The Chaperone BiP/GRP78 Binds to Amyloid Precursor Protein and Decreases Aβ40 and Aβ42 Secretion*

(Received for publication, July 14, 1998, and in revised form, August 18, 1998)

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Recent studies of cellular amyloid precursor protein (APP) metabolism demonstrate a β/γ-secretase pathway resident to the endoplasmic reticulum (ER)/Golgi resulting in intracellular generation of soluble APP (APPsβ) and Aβ42 peptide. Thus, these intracellular compartments may be key sites of amyloidogenic APP metabolism and Alzheimer’s disease pathogenesis. We hypothesized that the ER chaperone immunoglobulin binding protein (BiP/GRP78) binds to and facilitates correct folding of nascent APP. Metabolic labeling and immunoprecipitation of transiently transfected human embryonic kidney 293 cells demonstrated co-precipitation of APP with GRP78, revealing their transient interaction in the ER. Maturation of cellular APP was impaired by this interaction. Furthermore, the levels of APPs, Aβ40, and Aβ42 recovered in conditioned medium were lower compared with cells transfected with APP alone. Co-expression with APP of GRP78 T37G, an ATPase mutant, almost completely blocked cellular APP maturation as well as recovery of APPs, Aβ40, and Aβ42 in conditioned medium. The inhibitory effects of GRP78 and GRP78 T37G on Aβ40 and Aβ42 secretion were magnified by co-expression with the Swedish mutation of APP (K670N/M671L). Collectively, these data suggest a transient and direct interaction of GRP78 with APP in the ER that modulates intracellular APP maturation and processing and may facilitate its correct folding.

The major components of amyloid plaque in Alzheimer’s disease (AD) brain are Aβ peptides, including Aβ40 and Aβ42, that are derived from amyloid precursor protein (APP). APP is metabolized at or near the cell surface by an α-secretase that results in soluble APP (APPsα) secretion and precludes Aβ formation. APP is also metabolized by an endosomal/lysosomal (endocytic) pathway that results in Aβ secretion (1–3). Recent data with human NT2 neurons demonstrates that Aβ is found intracellularly (4) with kinetics identical to APP synthesis, suggesting that a fraction of nascent APP is immediately metabolized to Aβ (5). Subsequently, a β/γ-secretase pathway of APP metabolism resident to the endoplasmic reticulum (ER)/Golgi of neurons was identified, resulting in intracellular APPsβ and Aβ42 generation (6–11). This exocytic pathway may be specifically promoted by presenilin-1 and presenilin-2 mutations found in some pedigrees of familial AD, since these proteins localize to the ER/Golgi (12–14) and result in greater Aβ42 secretion (15–17). In fact, common to all mutations of APP and presenilins linked to early-onset familial AD is their ability to promote Aβ42 generation (1–3). Because Aβ42 is intrinsically more amyloidogenic than Aβ40 and deposits preferentially in brain, this relatively minor pathway of APP metabolism within the ER/Golgi may have major implications for AD pathogenesis.

All proteins destined for the cell membrane or secretion must first translocate into the ER. Newly translocated proteins are folded and assembled by a group of proteins, which include immunoglobulin-binding protein (BiP/glucose-regulated protein, 78 kDa (GRP78), glucose-regulated protein, 94 kDa (GRP94), peptidyl prolyl isomerase, calnexin, and protein disulfide isomerase. Paramount among these ER residents is the highly conserved ATP-binding protein GRP78, which associates transiently with many polypeptides and more stably with misfolded or incompletely assembled proteins (18). Association with GRP78 is hypothesized to prevent misfolding and aggregation of nascent polypeptides during synthesis and assembly in the ER. Misfolded proteins in the ER remain bound to GRP78 and are destined for degradation. In mammalian cells, GRP78 is detected noncovalently bound to a variety of proteins, including immunoglobulin heavy chain, nicotinic receptor, HIV-1 envelope protein, T cell receptor α chain variants, influenza hemagglutinin, and type I procollagen pro chain (for reviews, see Refs. 19 and 20). This wide variety of substrates of GRP78 suggests that the correct folding of APP may also require transient binding to GRP78 in the ER.

As is true for all Hsp70 proteins, the amino-terminal two-thirds of GRP78 comprise an ATP binding domain, and the carboxyl-terminal third contains a peptide binding domain. GRP78 binds ATP and has weak ATPase activity. Elucidation of the three-dimensional structure of this domain led to the development of mutants such as GRP78 T37G with impaired ATP-induced release of bound protein (21, 22). This ATPase mutant also blocks assembly and folding of immunoglobulin heavy chains (23). Thus the GRP78 T37G mutant functions as a molecular trap by stabilizing the normally transient interaction of newly synthesized polypeptide with GRP78. ATPase-defective GRP78 does not bind to and retard all secreted proteins. For example, Factor VIII secretion is reduced by ATPase-defective GRP78 co-expression, but monocyte/macrophage

kDa; HEK, human embryonic kidney; PAGE, polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay.
colony-stimulating factor is not (24). We hypothesized that: 1) GRP78 binds to APP in the ER, 2) this interaction has functional consequences on APP metabolism, and 3) the transient interaction of GRP78 with APP may be captured by co-expression with GRP78 T37G.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—Human embryonic kidney (HEK) 293 cells were cultured in Dulbecco’s modified Eagle’s medium containing 100 units of penicillin/ml and 100 μg/ml of streptomycin sulfate supplemented with 10% heat-inactivated fetal bovine serum and 2 mM glutamine (Life Technologies, Inc.). Human APP or APP Swe was cloned into pCI (25). The APP751 isoform was used exclusively in this study. Hamster GRP78 and GRP78 T37G were each cloned into pcDNA3 (Invitrogen). Hamster GRP78 is greater than 99% identical to human GRP78. HEK 293 cells were split 1 day prior to transfection (1 × 10^6 cells/6-cm dish) and transfected with 10 μg of DNA by the calcium phosphate procedure or LipofectAMINE (Life Technologies, Inc.) as described by the manufacturer.

**Metabolic Labeling and Immunoprecipitation**—Forty-four hours after transfection, cells were labeled with [35S]methionine and [35S]cysteine, and subsequently, proteins from cell lysates or conditioned medium were precipitated with 1 ml of lysis buffer (1% Nonidet P-40 in 50 mM Tris, 150 mM NaCl, 5 mM EDTA, pH 8.0). Cell lysates were centrifuged to precipitate insoluble material. The cleared supernatants were equally divided into a pair of tubes containing either Karen, a polyclonal anti-APP antiserum raised to the secreted amino terminus of APP, or anti-rodent GRP78 antibody (anti-GRP78), which does not cross-react with endogenous human GRP78. Protein-antibody complexes were incubated with 25 μl/sample) at 4 °C overnight. Excess anti-GRP78 was precipitated by anti-GRP78 (Fig. 1B, lanes 3 and 6), or conditioned medium (C) or anti-GRP78 (B) and detected by fluorography. Immunoprecipitation of APP co-precipitated a 78-kDa protein (labeled GRP78) (A), and immunoprecipitation of GRP78 co-precipitated a 95-kDa protein (labeled APP) (B).

**RESULTS**

To determine whether GRP78 was capable of binding to APP within the ER, HEK 293 cells were co-transfected with APP and either empty vector (pcDNA3), GRP78, or GRP78 T37G. Processing of the Swedish mutant APP (APP Swe; K670N/M671L) in HEK 293 cells results in elevated levels of Aβ40 and Aβ42 compared with wild type APP (26, 27). Similar co-transfections were also performed with APP Swe and GRP78. Following the co-transfections, cells were metabolically labeled with [35S]methionine and [35S]cysteine, and subsequently, proteins from cell lysates or conditioned medium were immunoprecipitated with the anti-APP antiserum Karen (2, 1A, lanes 2 and 5). Again, this transient interaction was stabilized by co-transfection of APP or APP Swe (lanes 3 and 6) with GRP78 T37G. A single protein corresponding in molecular weight to immature APP was detected in these lanes, suggesting that GRP78 and GRP78 T37G bound only to immature APP.

We hypothesized that the association of APP with GRP78 in the ER may impede the secretion of APPs into conditioned medium. To test this hypothesis, radiolabeled APPs in conditioned medium were co-transfected with GRP78 (Fig. 1A, lanes 2 and 5, respectively). This 78-kDa protein was not detected in co-transfections of APP or APP Swe with empty vector (Fig. 1A, lanes 1 and 4, respectively). As predicted, when the ATPase-defective mutant, GRP78 T37G, was co-transfected with either APP or APP Swe, a much greater amount of the 78-kDa protein co-precipitated from cell lysates with anti-APP antibody (Fig. 1A, lanes 3 and 6, respectively). Additionally, a decrease in the amount of mature APP and APP Swe was consistently detected in lysates of cells co-transfected with GRP78 T37G.
tioned medium was immunoprecipitated with Karen antibody (Fig. 1C). A moderate but reproducible decrease in APPs was detected in the media of cells co-transfected with GRP78 (lanes 2 and 5) compared with empty vector (lanes 1 and 4). A more significant reduction of APPs recovery was observed for cells co-transfected with GRP78 T37G and either APP or APP Swe (lanes 3 and 6).

The radiolabeled protein migrating above immature APP (Fig. 1A) may be either mature, fully glycosylated APP, or an unrelated co-precipitating protein. To distinguish between these possibilities, similar experiments were conducted and proteins in conditioned medium or cell lysates detected by immunoprecipitation and immunoblot (Fig. 2). The larger molecular weight protein above immature APP was recognized by the mouse monoclonal anti-APP antibody (22C11), verifying that it is mature, glycosylated APP (labeled M, Fig. 2A). Co-transfection of APP or APP Swe with GRP78 or GRP78 T37G impaired APP maturation (Fig. 2A, lanes 5, 6, 8, and 9). In addition, transfection with GRP78 T37G consistently resulted in accumulation of immature endogenously expressed APP in cell lysates (labeled I, Fig. 2A, lane 3). Recovery of APPs in conditioned medium was reduced by co-transfection with GRP78, and this inhibitory effect was more pronounced with the GRP78 T37G mutation (Fig. 2C), despite equivalent levels of GRP78 or GRP78 T37G expression (Fig. 2B).

We next hypothesized that the binding and retention of APP in the ER by GRP78 would modulate Ab40 and Ab42 secretion. HEK 293 cells were transfected with vector (pRK5), APP, or APP Swe in the absence or presence of vector (pcDNA3), GRP78, or GRP78 T37G. Ab40 and Ab42 were measured by ELISA (Fig. 3). Transfection with APP resulted in measurable and reproducible levels of Ab40 and Ab42 in conditioned medium. Analogous to results with APPs (Figs. 1C and 2C), the secreted Ab40 and Ab42 levels were reduced by co-transfection of APP with GRP78 and more strongly diminished with GRP78 T37G. The levels of total APP expression from all these cell lysates were equivalent (not shown). Thus, the decreases in secreted Ab peptides appear due to an effect of GRP78 on the catabolism of APP and not on APP synthesis. This conclusion is further supported by transfections with APP Swe, which results in greater concentrations of both Ab40 and Ab42 compared with normal APP (27, 28). When co-expressed with APP Swe, the inhibitory effects of GRP78 and GRP78 T37G on Ab40 and Ab42 secretion were magnified.

**DISCUSSION**

This study demonstrates co-precipitation of APP with GRP78 and functional consequences of this interaction on APP metabolism. Specifically, the maturation of cellular APP was impaired, and APPs, Ab40, and Ab42 recovery in conditioned medium was reduced. The binding of GRP78 to proteins is ordinarily transient and may be difficult to detect. Thus, co-precipitation and functional effects obtained with GRP78 and APP were magnified by the GRP78 T37G ATPase mutation, which fails to release transiently bound protein, and by the APP Swe mutation that results in greater Ab40 and Ab42.
secretion. The APP detected bound to GRP78 was of a single molecular weight corresponding to the immature form. The mature or intermediate forms of APP did not co-precipitate even with the mutant GRP78 T37G. Thus, the transfected APP bound to BiP was not saturating the system retaining it in the ER. These results also suggest that the membrane-spanning APP is normally transiently bound to and retained in the ER as a nascent polypeptide by the chaperone GRP78. GRP78 may subsequently interact with the KDEL receptor causing a net retention of the GRP78-APP complex in the ER lumen. Alternatively, because GRP78 binding impedes APP maturation in the ER, it may be retained via interaction with other chaperones such as calnexin (for review, see Ref. 29). Future experiments will distinguish between these possibilities.

Levels of APPs, Aβ40, and Aβ42 secretion were reduced by interaction of APP with GRP78. This transient interaction may impair access of APP to β-γ-secretases within the ER/Golgi or may influence APP metabolism by facilitating its correct folding. Because secreted Aβ42 is generated primarily in the ER, these data suggest that GRP78 binding to APP may directly or indirectly confer protection from β-γ-secretases within this cell compartment. One may speculate that immediate processing of nascent APP is exclusive to misfolded APP. However, the possibility remains that less Aβ42 is secreted, because it is bound to GRP78 or other proteins in the ER, such as ERAB (endo-}

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Acknowledgements—We express our thanks to Dr. N. Suzuki (Takeda) for antibodies BAN-50, BA-27, and BC-05, Dr. B. Greenberg (Cephalon) for the antisera Karen; and Dr. L. Hendershot (St. Jude Children’s Research Hospital) for the polyclonal anti-rodent GRP78 antibody.