. 1).

The Site of Degradation Is Determined by Assembly of µ with the Light Chain—It is well established that the thiol redox state plays a crucial role in the formation of intra- and intermolecular disulfide bonds (12, 13, 36, 37). Therefore, an alternative explanation for the distinct effects of ME, DTT, and BMS on IgM degradation could be their differential capacity to generate diverse degradation substrates by interfering with IgM folding and assembly. This was shown by monitoring, by nonreducing SDS-PAGE, the effects of different concentrations of these thiol on the process of IgM assembly (Figs. 2B and 3B) and the levels of IgM assembly intermediates (Figs. 2C and 3C). A dose-dependent reduction of IgM revealed that the disulfide bond between µ and the light (L) chain in µL hemimers was reduced at 5 mM DTT (Fig. 2C) or 10 mM BMS (Fig. 3C) but hardly by ME (Fig. 2C), whereas 1 mM DTT (Fig. 2C) or BMS (Fig. 3C) was sufficient to fully reduce the µ–µ bond in µL2 monomers. The assembly process of newly synthesized µ with L into µL hemimers and subsequently into µL2 monomers (Figs. 2B and 3B) (see also Amitay et al. (26)) was interrupted in 38C cells treated with 14.3 mM ME (Fig. 2B) or 1 mM BMS (Fig. 3B). Under these conditions, hemimers and free µ chains were observed during the entire chase, indicating that their subsequent assembly into monomers was inhibited, whereas the disulfide bond between µ and L in preexisting hemimers was relatively stable. On the other hand, in cells treated with 5 mM DTT (Fig. 2B) or 10 mM BMS (Fig. 3B), the majority of the µ chains were free, indicating their dissociation from the L chain. Moreover, these free µ chains exhibited a retarded electrophoretic mobility on nonreducing SDS-PAGE, suggesting that intrachain disulfide bonds were also reduced (38).

Although monomer formation was more prominent in SLO-permeabilized 38C cells, essentially similar effects of ME and DTT were observed in permeabilized or intact 38C cells (Fig. 2B). The higher level of monomers detected in permeabilized cells in the absence of any thiol could be caused either by SLO directly, by the 15-min incubation period at 37 °C required for the SLO treatment, or by the relatively oxidizing conditions generated upon permeabilization. The two former possibilities
were ruled out when higher levels of monomers were detected also in digitonin-permeabilized cells upon 5 min of incubation on ice (data not shown). Regardless of the permeabilization procedure, different patterns of assembly were observed with DTT, ME, or BMS. It was evident that these three thiols were capable of reducing disulfide bonds, yet with quite distinct reducing capacity. Reduction with ME and low BMS was limited to interchain disulfide bonds between \( \mu \)-chains in monomers and to a lesser extent between \( \mu \) and L in hemimers, while DTT and high BMS reduced completely interchain as well as intrachain disulfide bonds. It is interesting to note the hierarchy regarding the various interchain disulfide bonds in IgM; the \( \mu -L \) bond is readily formed to produce hemimers and is the one most resistant to reduction, while the subsequently formed \( \mu -\mu \) bond in monomers is the one more easily reduced (Figs. 2 and 3) (see also Fig. 5) (26). These results support our hypothesis that the distinct effects of DTT and ME and the dose-dependent effect of BMS on the degradation of IgM is the consequence, at least in part, of their differential effects on IgM assembly and folding. It appears that only DTT and BMS have the reducing capacity required to form free unfolded \( \mu \) chain which is in turn a suitable substrate for retention and degradation in the ER by the quality control mechanism.

Degradation in Permeabilized Cells Is Not Restricted to B Lymphocytes and Also Occurs in Hybridoma Cells—We have previously shown that in vivo the degradation of slgM is restricted to 38C B lymphocytes, while the 38C-derived D2 hybridoma efficiently secretes this slgM (26). However, we have recently provided evidence that, in D2 cells, slgM was largely diverted to the post-ER degradation when its sorting was perturbed (39). Here we show that the ER quality control mechanism, that retains and degrades free unfolded \( \mu \) chains, also functions in D2 cells. Upon permeabilization of D2 cells with SLO, \( \mu \)-slgM was relatively stable for up to 3 h of \textit{in vitro} incubation in the absence of thiols, but was rapidly degraded with a half-life of about 1.5 h when DTT was added to the incubation medium (Fig. 5A).

This conclusion was corroborated in whole cells, when intracellular transport from the ER was blocked by BFA. As previously shown, the efficient secretion of slgM from D2 cells was abolished by BFA (28). A dose-dependent inhibition of secretion was also exerted by DTT, concomitant with stimulation of degradation (Fig. 5B). However, this DTT-induced degradation was not prevented by BFA, indicating an ER process (Fig. 5C). Interestingly, as we have previously shown, in the presence of ME secretion was hampered and degradation was promoted, yet the ME-induced degradation was inhibited by BFA (39). The patterns of assembly process of newly synthesized \( \mu \) (Fig. 5D) (see also Amitay et al. (26)) and the levels of assembly intermediates (Fig. 5E) indicated that in D2 cells \( \mu \)-slgM was rapidly assembled into \( \mu _2 \)L\( _2 \) monomers and mostly accumulated as \( \mu _2 \)L\( _2 \)L\( _g \)-polymers. The hierarchy of the various interchain disulfide bonds in IgM reported above for 38C cells (see Figs. 2 and 3), was even more prominent in D2 cells. As revealed by a dose-dependent slgM reduction with DTT, the readily formed \( \mu -L \) bond required 5 mM DTT for its complete reduction, the subsequent \( \mu -\mu \) bond was reduced at 2 mM, and the last disulfide bond formed upon polymerization was reduced at as low as...
After 1 h of chase, intact D2 cells, similar to their effects in 38C cells (see Fig. 2),
distinct effects on the assembly process in permeabilized and
deglycosylated IgM (the pulse labeling, as described under “Materials and Methods.”

In conclusion, it appears that, in both 38C and D2 cells, or into monomers that are
degraded. Moreover, it functions in the 38C B cells and D2
degraded. Moreover, it functions in the 38C B cells and D2

1 mM DTT (Fig. 5E). Both ME and DTT exerted significant yet
distinct effects on the assembly process in permeabilized and
unlabeled 38C cells, incubated for 1 h with the indicated concentration of BMS (C). Total IgM was immunoprecipitated by GAM IgM from cell lysates with equal
amounts of protein and with similar amounts of trichloroacetic acid-precipitable material, treated with (A) or without (B and C) endo H and
resolved by 10% SDS-PAGE under reducing conditions (A) or 3–12% SDS-PAGE under nonreducing conditions (B and C). Quantification by
Imaging Plate densitometry is presented as percent of

FIG. 4. A cysteine protease is involved in the DTT-induced ER
degradation of IgM in 38C cells. 38C cells were starved for 1 h,
pulse-labeled for 7 min with [35S]methionine, and chased for the indicated time (h) in the absence
(–) or presence (+) of ALLN (60 μg/ml). The cells were chased for the
indicated time (h) in the absence (−) or presence (+) of DTT (6 mM).
Total IgM was immunoprecipitated by GAM IgM from cell lysates with similar amounts of trichloroacetic acid-precipitable material and re-
solved by 10% SDS-PAGE under reducing conditions. Quantification by
Imaging Plate densitometry is presented as percent of μ at the end of the pulse labeling, as described under “Materials and Methods.”
deglycosylated μ; dg-μ, deglycosylated μ; μ heavy chains; μL, hemimers; μL, monomers; arrow, unfolded μ.

DISCUSSION

In this article we show that the μ heavy chains undergo
degradation by two locally distinct mechanisms. Although in
most cases degradation of a certain protein along the secretory
pathway is confined to a single site, degradation at multiple
sites has been recently reported for apoB (9, 10). The degrada-
tion of IgM at both sites can be reconstituted in vitro in per-
meabilized cells. The first retention/degradation mechanism is
a bona fide ER quality control that has been documented in
many cell types and here is demonstrated in both 38C and D2
cells. Based on insensitivity to BFA and persistence in perme-
abilized cells, this degradation is transport-independent, thus
occurs within the ER. Our results demonstrate that a suitable
substrate for this rapid ER degradation is unassembled free
unfolded μ heavy chains that are not in association with L
chains. However, once assembled with L chains, μ is no longer
recognized by the quality control mechanism. Here we show
that upon reduction of inter- and intramolecular disulfide
bonds by DTT or BMS, the resulting free unfolded μ heavy chains can be recognized by this ER mechanism. Similarly, the
relatively stable chimeric γ2bμp heavy chain (40, 41) is also
rapidly degraded upon DTT treatment of transfected myeloma
cells (42). Also, in the absence of L chains in either B cell
variants (43) or transfectants (44), the unassembled heavy
chains are recognized by the quality control mechanism, prob-
ably reflecting the situation in the pre-B cells. This ER quality
control mechanism is not selective to μs and μm is also rapidly
degraded. Moreover, it functions in the 38C B cells and D2

1 mM DTT (Fig. 5E). Both ME and DTT exerted significant yet
distinct effects on the assembly process in permeabilized and
unlabeled 38C cells, similar to their effects in 38C cells (see Fig. 2).
After 1 h of chase, μL hemimers and free μs chains were
detected in ME-treated cells, suggesting that the subsequent
process was inhibited (Fig. 5D). However, in the presence of DTT, only free unfolded μs chains were detected
indicating complete reduction of interchain as well as intrachain disulfide
bonds.

In conclusion, it appears that, in both 38C and D2 cells, μ
chains that are assembled with L chains do not undergo de-
gration within the ER. They are assembled into polymers,
which are secreted in D2 cells, or into monomers that are
diverted to degradation in a post-ER compartment in 38C cell.
Unfolded free μ chains, on the other hand, are retained and
degraded within the ER by the quality control mechanism that functions efficiently in both cell types.
The second IgM retention/degradation mechanism we describe functions only when the first one is rendered nonfunctional, probably by association with L chain. This mechanism takes place in a post-ER compartment and is transport-dependent, as judged from its sensitivity to BFA and abolishment in permeabilized cells. The substrate for this degradation is μ heavy chain in association with L chain, namely hemimers and most probably monomers (see also Shachar et al. (27)). This post-ER mechanism is selective to μ and does not degrade γμ, and it functions only in the 38C B cells. However, when sorting is impaired either by ME or by perturbation of calcium sequestration, the post-ER retention/degradation functions also in the D2 hybridoma cells (39). The sorting to this post-ER mechanism is probably mediated by Cys-575, since it is hampered by mutation of this residue as well as by ME (39–41). Exposed thiol groups appear to participate also in the trans-Golgi network sorting of chromogranin B, which is diverted from the regulated to the constitutive secretory pathway upon DTT treatment (51).

Although cysteine proteases are implicated in degradation within the ER (8, 9, 16, 35, 52, 53), they are not the characteristic of this process and have been shown also in post-ER degradations (10, 28, 54). Indeed, here we show that cysteine protease(s) are involved, at least in vivo, in both the ER and the post-ER degradations (see also Amitay et al. (28)). Importantly, degradation by cysteine proteases has not been directly demonstrated but rather, as shown here, deduced from inhibition by ALLN (8, 9, 28, 35, 53, 55) and additional inhibitors (10, 52) or from stimulation by DTT (16). Among the proteolytic enzymes implicated in degradation along the secretory pathway, two cysteine proteases, ER60 (52) and ERp72 (53) have been purified from the rough ER of rat and mouse livers. Interestingly, the cysteine protease involved in the post-ER degradation of sIgM does not require any exogenous thiols for its function either in vivo or in vitro. Moreover, it appears that both degradation processes per se require neither cytosolic components nor exogenous ATP.

The ER quality control mechanism correlates transport of membrane and secretory proteins with their adequate folding and assembly within the ER lumen. Among the myriad of chaperones, which assist translocation, folding, and assembly (12, 13), BiP/GRP78 competes with L chain for the CH1 domain of the heavy chain (56) and is displaced by L chain (57). It remains to be established whether BiP/GRP78 participates in the ER quality control retention/degradation of the μ heavy chains. It is possible that other chaperones such as GRP94, protein disulfide isomerase, and calnexin, which have been shown in association with a variety of misfolded and unassembled proteins including DTT-reduced ones (22, 46, 58–60), are involved. The ER lumen oxidized redox state (36) and protein disulfide isomerase (61) contribute to the formation of intra- and intermolecular disulfide bonds abundant in IgM and in other cysteine-rich proteins that are folded within the ER (37). The orderly formation of these bonds is shown here for IgM and is required for folding progression and protein stability (13). Folding intermediates, incompletely assembled proteins such as free μ chains, misfolded proteins, and aggregated side products are selectively retained and degraded by the ER quality control (2, 13, 62). Among them are many newly made proteins which are retained within the ER upon DTT treatment (13, 38, 45–49), resembling the unfolded free μ chains described here.

The most susceptible to reversible reduction of disulfide bonds are nascent proteins, folding intermediates, and misfolded proteins in the ER, whereas DTT hardly unfolds stabilized molecules that have already acquired mature oligomeric
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conformation (38, 45, 46, 48, 49). Moreover, here we show an

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