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# ATP-induced loss of Alz-50 immunoreactivity with the A68 proteins from Alzheimer brain is mediated by ubiquitin

(Alzheimer disease/paired helical filament/ATP-dependent proteolysis)

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ABSTRACT **The Alz-50 immunoreactive proteins, designated A68, are detected by electrophoretic blot analysis of 100,000 × *g* pellet fractions of brain tissue from individuals with Alzheimer disease (AD). In exploring the biochemical nature of these proteins, we have found that a preincubation of such fractions with 5 mM ATP results in loss of Alz-50 immunoreactivity on immunoblots. The loss of antigenicity is complete after a 1-hr incubation at 37°C and is stringently dependent on ATP. Hydrolysis of ATP is required, since the inhibition is not supported by the nonhydrolyzable analog adenosine 5′-[γ-thio]triphosphate (ATP[γS]) and is prevented when the ATPase inhibitors *o*-vanadate and oligomycin are present. Upon further characterization, it was found that certain protease inhibitors, phenylmethylsulfonyl fluoride, antipain, tosylphenylalanine chloromethyl ketone, and aprotonin prevent the loss of the epitope. This suggests that hydrolysis of ATP is coupled with proteolysis of A68, leading to loss of Alz-50 immunoreactivity. Since a variety of proteins are believed to be degraded by an ATP/ubiquitin-dependent pathway, a possible role for ubiquitin (Ub) in this effect was investigated. Two polyclonal antibodies against Ub protected A68 from proteolysis and were also effective in immunoprecipitating A68 after incubation with ATP in the presence of Ub and phenylmethylsulfonyl fluoride. The proteolysis of A68 was also blocked by hemin, an inhibitor of the protease that cleaves Ub–protein conjugates. Taken together, these findings indicate that loss of Alz-50 immunoreactivity with A68 is due to ATP-dependent/Ub-mediated proteolysis. This mechanism may be relevant to the physiological role for A68 in AD or it may simply represent an attempt to abort an aberrant protein.**

A voluminous amount of data regarding protein abnormalities in the brains of individuals with Alzheimer disease (AD) has emerged in the past decade (1). The spectrum ranges from posttranslational modification to changes in concentration of normal cellular proteins. What remains to be clarified is a casual relationship between any of these abnormalities and the two characteristic lesions of AD—i.e., the neurofibrillary tangle (NFT) and the neuritic plaque (NP). Since the abundance of these pathological structures is correlated with the severity of dementia (2–5), identification of a biochemical abnormality that precedes the formation of NFTs and NPs may be crucial to the understanding of the pathogenesis of AD.

An example of a biochemical abnormality that is detected prior to structural changes in AD is a group of proteins called A68, which are identified by the monoclonal antibody (mAb) Alz-50 (6, 7). The Alz-50-reactive proteins are localized in neurites of NPs, in NFT-bearing neurons and in neurons susceptible to the development of these pathological structures (7–9). They also occur in substantial quantities in the brains of patients with AD but are barely detectable in normal individuals and in other neurological diseases (7, 8). Since A68 appears to be an early marker for Alzheimer-like pathology, elucidating the nature of the antigen may provide some insight into the formation of NFTs and NPs.

After the discovery of the A68 proteins with Alz-50, this antibody was found to cross-react with tau protein, but only after denaturation with 2-mercaptoethanol and SDS (10, 11). There are also reports that the A68 proteins share additional epitopes with tau, as well as epitopes with certain other cytoskeletal elements (10, 12). It has therefore been suggested that these proteins are modified tau proteins that accumulate in AD (10, 11). Apart from this limited knowledge of their antigenic characteristics, virtually nothing is known about the biochemical properties of these proteins. Based on their ability to bind to cibacron blue, it was suggested that the proteins had an affinity for ATP (1). Subsequently, a phosphorylating activity was observed in enriched preparations of A68 (13), although upon further scrutiny, this activity was found to be associated with a comigrating contaminant in the preparation (39). However, these studies led to the finding that A68 undergoes an ATP-induced loss of Alz-50 immunoreactivity. The present paper describes the biochemical characteristics of this ATP-induced loss of Alz-50 reactivity with A68. The results suggest that A68 is processed by an ATP-dependent ubiquitin (Ub)-mediated proteolytic pathway.

## EXPERIMENTAL PROCEDURES

**Materials.** Adenosine 5′-[γ-thio]triphosphate (ATP[γS]) was from Boehringer Mannheim. Pyrophosphate and *o*-vanadate were purchased from Fisher. All reagents including nucleotides, protease inhibitors, hemin, Ub, and oligomycin were obtained from Sigma.

Antibodies 30 and 42 are IgGs cloned from Alz-50 (IgM) following class switching. Antibodies 38, 126, 147, and 58 are mAbs prepared by immunization of mice with highly purified A68 (14). NP8 is a neurofilament mAb (15) that shows cross-reactivity with A68. UH-11, a polyclonal antibody (pAb) to Ub was a gift from S.-H. Yen. Another pAb to Ub (Ubp) was provided by A. Haas. Secondary goat anti-mouse antibodies were purchased from Fisher.

**Preparation of A68-Enriched Fractions.** AD cases were selected by clinical and neuropathological criteria. Tissue dissected from frozen cortical specimens of AD brain was homogenized in 10 vol of buffer containing 10 mM Tris·HCl, pH 7.4/0.8 M NaCl/3 mM EGTA/1 mM phenylmethylsulfonyl fluoride (PMSF). Final selection of cases was based on titers of these homogenates with Alz-50 by ELISA. The

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Abbreviations: PMSF, phenylmethylsulfonyl fluoride; Ub, ubiquitin; AD, Alzheimer disease; NFT, neurofibrillary tangle; NP, neuritic plaque; PHF, paired helical filament; mAb, monoclonal antibody; pAb, polyclonal antibody; ATP[γS], adenosine 5′-[γ-thio]triphosphate.

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selected homogenates were centrifuged at 27,000 × g for 20 min and the supernatants were recentrifuged at 27,000 × g for 20 min. The supernatants were then spun at 100,000 × g for 35 min, and the resulting pellets, enriched in A68, were resuspended in 1 vol of Tris-buffered saline (TBS; 0.01 M Trizma-base/0.14 M NaCl, pH 7.4). The protein concentration of the pellet suspension was determined by the Bio-Rad protein assay, and aliquots of 25 μg of protein were stored at −70°C until used. According to the above scheme, the tau proteins remain in the high-speed supernatant fraction and aliquots of this supernatant were used as a source of tau protein.

**Incubation with ATP.** Aliquots (25 μl) of the A68 preparation of TBS were incubated with 5 mM ATP in a final vol of 30 μl at 37°C for 1 hr. From preliminary experiments, it was determined that the concentration of ATP required for optimal inhibition of Alz-50 immunoreactivity was 5 mM. This concentration is consistent with that used in various studies of ATP-dependent proteolysis (16, 17). The ATP reaction was terminated by addition of 5 μl of electrophoresis sample buffer containing 1 M Tris·HCl, pH 6.8/5% SDS/5% 2-mercaptoethanol/50% (vol/vol) glycerol/0.5% bromophenol blue.

**SDS/PAGE and Electrophoretic Blotting.** Samples were boiled for 5 min prior to electrophoresis on SDS/10% polyacrylamide gel with a 4% stacking gel (18). The resolved proteins were transferred from gel to nitrocellulose paper (19). Alz-50 staining of blotted proteins was performed as described (7). Briefly, nonspecific binding was blocked by 5% milk in TBS; the blot was then incubated for 2 hr with Alz-50 at a 1:10 dilution; bound antibody was detected by incubation with peroxidase-conjugated goat anti-mouse IgM antibody using 4-chloronaphthol as the chromogen. Immunoblotting with other mAbs (see Fig. 6) was conducted with 1:10 dilutions of primary antibody in 5% milk/TBS according to the same protocol.

**Immunoprecipitation with Ub Antibodies.** After incubation of samples with 5 mM ATP, 2 μg of affinity-purified Ub pAb was added and the samples were incubated at 37°C for 1 hr. Protein A-Sepharose (50 μl) (1 ml of gel in 2 ml of TBS) was then added and the samples were incubated at 37°C for an additional hour. The immunoprecipitates were collected by centrifugation, washed three times with 200 μl of TBS, and then boiled in sample buffer to release the bound antigen.

## RESULTS

Incubation of A68-enriched preparations with 5 mM ATP resulted in a loss of Alz-50 immunoreactivity with A68 as determined by subsequent electrophoretic blot analysis of the treated preparations (Fig. 1*A*). The loss occurred in a time-dependent manner, beginning after 15 min of incubation and requiring 1 hr for completion (Fig. 1*A*). Incubation of A68 preparations in the absence of ATP did not result in such a loss of Alz-50 immunoreactivity (Fig. 1*C*, lane 1). Since Alz-50 cross-reacts with the tau proteins, the effect of ATP on the reactivity of the antibody with tau protein was also tested. The results in Fig. 1*B* indicate that Alz-50 immunoreactivity with tau protein is not sensitive to ATP after incubation for 15, 30, or 60 min. The effect of temperature on the action of ATP was investigated by comparing Alz-50 immunoreactivity with A68 after incubation at 4°C, 25°C, or 37°C for 1 hr (Fig. 1*C*, lanes +). Incubation with 5 mM ATP at 4°C produced no effect on the subsequent labeling of A68 with Alz-50. However, incubation with 5 mM ATP at 25°C resulted in a partial loss of Alz-50 immunoreactivity with the protein, and at 37°C Alz-50 reactivity was totally abolished (Fig. 1*C*). The effect of ATP at 37°C was prevented when the A68 preparation was boiled for 5 min before incubation with ATP (Fig. 1*C*, lane *). In the absence of ATP (Fig. 1*C*, lanes −), there were no detectable changes in reactivity of the antibody with A68 at either 4°C or 37°C.

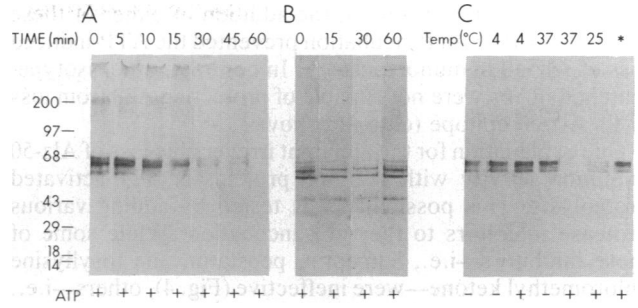


FIG. 1. Time course and temperature dependence of the ATP-induced loss of Alz-50 immunoreactivity. For the time course studies, A68 (*A*) and tau (*B*) preparations were incubated with 5 mM ATP at 37°C for the indicated durations. The incubation was terminated by addition of electrophoresis sample buffer and boiling. Samples were electrophoresed and the gels were transferred to nitrocellulose. The blots were then immunostained with Alz-50. For the temperature-dependence studies (*C*), A68-enriched preparations were incubated for 1 hr in the absence (lanes −) or presence (lanes +) of 5 mM ATP at the indicated temperatures. Samples were then subjected to electrophoresis and Alz-50 immunoblotting according to the routine protocol. The first lane represents an unincubated sample and the lane marked with an asterisk represents a sample that was boiled for 10 min before incubation with ATP. Numbers on left are kDa.

A68 preparations were incubated with various ribonucleotide triphosphates and Alz-50 immunoreactivity with the proteins was subsequently examined by electrophoretic blot analysis (Fig. 2). Of the various nucleotide triphosphates examined, only ATP was effective in inhibiting Alz-50 immunoreactivity with A68. To test whether the hydrolysis of ATP was necessary for the effect, the incubation was conducted in the presence of the ATPase inhibitors *o*-vanadate and oligomycin. In these conditions, nucleotide-induced loss of Alz-50 antigenicity was prevented (Fig. 2). Furthermore, incubation with a nonhydrolyzable analog of ATP—i.e., ATP[γS]—was not effective in reducing subsequent Alz-50 immunoreactivity (Fig. 2).

It was of interest to determine whether incubation with ATP in the presence of antibody would result in protection of the antigen from loss of its Alz-50 epitope. Affinity-purified mAbs Ab42 and Ab30 (IgGs), which were derived from Alz-50 (IgM) after class switching, were used for this pur-

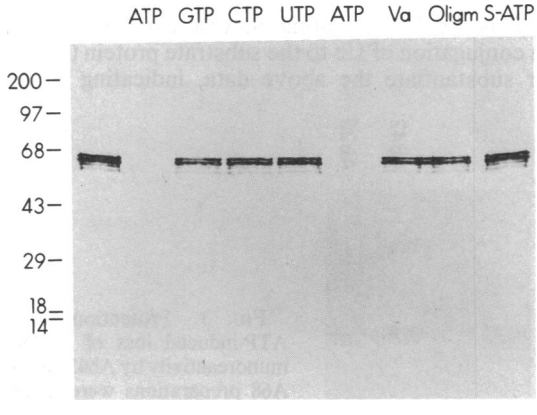


FIG. 2. Nucleotide specificity and dependence on hydrolysis of ATP. A68 preparations were incubated with 5 mM concentrations of the indicated nucleotides in TBS for 1 hr at 37°C. The effects of 200 μM *o*-vanadate (Va) and 100 μM oligomycin (oligm) were examined in the presence of 5 mM ATP. Electrophoresis and immunoblotting with Alz-50 were conducted according to the routine protocol. Numbers on left are kDa.

pose. As indicated in Fig. 3, the addition of either of these antibodies to the ATP incubation prevented the ATP-induced loss of Alz-50 immunoreactivity. In contrast, other isotype-matched mAbs were not capable of protecting A68 from loss of its Alz-50 epitope (data not shown).

One explanation for the apparent irreversible loss of Alz-50 immunoreactivity with the A68 proteins is ATP-activated proteolysis. This possibility was tested by adding various protease inhibitors to the ATP incubation. While some of these inhibitors—i.e., leupeptin, pepstatin, and tosyllysine chloromethyl ketone—were ineffective (Fig. 4), others—i.e., PMSF, antipain, tosylphenylalanine chloromethyl ketone, and aprotonin—did prevent the loss of Alz-50 immunoreactivity (Fig. 4).

The polypeptide Ub is known to play a role in the nonlysosomal ATP-dependent degradation of abnormal and certain normal cellular proteins (20–22). With this in mind, the involvement of Ub in the apparent ATP-stimulated proteolysis of the A68 proteins was investigated. Addition of Ub to the ATP incubation was found to partially protect A68 from loss of its Alz-50 epitope (Fig. 5, lane Ub). When A68 preparations were incubated with 5 mM ATP in the presence of either of two independently derived pAbs against Ub—i.e., UH-11 and Ubp—the loss of Alz-50 reactivity was totally prevented (Fig. 5, lanes UH-11 and Ubp, respectively). Since ATP promotes ligation of Ub with its substrate proteins (16, 21, 23), the possibility of *in vitro* conjugation of A68 was tested by subjecting the ATP-treated preparations to immunoprecipitation with UH-11 or Ubp. In these experiments, PMSF, which blocks subsequent conjugate degradation, was added to the ATP incubation. When the UH-11 and Ubp precipitate and supernatant fractions were electrophoresed and immunoblotted with Alz-50, the A68 proteins were found to be completely recovered in the immunoprecipitates (Fig. 5, lanes UH-11ppte and Ubp ppte). Under similar conditions, a nonspecific pAb (RbIgG) was ineffective in precipitating the Alz-50 antigen (Fig. 5, lane RbIgG). Neither of the two Ub pAbs precipitated the tau proteins after they were similarly incubated with ATP in the presence of PMSF (data not shown).

One of the characteristic features of the ATP/Ub system is the inhibition by hemin of the protease that cleaves the Ub–protein conjugates (24, 25). Another feature is the isotopic exchange mechanism between ATP and AMP, and ATP and $PP_i$ according to the following reaction:

$$2ATP + 2Ub + E \leftrightarrow AMP + 2PP_i + Ub\text{–}E\text{–}AMP\text{–}Ub,$$

where E is the Ub-activating enzyme catalyzing the initial step in conjugation of Ub to the substrate protein (26, 27). To further substantiate the above data, indicating a possible involvement of Ub in the ATP-induced loss of Alz-50 immunoreactivity, experiments were conducted to determine the effects of hemin on the ATP-induced proteolysis of A68 and to examine the effects of isotopic exchange (Fig. 5). In agreement with the established action of hemin, this compound inhibited the loss of Alz-50 reactivity induced by ATP (Fig. 5). It was also found that the presence of AMP or $PP_i$/NaF abolished the ATP-induced cleavage of A68,

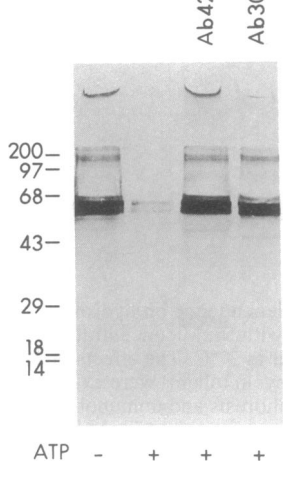


FIG. 3. Protection from the ATP-induced loss of Alz-50 immunoreactivity by Ab42 and Ab30. A68 preparations were incubated at 37°C for 1 hr with 5 mM ATP (lanes +) in the absence or presence of 1 μg of affinity-purified Ab42 or Ab30. Samples were electrophoresed and immunoblotted with Alz-50 according to the routine protocol. Numbers on left are kDa.

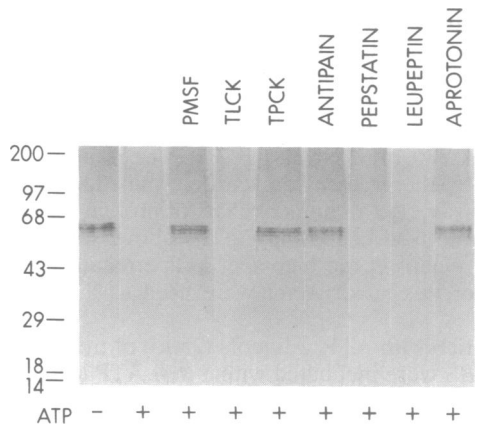


FIG. 4. Effects of protease inhibitors on the ATP-induced loss of Alz-50 immunoreactivity with A68. A68 preparations were incubated at 37°C for 1 hr with 5 mM ATP (lanes +) in the presence of the indicated protease inhibitor. Samples were processed as described in the legend to Fig. 1 and the blots were stained with Alz-50. The concentrations of the inhibitors were as follows: PMSF, 2 mM; tosyllysine chloromethyl ketone (TLCK), 500 μM; tosylphenylalanine chloromethyl ketone (TPCK), 500 μM; antipain, 100 μM; pepstatin, 2 mM; leupeptin, 30 μM; aprotonin, 0.80 mg of protein per ml. Numbers on left are kDa.

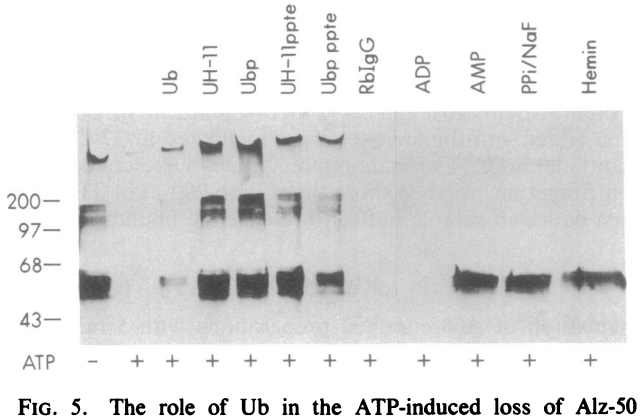


FIG. 5. The role of Ub in the ATP-induced loss of Alz-50 immunoreactivity with A68. A68 preparations were incubated at 37°C for 1 hr with 5 mM ATP (lanes +) plus Ub (5 μg), polyclonal Ub antibody UH-11 (3 μg), or polyclonal Ub antibody Ubp (3 μg). In other experiments, A68 preparations were incubated with 3 mM ATP plus ADP (3 mM), AMP (3 mM), $PP_i$ (3 mM)/NaF (2 mM), or 100 μM hemin. Lanes UH-11ppte, Ubp ppte, and RbIgG represent immunoprecipitates of ATP-treated A68 preparations with antibodies UH-11, Ubp, or RbIgG (an irrelevant pAb). In these experiments, samples were incubated with 5 mM ATP in the presence of 5 μg of Ub and 2 mM PMSF to promote accumulation of conjugates. UH-11, Ubp, or RbIgG (3 μg) was added to respective samples and incubation was continued for 1 hr at 37°C. Fifty microliters of a 50% suspension of protein A-Sepharose in TBS was then added to each tube and the samples were incubated for an additional hour. The resulting immunoprecipitates bound to the Sepharose beads were collected by centrifugation and washed with TBS. Bound antigen was released from the beads by boiling in electrophoresis sample buffer. The samples were centrifuged and aliquots of the supernatants were loaded onto SDS/polyacrylamide gels. Electrophoresis and immunoblotting with Alz-50 for all the above data were conducted according to the routine protocol. Numbers on left are kDa.

whereas ADP, which does not participate in isotopic exchange, did not (Fig. 5). Similar to previously described findings (26), $PP_i$ was only effective in inhibiting ATP-dependent protein breakdown in the presence of NaF, an inhibitor of endogenous pyrophosphatase activity.

Since loss of an epitope does not necessarily imply total loss of protein, detection of possible reaction products of the ATP-activated cleavage was attempted by using other mAbs that cross-react with A68. Thus, ATP-treated preparations were electrophoresed and immunoblotted with the mAbs 126, 147, 58, and 38 (mAbs against purified A68) and NP8 (an antibody against neurofilament protein). Every one of these mAbs identified the same reaction products that were found to have a higher electrophoretic mobility than the native Alz-50 reactive proteins (Fig. 6). In particular, the proteolytic products lacking Alz-50 immunoreactivity consistently migrated 2–3 kDa faster than the native A68 proteins (Fig. 6).

## DISCUSSION

These experiments describe a loss of Alz-50 immunoreactivity with A68 in enriched preparations from AD brain after incubation with 5 mM ATP. The ATP effect is only observed when the incubation is conducted at temperatures above 4°C, and it is prevented when certain protease inhibitors (PMSF, tosylphenylalanine chloromethyl ketone, antipain, and aprotonin) are present. These observations suggest that the loss of antigenicity is a result of an ATP-dependent proteolytic activity. The proteolysis requires hydrolysis of ATP, since it is not supported by the nonhydrolyzable analog ATP[$\gamma$S] or other ribonucleotide triphosphates. Additional evidence in favor of ATP cleavage is the finding that the ATPase inhibitors *o*-vanadate and oligomycin both abolish the ATP-induced loss of Alz-50 immunoreactivity with A68. In this regard, the observed ATP-induced proteolytic activity resembles that of the *Escherichia coli* ATP-dependent proteases (28–30) and the mammalian mitochondrial ATP-activated protease (31, 32). However, in contrast with these ATP-dependent proteolytic activities, the ATP-induced loss of Alz-50 immunoreactivity is not inhibited by 1 mM EGTA or 1 mM EDTA, nor is it affected by addition of $Mg^{2+}$ or $Ca^{2+}$ (data not shown). Thus, there appears to be no metal ion requirement for ATP binding, the reasons for which are presently unclear.

Electrophoretic blot analysis of the ATP-treated preparations with other mAbs that cross-react with A68 identified proteolytic by-products migrating 2–3 kDa faster than the native proteins. While it is recognized that other ATP-mediated posttranslational modifications such as phosphorylation or ADP-ribosylation may produce similar changes in electrophoretic mobility of proteins, attempts to demonstrate such modifications with A68 under these conditions were unsuccessful (unpublished observations). Instead, the action of the various protease inhibitors on the ATP-induced loss of Alz-50 reactivity provides convincing evidence for the occurrence of a lytic event. The relatively small electrophoretic shift indicates cleavage from either end of the molecule, suggesting that the Alz-50 epitope in A68 is located within a terminal region of the protein. Interestingly, the Alz-50 epitope in tau protein has also been mapped to a terminal region, although there is some conflict as to whether it exists at the amino (33) or the carboxyl terminus (34). Yet, in contrast to the results with the A68 proteins, the Alz-50 epitope on tau protein does not appear to be susceptible to ATP-activated proteolysis. The specificity of the ATP-induced cleavage for A68 therefore suggests the existence of some recognition signal in these proteins (but not found in normal tau) that targets them for energy-dependent proteolysis. The recognition signal may consist of either a unique amino acid sequence or a posttranslational modification.

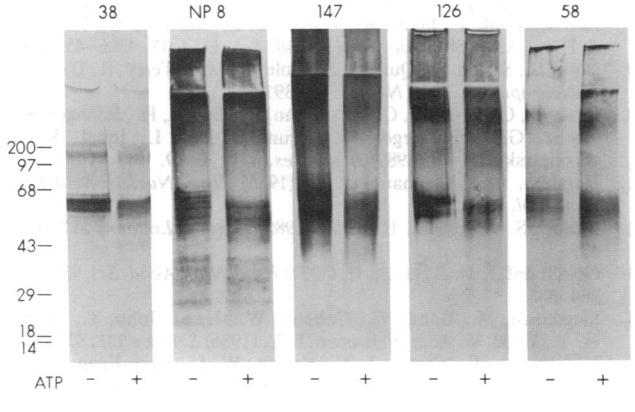


FIG. 6. Detection of proteolytic by-products. A68-enriched preparations were incubated at 37°C for 1 hr in the absence (lanes −) or presence (lanes +) of 5 mM ATP. Samples were processed as described in the legend to Fig. 1. The blots were immunostained with the indicated antibodies as described. Numbers on left are kDa.

Another observation that supports the specificity of the ATP-induced proteolysis of A68 is its resistance to further or complete degradation at the expense of ATP. After similar incubations of A68-enriched preparations with trypsin or chymotrypsin, virtually no proteolytic fragments are detectable with any of the antibodies against the proteins (data not shown). Thus, the limited sensitivity to the ATP-activated protease is not likely to be due to protection of potential cleavage sites by a robust secondary and tertiary conformation. Rather, the ATP-dependent protease appears to cleave A68 in a limited fashion. Proteolysis introducing only limited cuts into proteins (and thus being very specific) generally provides a means for activation or inactivation of proteins (35). While a biological action has not yet been assigned to A68, such a mechanism may serve as a useful tool in determining the role of these proteins in the pathophysiology of AD.

Another intriguing aspect of the ATP-activated proteolysis of A68 is the involvement of Ub in the mechanism. This polypeptide is covalently attached to free amino groups on proteins by a multicomponent enzyme system (36, 37) and serves as a marker for proteins destined for nonlysosomal degradation (25, 38). The demonstration of isotopic exchange suggests that the presence of the enzymatic machinery in the A68 preparation. However, it is unlikely that these proteins undergo conjugation with Ub *in vitro* since there is no alteration in their electrophoretic mobility. Most ATP-dependent proteases, although soluble, have been reported to exist as large (>500 kDa) complexes in both bacterial and mammalian systems (40, 41). It is therefore reasonable that some ATP-dependent proteolytic activity is recovered in a 100,000 × *g* pellet, such as the one used in our studies.

The association of Ub with intraneuronal pathological inclusions is a feature commonly observed in several neurodegenerative diseases (42). The target proteins for Ub conjugation in these dementing illnesses have not yet been identified, although it is clear that the various lesions differ with respect to structural and antigenic characteristics (42). One explanation for the formation of ubiquitinated intermediates in neurodegenerative diseases is activation of the Ub system as a general response to a variety of damaging insults that ultimately lead to neuronal cell death. Recently, it has been reported that Ub is a normal component of the microtubular network in cells (43). In light of this discovery, it is also possible that association of Ub with pathological inclusions is a result of reorganization of already existing Ub–protein conjugates.

In AD, Ub has been definitively identified (44) as an integral component of paired helical filaments (PHFs), which

are a major constituent of NFTs. Ub is also associated with the neuritic elements of senile plaques (45, 46). While the compositional analysis of PHFs is still incomplete, there is evidence that at least a portion of tau protein is another integral element (47, 48). However, it is not known whether these two components are covalently linked to each other in PHFs. In this regard, it has been reported that tau protein purified from bovine brain does not serve as a substrate for the ATP/Ub system (34). The resistance of the Alz-50 epitope in tau protein from Alzheimer brain to ATP-induced Ub-mediated proteolysis in the present study is consistent with this finding. Other observations reported here do, however, suggest the involvement of Ub in the processing of the Alz-50-reactive A68 proteins from Alzheimer brain. Moreover, they suggest that A68 may be a Ub acceptor protein in AD brain. At the immunocytochemical level, Alz-50 reactivity is detected in neurons prior to structural pathology (7, 8), but the appearance of Ub has been correlated with the deposition of fibrillar material (46, 49, 50). Taken together, these findings suggest that the association of Ub with Alz-50 immunoreactive proteins may be an important event in PHF formation and may be responsible for some of the staining of AD brain tissue by antibodies to Ub. Ub also appears to be associated with the proteolytic by-products derived from ATP-induced cleavage of the A68 proteins (unpublished data). While sequestration of abnormal proteins in response to heat shock and stress is the predominant function of the Ub system (25, 51, 52), an alternative nonproteolytic role has been suggested whereby reversible attachment of Ub to an acceptor protein modulates protein function (53–55). Whether the ATP-induced Ub-mediated proteolysis of A68 is an abortive effort to degrade abnormal neuronal proteins or whether it serves a more distinctive role in the pathogenesis of the disease is an important issue that remains to be determined.

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