Structure of Substrate-free Human Insulin-degrading Enzyme (IDE) and Biophysical Analysis of ATP-induced Conformational Switch of IDE*§

Received for publication, February 22, 2007, and in revised form, May 22, 2007 Published, JBC Papers in Press, July 5, 2007 DOI 10.1074/jbc.M701590200

Hookam Im†, Marika Manolopoulou†, Enrico Malito†,†, Yuequan Shen†, Ji Zhao†, Marie Neant-Fery†, Ching-Yu Sun†, Stephen C. Meredith†, Sangram S. Sisodia§, Malcolm A. Leissring†, and Wei-Jen Tang†,‡

From the †Ben-May Department for Cancer Research, the University of Chicago, Chicago, Illinois 60637, the ‡Department of Biochemistry, The Scripps Research Institute, Jupiter, Florida 33458, and the §Department of Neurobiology and Department of Pathology, the University of Chicago, Chicago, Illinois 60637

Insulin-degrading enzyme (IDE) is a zinc metalloprotease that hydrolyzes amyloid-β (Aβ) and insulin, which are peptides associated with Alzheimer disease (AD) and diabetes, respectively. Our previous structural analysis of substrate-bound human 113-kDa IDE reveals that the N- and C-terminal domains of IDE, IDE-N and IDE-C, make substantial contact to form an enclosed catalytic chamber to entrap its substrates. Furthermore, IDE undergoes a switch between the closed and open conformations for catalysis. Here we report a substrate-free IDE structure in its closed conformation, revealing the molecular details of the active conformation of the catalytic site of IDE and new insights as to how the closed conformation of IDE may be kept in its resting, inactive conformation. We also show that Aβ is degraded more efficiently by IDE carrying destabilizing mutations at the interface of IDE-N and IDE-C (D426C and K899C), resulting in an increase in Vₘₐₓ with only minimal changes to Kₘ. Because ATP is known to activate the ability of IDE to degrade short peptides, we investigated the interaction between ATP and activating mutations. We found that these mutations rendered IDE less sensitive to ATP activation, suggesting that ATP might facilitate the transition from the closed state to the open conformation. Consistent with this notion, we found that ATP induced an increase in hydrodynamic radius, a shift in electrophoretic mobility, and changes in secondary structure. Together, our results highlight the importance of the closed conformation for regulating the activity of IDE and provide new molecular details that will facilitate the development of activators and inhibitors of IDE.

Insulin and amyloid-β (Aβ)³ play a central role in diabetes and Alzheimer disease (AD), respectively (1–5), and both are avidly degraded by insulin-degrading enzyme (IDE) (3, 5–7). Consistent with a role for IDE in controlling the levels of insulin and Aβ endogenously, loss-of-function mutations of the IDE gene in rodents lead to elevated circulating insulin and cerebral Aβ levels (8–10). Conversely, enhancement of IDE activity in neurons effectively reduces Aβ accumulation in AD mouse models (11). Human genetic analysis also suggests a link between late-onset AD and genetic variations in and around the IDE gene on chromosome 10q (12, 13). Furthermore, IDE activity was recently found to be reduced in lymphoblasts from chromosome 10q-linked AD patients (14). Type 2 diabetes has also been linked to variations in the IDE region on chromosome 10q (15, 16). However, the linkage between IDE and type 2 diabetes remains in dispute (17, 18).

IDE belongs to an evolutionarily distinct family of zinc metalloproteases, and its activity is regulated at many levels. The subcellular localization of IDE may differ depending on the specific cell type, where it can be found in the cytosol, on the cell surface, in endosomes, or as part of the extracellular milieu (1, 13, 19–21). The catalytic activity of IDE has been reported to be regulated by oligomerization (22). Moreover, the catalytic activity of IDE can be regulated by physiologically relevant ATP concentrations, long chain fatty acids, oxidative stress, and endogenous peptide inhibitors such as ubiquitin (23–29).

IDE prefers to degrade <6-kDa bioactive peptides such as insulin, Aβ, amylin, glucagon, atrial natriuretic peptides, and transforming growth factor-α (1). Paradoxically, even though IDE has a broad range of substrates, it exhibits a remarkable capacity to selectively cleave certain hormones without degrading related family members. We recently solved structures of human IDE in complex with Aβ-(1–40), insulin B chain, amylin, and glucagon (4). These structures reveal that IDE has ~56-

---

* This work was supported by National Institutes of Health Grant GM81539, the University of Chicago Diabetes Center pilot and feasibility grants (to W.-J.T.), American Health Assistance Foundation (to E.M.), American Heart Association postdoctoral fellowship (to Y.S.), and Ellison Medical Foundation (to S.S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The atomic coordinates and structure factors (code 2jg4 and 2jbu) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

‡ The on-line version of this article (available at http://www.jbc.org/) contains supplemental Methods and Figs. 1–5.

§ These authors contributed equally to this work.

1 To whom correspondence should be addressed: Tel: 773-702-4331; Fax: 773-702-4476; E-mail: wtang@uchicago.edu.

3 The abbreviations used are: Aβ, amyloid-β; IDE, insulin-degrading enzyme; IDE-N, N-terminal domain of IDE; IDE-C, C-terminal domain of IDE; AD, Alzheimer disease; FAPβ, fluoroscein-Aβ-(1–40)-Lys-biotin; AtfPreP, A. thaliana prerequisite peptidase; MMP, mitochondrial processing peptidase; PPP, inorganic triphosphate; PDB, Protein Data Bank; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; BSA, bovine serum albumin; r.m.s.d., root mean square deviation; HPLC, high performance liquid chromatography; TNP-ATP, 2’(3’)-O-(2,4,6-trinitrophenyl)adenosine 5’-triphosphate; APBS, adaptive Poisson-Boltzmann solver.
Substrate-free Human Insulin-degrading Enzyme

kDa N- and C-terminal domains, IDE-N and IDE-C, that are joined by a 26-amino acid loop (4, 30). The catalytic site is present within IDE-N, but this domain alone has <2% activity of the wild-type enzyme (30). The addition of IDE-C can partially restore the catalytic activity of IDE (30). Our structures reveal that IDE contains an unusually large internal chamber, formed jointly by IDE-N and IDE-C. This atypical configuration suggests that the substrate selectivity of IDE is determined only partially by the classical protease-substrate recognition at the catalytic site. Such selectivity is in fact more strongly influenced by the size, shape, and charge distribution within the large internal chamber of IDE. Intriguingly, our substrate-bound IDE structures reveal that IDE substrates need to undergo a conformational switch to form β-strands. This requirement may explain its known preference for amyloidogenic substrates (2).

Our structural and biochemical analyses suggest that IDE can adopt at least two conformations, designated as “open” and “closed” (4). In the open state, substrates can freely diffuse in and out of the catalytic chamber. In the closed state, previously bound substrates are entrapped inside the catalytic chamber and repositioned to allow hydrolysis. The closed conformation is also significant to the catalytic function of IDE because new substrates cannot gain access to the internal chamber in this state. Thus, factors that tend to stabilize the closed state will slow the activity of the protease. Our previous structures revealed extensive interactions between IDE-N and IDE-C that are predicted to act like a “latch,” which stabilizes the closed conformation. Consistent with this model, mutations that destabilize the interactions between IDE-N and IDE-C were found to significantly increase the catalytic rate of IDE (4).

However, consideration of the known structures of IDE and related homologs casts doubt on the stability of the closed state in the absence of substrate. Crystal structures of human IDE and a homolog from Arabidopsis thaliana, presequence peptidase (AtPepP), were both solved in their closed conformation; however, these structures also contained substrates bound within their catalytic chambers (4, 31). Furthermore, the structure of Escherichia coli pitrilysin, which does not have an associated substrate, is in the open conformation (32). To demonstrate that the closed state of IDE is stable in the absence of substrate, and to obtain the molecular details of this conformational state, we report here a 2.8 Å crystal structure of substrate-free IDE in the closed conformation. This is the first structure of catalytically active IDE, revealing the natural configuration of the active site. Furthermore, using a variety of functional and spectroscopic approaches, we provide evidence that ATP might facilitate the transition from the closed to the open state.

EXPERIMENTAL PROCEDURES

IDE Expression and Purification—Wild-type human IDE and its mutants, IDE-Y831F, R824K, D426C/K899C, N184C/Q828C, and S132C/E817C, were purified as described previously (4). The purification of IDE-E111Q with its co-purified peptides is described in the supplemental Methods. Recombinant IDE used for circular dichroism and native gel electrophoresis experiments was purified as described previously (4, 33).

IDE Crystallization—To crystallize catalytically active IDE-Y831F, protein was purified by Superdex 200 columns (GE Healthcare) five times and used immediately after purification. 1 μl of protein (15–20 mg/ml) and 1 μl of crystallization solution (10–13% PEG MME 5000, 100 mM HEPES, pH 7.0, 4–14% Tacsimate, 10% dioxane) were mixed and equilibrated with 500 μl of well solution at 18 °C by the hanging drop method. Clusters of needle crystals appeared in 3–5 days. For data collection, crystals were sequentially equilibrated in 15 and 30% glycerol cryo-protective solutions containing reservoir buffer and flash-frozen in liquid nitrogen.

X-ray Diffraction Data Collection and Structure Determination—Diffraction data were measured at 100 K on the beamline 19-ID at the Structural Biology Center, Argonne National Laboratory. The data sets were processed using HKL2000 (34) and the CCP4 suite of programs (35). Structure determinations were performed by molecular replacement using the previously known IDE-E111Q/Aβ structure as a search model (PDB ID 2G47) (4) with the program Phaser (36). Structure refinement and rebuilding were performed by CNS (37), REFMAC (38), and Coot (39). For the structure of substrate-free IDE-Y831F, the absence of extra electron density in the catalytic chamber was assessed by inspection of both σA-weighted 2Fo − Fc and Fo − Fc difference electron density maps, as well as by inspection of the 2Fo − Fc-simulated annealing omit map. The final model had Rwork and Rfree of 18.3 of 22.7%, respectively. For the structure of IDE-E111Q in complex with the co-purified peptide, the extra electron density in the catalytic chamber was fitted by two polyalanine peptide chains. The final model had Rwork and Rfree of 18.6 and 24.0%, respectively. The refinement statistics are summarized in Table 1.

Synthesis and Purification of Aβ Peptide—Fluorescein-Aβ(1–40)-Lys-biotin (FAβB) was synthesized by Anaspec (San Jose, CA) as described (40). Biotin was attached to the C-terminal lysine side chain via an aminoacapo acid linker, and 5(6)-carboxyfluorescein (Sigma) was attached to the N terminus via a peptide bond. Aβ(1–40) was synthesized and purified as described (41). Briefly, modified Fmoc (N-(9-fluorenylmethoxycarbonyl) and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethylenuronium hexafluorophosphate/N-hydroxybenzotriazole (Fastmoc) chemistry on an Applied Biosystems (Foster City, CA) model 433A instrument was used. Peptides were cleaved from the resin using 9 ml of trifluoroacetic acid plus 0.5 ml of thioanisole, 0.3 ml of ethanedithiol, and 0.2 ml of anisole for 1.5 h at 22 °C. Peptides were purified by reverse phase-HPLC on a preparative C18 (Zorbax) column at 60 °C. Peptide purity was greater than 98% by analytical HPLC. The molecular mass of the peptide was verified by electrospray ionization and matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

Enzymatic Assays—Substrate V (7-methoxy coumarin-4-yl-acetyl-RPPGFSAKF-2,4-dinitrophenyl; R&D Systems), a fluorogenic peptide substrate of IDE, was used to monitor the effect of ATP and bacitracin as described (4, 23, 30). To this end, 17.4 μM substrate V for 5 μg of wild-type IDE and 8.7 μM sub-
substrate V for 0.16 μg of IDE mutant were used, respectively, in the presence of indicated concentrations of bacitracin (0–5.5 mM) and 2 mM ATP in 115 μl of buffer containing 50 mM Tris-HCl, pH 7.3, and 0.13% BSA at 37 °C for 3–5 min. The hydrolysis of substrate V was measured based on the increase of fluorescence (λex = 327 nm, λem = 395 nm) using a Tecan Safire microplate reader. For a subset of experiments analyzing activation by various nucleotides, IDE activity was determined from the rate of cleavage of a second fluorogenic peptide substrate, o-aminobenzoic acid-GGFLRKHGQ-ethylendiamine 2,4-dinitrophenyl (5 μM) by recombinant IDE (2 nM) at 22 °C in either 20 mM Tris-HCl, pH 7.4, or Buffer B (50 mM HEPES, 100 mM NaCl, 0.1% BSA, pH 7.4). For kinetic analysis of Aβ degradation by IDE, the indicated Aβ-(1–40) concentrations (6.3–100 μM) were used in presence of 0.25 μM FAβB. The reaction was performed with the indicated concentrations of IDE in Buffer A (50 μl 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, and 0.05% BSA) at 37 °C. At the appropriate times, the reaction was stopped by adding 540 μl of Buffer A containing 2 mM 1,10-phenanthroline. NeutravidinTM-coated agarose (10 μl, Pierce) was added and gently rocked for 30 min. The mixture was centrifuged at 14,000 × g for 15 min, and supernatant solutions were transferred in three 100-μl aliquots to black 96-well plates (Nunc). Fluorescence intensity (λex = 488 nm, λem = 535 nm) was measured at 37 °C using a Wallac multilabel plate reader (PerkinElmer Life Sciences). The background fluorescence was measured using 0.25 μM FAβB in the absence of enzyme, and this signal was subtracted. The maximum possible fluorescence intensity was determined based on the fluorescence signal from 0.25 μM FAβB and 25 μM Aβ-(1–40) reacted with excess IDE-D426C/K899C for 30 min.

Degradation of Aβ in the Conditioned Medium of HEK293swe.3 Cells by IDE—HEK293APPswe.3 cells (42) were plated at ~50% confluency in 60-mm dishes and maintained in 2 ml of Dulbecco’s modified Eagle’s medium supplemented with 1% fetal bovine serum under normal cell culture conditions for 18 h. The conditioned medium was collected and centrifuged at 100,000 × g for 15 min to remove cell debris and membranes, and the supernatant fraction was frozen in aliquots at −20 °C without added protease inhibitors. The conditioned medium (40 μl) was incubated with purified IDE enzyme at 37 °C for the indicated times, and reactions were terminated by addition of a mixture of 3× Laemmli sample buffer containing 2 mM 1,10-O-phenanthroline. The resulting mixtures were boiled, fractionated on 16% Tris-Tricine gels, and transferred to nitrocellulose membranes. APPsα derivatives and Aβ peptides were detected using the Aβ-specific monoclonal antibody 26D6 (43). Bound antibodies were visualized by enhanced chemiluminescence (PerkinElmer Life Sciences). For quantification of Western blots, we used a Bio-Rad XRS Chemidoc imager and Bio-Rad Quantity One software. Boltzman fits were determined using Prism software.

Dynamic Light Scattering—Recombinant IDE was diluted to 1 μM in 20 mM Tris-HCl, pH 7.4, in the presence or absence of ATP or PPP, (Sigma). Dynamic light scattering measurements were performed on a DynaPro Titan instrument equipped with temperature-controlled micro-sampler (Wyatt Technology Co., Santa Barbara, CA).

Circular Dichroism Spectroscopy—Recombinant IDE was diluted to 1 μM in 10 mM NaPO₄, pH 7.4, in the absence or presence of ATP or PPP. Samples were examined using 1-mm quartz cuvettes (Hellma, Forest Hills, NY) in a Jasco J-815 CD spectrometer equipped with a Jasco JWTC-484 temperature control unit (Jasco Inc., Easton, MD). Spectra were recorded at 37 °C at 1 nm resolution with a scan rate of 0.25 nm/s. Five scans were acquired and averaged for each sample.

Native Gel Electrophoresis—Equal amounts of recombinant IDE (1 μg) were electrophoresed on Novex® Tris-Glycine Mini Gels using Tris-Glycine Native Running Buffer (Invitrogen) in the presence or absence of 3 mM ATP or 3 mM PPP, (Sigma) at pH 8.3.

RESULTS

Structural Analysis of Substrate-free IDE-Y831F—Within the active site of IDE, glutamate 111 served as a base to activate a catalytic water molecule that mediates peptide hydrolysis, and mutation of this residue to glutamine renders IDE inactive (4). Our laboratory recently reported structures of IDE-E111Q in complex with four different substrates, Aβ-(1–40), insulin B chain, amylin, and glucagon (4). These structures reveal how IDE uses the catalytic chamber formed by the IDE-N and IDE-C domains to entrap these substrates within its closed conformation. The extensive interaction between IDE-N and IDE-C (11,496-Å² buried surface) suggests that IDE could exist in its closed conformation without requiring the binding energy contributed by its substrate. We postulated that, in the absence of substrate, the closed conformation is the resting and inactive state of IDE, because substrates cannot gain access to the catalytic chamber when the protease is in this state (4). Thus, the closed conformation is critical for regulating the catalytic cycle of IDE.

Paradoxically, we failed to obtain a substrate-free structure of the catalytically inactive IDE mutant, IDE-E111Q. Although crystals were obtained in the absence of exogenously added substrate, they all diffracted poorly. We then attempted to obtain substrate-free IDE structures using a total of six catalytically active IDE proteins, including wild-type IDE as well as five IDE mutants as follows: IDE-Y831F, R824K, D426C/K899C, N184C/Q828C, and S132C/E817C. Only IDE-Y831F grew sizeable crystals, which diffracted at best to 2.8 Å (Table 1). Using a short peptide substrate, substrate V, IDE-Y831F was found to have a catalytic rate comparable with that of wild-type IDE (4).

The structure of IDE-Y831F superimposes well with the structure of Aβ-bound IDE (r.m.s.d. = 0.18 Å) and thus represents the closed conformation of IDE (Fig. 1A). No obvious electron density was found either in the catalytic site (Fig. 1B) or at the N-terminal peptide anchoring site of IDE domain 2 (Fig. 1C). For comparison, Fig. 1, B and C, shows a model of the Aβ peptide (yellow sticks) to indicate where the peptide anchoring sites are. These results indicate that our structure indeed represents substrate-free IDE in its closed conformation. Thus, substrate-free IDE is likely to be a stable state in the catalytic cycle of this enzyme, and this structure provides the first insight into the molecular details of this state.
IDE-Y831F has catalytic activity similar to wild-type IDE (4). A water molecule is clearly visible in the active site of IDE-Y831F. No such water molecule was observed in our previous substrate-bound catalytically inactive IDE mutant IDE-E111Q. Comparison of this substrate-free IDE structure with that of a related M16A member, E. coli pitrilysin (31), and the M16B member, mitochondrial processing peptidase (MPP) (44), reveals that the positions of the residues involved in activating the water molecule and coordinating the zinc ion are structurally conserved (Fig. 1D).

**Structural Comparison of IDE and AtPreP—Presequence protease (PreP), a member of M16C family of metallopeptases, resides in mitochondrial matrix and is responsible for the degradation of mitochondrial signal peptides that are cleaved off by mitochondrial processing peptidase (31, 45). Similar to human IDE, human PreP also prefers to degrade short peptides such as amyloid-β (45). The structure of PreP from A. thaliana, AtPreP, was recently solved (31). Comparison of the IDE structure, which belongs to the different subfamily (M16A), with AtPreP reveals that these enzymes share substantial similarities. Both IDE and AtPreP have four homologous domains within each monomer (Fig. 2A). Each domain also shows similar secondary structure (supplemental Fig. 1). The first two domains (domains 1 and 2) of these two enzymes make up a αβαβα sandwich for their N-terminal domain, as do domains 3 and 4 within their C-terminal domains. The N- and C-terminal domains of IDE and AtPreP form an enclosed chamber that is similar in size (~13,000 Å³) in which substrates are entrapped for hydrolysis.

The crystal structures of IDE and AtPreP were both solved in their closed conformation. In this state, substrate cannot enter the catalytic chamber nor can products exit. Similar to substrate-bound IDE, we found that the IDE-N and IDE-C domains of substrate-free IDE make extensive interactions that bury a large surface (11,200 Å²) with good shape complementarity (shape complementarity score = 0.65) (46) (Fig. 2, B and C). Using the structure of AtPreP, we also found that the interaction between PreP-N and PreP-C is also extensive (buried surface = 11,150 Å² with shape complementarity score of 0.60) (Fig. 2, B and C) (46). Electrostatic potential analysis by APBS (47) reveals that the extensive interaction between the N and C domains of IDE and PreP is mediated by charge complementarity and van der Waals interactions (Fig. 2B). Furthermore, sequence comparison among the IDE homologs from fungi to humans as well as comparison among PreP homologs from bacteria to human using Consurf (48) reveals that the residues involved in the interface interaction of these enzymes are relatively conserved (Fig. 2C). Together, our analysis suggests that the closed state of IDE and AtPreP is a stable state, and the interaction between the N- and C-terminal domains of both enzymes is evolutionarily preserved.

Despite the similarities described above, IDE and AtPreP show significant structural differences. First, they differ in the length and location of the linkage between the N-terminal domain and the C-terminal domain (Fig. 2A). IDE-N and IDE-C are connected by a 26-amino acid loop (amino acids 516–527). This loop interacts with both domains 2 and 3 but does not contact domain 1 or 4. In contrast, AtPreP-N and AtPreP-C are joined by a much longer hinge region (amino acids 485–533). This hinge is formed by two helices (amino acids 485–526) that contact domain 1 and 4 and a random coil punctuated by two short helices (amino acids 527–533), which wraps around domain 4 (Fig. 2A). In addition, analysis of the electrostatic potential of these two proteases, as calculated by APBS (47), reveals that the substrate chambers of the C-terminal domains of these two enzymes are quite different (Fig. 2B). The inner chamber of IDE-C is largely positive, whereas that of AtPreP has a weak positively charged surface with a strongly negatively charged patch at the interface between domain 3 and 4.

Another key difference between these two enzymes is that IDE contains a critical exosite that is absent in AtPreP. The structures of IDE in complex with four different substrates reveal that, apart from the catalytic site formed by domain 1 and 4, IDE contains an exosite at domain 2 that accommodates the N terminus of substrates (Fig. 2C) (4). Consistent with this notion, sequence comparison of IDE homologs using Consurf (48) reveals that the amino acids comprising the exosite are highly conserved (Fig. 2C). However, the structure of AtPreP in complex with a co-purified peptide only revealed an interaction of the substrate with the catalytic site, and no exosite is observed in domain 2 (31). Accordingly, the sequence within domain 2 of AtPreP corresponding to the exosite of IDE is indeed not conserved evolutionarily even though the catalytic site formed by domains 1 and 4 of PreP is highly conserved as

### Table 1: Crystallographic statistics

|                  | IDE-Y831F | IDE-E111Q-peptide |
|------------------|-----------|-------------------|
| **Data collection** |           |                   |
| **Beamline**     | APS 19-ID | APS 19-ID         |
| **Wavelength (Å)** | 0.9793   | 0.9793            |
| **Space group**  | P6₅      | P6₅               |
| **Cell dimension (Å)** |         |                   |
| a                | 263.2     | 263.2             |
| b                | 263.2     | 263.3             |
| c                | 90.4      | 90.7              |
| **Resolution (Å)** | 30-2.8    | 30-3.0            |
| **Rmerge (%)**   | 14.1 (66.6) | 12.0 (49.6)     |
| **I/σ (I)**      | 14.5 (9.2) | 19.9 (4.2)        |
| **Redundancy (%)** | 11.4 (11.4) | 11.4 (11.4)     |
| **Completeness (%)** | 100 (100) | 100 (100)         |
| **Unique reflections** | 88,131 (8,750) | 72,886 (7,193) |
| **Refinement**   |           |                   |
| **Rwork (%)**    | 18.3      | 18.6              |
| **Rfree (%)**    | 22.7      | 24.0              |
| **No. of atoms** | 15,656    | 15,705            |
| **B factor(Å²)** | 427       | 80                |
| **Rmerge (%)**   | 11.1 (9.2)| 11.1 (11.4)       |
| **I/σ (I)**      | 14.5 (9.2) | 19.9 (4.2)        |
| **Redundancy (%)** | 11.4 (11.4) | 11.4 (11.4)     |
| **Completeness (%)** | 100 (100) | 100 (100)         |
| **Unique reflections** | 88,131 (8,750) | 72,886 (7,193) |
| **r.m.s.d./**    |           |                   |
| Bond lengths (Å) | 0.009     | 0.009             |
| Bond angles (°)  | 1.2       | 1.5               |
| **Ramachandran plot (%)** |         |                   |
| Favorable region | 90.7      | 87                |
| Allowed region   | 9.2       | 12.4              |
| Generously allowed region | 0.1    | 0.6               |
| Disallowed region | 0.0      | 0.0               |
| **PDB accession code** | 2ig4 | 2ibu            |

### Image 360x26 to 388x38

---

25456 JOURNAL OF BIOLOGICAL CHEMISTRY VOLUME 282•NUMBER 35•AUGUST 31, 2007
expected (Fig. 2C). Instead, the catalytic site of AtPreP is connected with a series of conserved residues, which leads to the interface between domain 2 and 3 of AtPreP; the significance of these conserved residues remains elusive.

Consistent with the idea that domain 2 of IDE contains a bona fide exosite involved in the binding of substrate(s), our structure of IDE-E111Q was found to contain a co-purified peptide at this site, in addition to another peptide bound to the active site of IDE (Fig. 2D). In the course of preparing crystals of IDE-E111Q that could diffract better, we modified the purification scheme by incorporating a purification step using a derivatized Aβ-(1–40) peptide containing fluorescein at the N terminus and biotin at the C terminus (FA-BB). Hydrolysis of this substrate separates the fluorescent label from the biotin tag. The fraction of hydrolyzed substrate can be determined by

From the composite omit map, we found two regions that contained extra continuous electron density. One was located at the catalytic site, and the other was at the exosite (supplemental Fig. 2). Thus, we speculated that these densities corresponded to peptides co-eluting with the IDE protein. Mass spectrometry analysis confirmed that the purified IDE-E111Q contained two co-purified peptides with the molecular mass of 1114.4 and 1608.6 Da (supplemental Fig. 3). From tandem mass spectrometry analysis of these peaks, the peptide sequences were predicted to be rich in histidines, suggesting that they were enriched by the metal affinity chromatographic step used for initial steps of purification. The identity of the side chains of the co-purified peptide(s) was not clear because of the low to medium resolution of this structure. Thus, we modeled two polyalanine peptides with 7 and 3 amino acid residues into the active site and exosite of IDE, respectively. It is worth noting that the structure of AtPreP also contained continuous electron density in its active site, which was modeled as a hexapeptide (31) (Fig. 2D). The orientation of the unknown peptide in the active site of AtPreP is identical to that of the peptides within the active site of IDE (Fig. 2D).

Aβ Degradation by IDE Mutant, D426C/K899C—Using our previous substrate-bound IDE structures (4), we found that aspartate 426 forms a salt bridge with lysine 571. Based on this, we mutated Asp-426 and a nearby residue Lys-899 to cysteines and found that this IDE mutant, D426C/K899C, degraded a bradykinin-based substrate more efficiently than wild-type IDE under reducing conditions (4). This is consistent with the notion that mild disruption of the interface between IDE-N and IDE-C could promote the opening of the IDE catalytic chamber and thereby facilitate catalysis. Our substrate-free IDE structure shows the same interaction between Asp-426 and Lys-899. To determine whether this mutation would also affect the processing of longer substrates, we performed kinetic analysis using the physiologically relevant substrate Aβ-(1–40).

To this end, we examined the kinetic parameters of Aβ degradation using a modification of a fluorescence-based Aβ degradation assay we published previously (40). This assay is based on a derivatized Aβ-(1–40) peptide containing fluorescein at the N terminus and biotin at the C terminus (FA-BB). Hydrolysis of this substrate separates the fluorescent label from the biotin tag. The fraction of hydrolyzed substrate can be determined by
first removing the intact substrate by avidin-agarose precipitation and then quantifying the remaining fluoresceinated Aβ fragments (40). Here, we implemented a modification of the published assay by using unmodified Aβ as the substrate and FAAβ as a tracer to monitor degradation. This allowed a better assessment of the kinetics of Aβ degradation (instead of FAAβ degradation) by IDE. We found that the $k_{cat}$ of D426C/K899C was 2.5-fold higher than that of wild-type IDE, whereas no significant changes were observed in the values of $K_m$ or the Hill coefficient (Fig. 3, A and B).

To examine the relative catalytic efficiency of wild-type IDE versus D426C/K899C in a more physiological setting, we com-
IDE had no effect on the secreted ectodomain of the amyloid precursor protein derivative generated by α-secretase (APPsα) (49, 50), which retains the epitope recognized by the 26D6 antibody and was used as a loading control (Fig. 3C). These results confirm that the D426C/K899C mutation increases the catalytic efficiency of IDE against natural substrates, lending strong support to the hypothesis that the closed (inactive) state of IDE is the default state in an endogenous context.

We then examined the interplay between the hyperactive IDE mutation D426C/K899C and a known activator, ATP, and also an inhibitor, bacitracin. For short peptide substrates, physiologically relevant ATP concentrations can greatly enhance IDE activity (>20-fold) (23). However, no enhancement was found when larger peptide substrates such as insulin or Aβ were used (23). Under conditions in which ATP is known to enhance the activity of IDE (50 mm Tris•HCl, pH 7.3, and 0.15% BSA), we found that ATP was able to increase the activity of wild-type IDE by ~300%, as determined from the rate of hydrolysis of substrate V, a bradykinin-based fluorogenic substrate (Fig. 4A). Interestingly, ATP enhanced the activity of IDE-D426C/K899C by only ~50% (Fig. 4B). These results suggest that ATP and the hyperactivity-inducing mutations act through a shared mechanism, namely by promoting the open conformation of IDE.

The latter hypothesis was also supported by experiments examining the interplay between ATP and a low affinity IDE inhibitor, bacitracin. For wild-type IDE, the IC_{50} for bacitracin was decreased from 400 ± 100 μM in the absence of ATP to 110 ± 10 μM in the presence of ATP. By contrast, for the hyperactive IDE mutant, the IC_{50} values of bacitracin were similar in the absence or presence of ATP (200 ± 50 versus 180 ± 30 μM, respectively). Because the affinity of bacitracin for IDE is likely

AUGUST 31, 2007•VOLUME 282•NUMBER 35
JOURNAL OF BIOLOGICAL CHEMISTRY 25459

FIGURE 2. Structural comparison of IDE and AtPreP. A, monomer of substrate-free IDE and AtPreP (PDB code 2FGE) showing the N-terminal domains (domain 1 and domain 2) and C-terminal domain (domain 3 and domain 4). The N-terminal and C-terminal domains of AtPreP are connected by a hinge between domain 2 and domain 3 (yellow). B, surface representation of substrate-free IDE and AtPreP. The joining loop of IDE and the hinge of AtPreP are shown in yellow. The molecular surface is color-coded by electrostatic potential, as calculated by APBS (47). Potentials less than −6 kT are red, those greater than +6 kT are blue, and neutral potentials (0 kT) are white. The interaction surface between the N- and C-terminal domains of IDE and AtPreP is marked based on the contact residues displayed using CCP4 molecular graphics (54). C, representation of surface sequence conservation of the inner chamber of IDE and PreP. Colors represent the 9 graded scales of conservation as follows: dark blue represents the highest conservation; dark red represents the least conservation; and rainbow colors between red and blue represent the intermediate scales of conservation, of which green represents the average conservation. The sequence analysis includes IDE homologs from vertebrates as follows: human (NP004960); chimpanzee (XP0707922), mouse (AAH41675), rat (NP037291), Zebrafish (XP684736 and XP707623); insects (XP396981, P22817, and EA07246); roundworm (CAE64571 and AAM45374); plants (NP181710, XP477870, and CAC67408); and fungi (EA676500, XP956166, XP759404, CAG60009, XP719241, EAL18851, XP454175, AAB82351, CAA20142, and XP505854). PreP from invertebrates (CAI40001, AAC67244, XP001102165, AAX11355, NP98632, and XP535200); plants (CAL56365, XP001420882, CAL53630, Q08L33, and EA224704); insects (XP316646 and XP397099); fungi (XP660250); protozoa (XP654849); and bacteria (ZP01999330, ZP00769237, NP801741, ZP007869227, ZP01999330, ZP801683, and YP859525). The analysis was performed using program ConSurf (48). The interaction surface between the N- and C-terminal domains of IDE and AtPreP is marked based on the contact residues displayed using CCP4 molecular graphics (54). D, view of internal catalytic chamber of IDE-N and PreP-N shows the position of co-purified peptides (space-filling model).
to be greatly increased by entrapment within the internal chamber of IDE (i.e. in the closed state), these results are consistent with the idea that ATP promotes the open state.

Conformational Changes in IDE Induced by ATP—IDE has been shown to form homodimers and tetramers by several methods, including native gel electrophoresis (33), size-exclusion chromatography (30), sedimentation equilibrium centrifugation (30), and crystallography (4). Tetrameric forms of IDE show reduced specific activity (22), which crystallographic analysis suggests is caused by stabilization of the closed conformation through intermolecular steric hindrance (4). Previous studies have suggested that ATP exerts its activation effects by promoting the transition from tetrameric to dimeric forms of IDE (51). However, based on the preceding kinetic analyses, we postulated that ATP might enhance IDE activity by promoting the open state of the enzyme via intramolecular rather than intermolecular conformational changes.

To investigate this hypothesis, we used several independent methods that are sensitive to intramolecular conformational changes. First, we used dynamic light scattering, a spectroscopic method that can estimate the hydrodynamic radius of proteins in solution at relatively low concentrations and at physiological temperatures. As shown in Table 2, ATP increased the predicted size of wild-type IDE dose-dependently, from a minimum of ~250 kDa to a maximum of ~370 kDa, and these changes correlated with the ability of ATP to activate the hydrolysis of a short peptide substrate.

As an independent measurement of possible conformational changes induced by ATP, we also analyzed the migration of IDE by native gel electrophoresis. The electrophoretic mobility of IDE was slowed appreciably by the inclusion of 3 mM ATP in the sample and running buffer (Fig. 5A). This result is not consistent with the idea that ATP promotes a transition from an oligomeric to a monomeric state (51), which would be predicted to accelerate rather than impede the electrophoretic mobility of IDE. Instead, it is consistent with a direct conformational change induced by ATP within individual IDE molecules.

To further investigate possible intramolecular changes in IDE induced by ATP, we determined the circular dichroism spectra of recombinant IDE in the presence or absence of ATP. We found that ATP induced dose-dependent changes in dichroism spectra, indicative of a conformational switch (Fig. 5B). Together with our kinetic analyses, these results suggest that ATP activates the catalytic activity of IDE through intramolecular conformational changes that promote the open conformation of IDE.

Song et al. (23) found that the activating effect of ATP is mediated primarily through its triphosphate moiety and moreover that PPPi alone could activate IDE. Consistent with this result, we found that the activating effect of ATP is greater than that of ADP, which in turn is much greater than that of AMP (supplemental Fig. 5A). Moreover, all four nucleotide triphosphates, ATP, GTP, CTP, and TTP, are capable of activating IDE, although purine nucleotide triphosphates are better activators than pyrimidine nucleotide triphosphates (supplemental Fig. 5B). However, the fact that PPPi, alone can activate IDE, together with the observation that the activating effect of ATP is influenced by buffer conditions (23), raises the possibility that the activation of IDE by ATP might be attributable to nonspecific solvent effects rather than to specific interactions with a bona fide nucleotide binding domain.

**TABLE 2**

Effects of ATP and PPPi on hydrodynamic radius of IDE as detected by dynamic light scattering

| Condition | Hydrodynamic radius (nm) | Polydispersity | Predicted mass (kDa) | Corresponding % activation |
|-----------|--------------------------|----------------|----------------------|---------------------------|
| IDE alone | 6.3 ± 0.2 | 10 ± 2 | 250 ± 10 | 0 |
| 0.1 mM ATP | 6.4 ± 0.3 | 8 ± 2 | 260 ± 30 | 310 ± 30 |
| 1 mM ATP | 6.9 ± 0.2 | 10 ± 1 | 310 ± 20 | 590 ± 95 |
| 10 mM ATP | 7.3 ± 0.4 | 7.8 ± 0.5 | 370 ± 30 | 640 ± 98 |
| 10 mM PPPi | 6.4 ± 0.1 | 9.5 ± 0.4 | 260 ± 10 | 330 ± 20 |

* Corresponding activation was measured by the rate of hydrolysis of o-aminobenzoic acid-GGFLRKHGQ-ethylenediamine 2,4-dinitrophenyl (5 μM) by recombinant IDE (2 μM) at 22 °C in 20 mM Tris-HCl pH 7.4.
To address this important question, we analyzed the effects of PPP, using the same set of conformation-sensitive methods used for ATP. By dynamic light scattering, we found that 10 mM PPP induced no significant change in the apparent hydrodynamic radius of IDE, whereas the same concentration of ATP induced a large increase (Table 2). Furthermore, in marked contrast to ATP, which causes IDE to migrate more slowly, but in a distinct band by native gel electrophoresis (Fig. 5A), PPP caused IDE to migrate in a poorly defined smear (supplemental Fig. 5C). Moreover, the changes in the CD spectra of IDE induced by ATP (Fig. 5B) were not evidenced by PPP, (supplemental Fig. 5D). Thus, although the triphosphate moiety within ATP does indeed play an important role in mediating the activating effect, the full complement of changes induced by ATP and other nucleotide triphosphates is not recapitated by PPP, alone. Taken together, these results suggest that IDE possesses a distinct nucleotide binding domain that mediates intramolecular conformational changes.

**DISCUSSION**

IDE belongs to the M16 family of zinc metalloproteases, within the ME clan (51). Structures of four members of this family have been solved, including human IDE and *E. coli* pitrilysin (M16A members), yeast MPP (M16B member), and AtPreP (M16C member) (4, 31, 32, 44). These structures show that this family of proteases exhibits a characteristic two-domain organization. The N-terminal domains of M16A/C members and the β-subunit of MPP contain the catalytic site. Comparison of the catalytic site of our substrate-free catalytically active IDE-Y831F structure with substrate-free MPP and pitrilysin reveals near identical zinc ion coordination by two histidines, one glutamate, and a catalytic water with a distorted tetrahedral geometry (Fig. 1D). The catalytic water that is coordinated by a zinc ion also interacts with a glutamate residue, which serves as the general acid/base catalyst.

Among the four M16 proteases for which structures have been obtained, both human IDE and PreP can effectively degrade Aβ (7, 45). Interestingly, both structural and mutational analyses suggest that IDE and AtPreP have at least two conformations, open and closed (4, 31). The closed conformation is the primary state for catalysis after substrate is bound, but transition to the open conformation is required to permit the exit of the degraded products and binding of new substrates. Consistent with this model, the introduction of pairs of cysteines that lock the N- and C-terminal domains of IDE and AtPreP in the closed state can specifically inactivate these enzymes (4, 31).

Substrate-free IDE in its closed conformation likely plays a key role in controlling the catalytic rate of IDE. In this state, substrate cannot access the catalytic chamber, thus IDE is in a catalytically inactive state. IDE can bind its substrate only when it switches from the closed state to the unbound open state. The extensive contact surface with good complementarity between IDE-N and IDE-C as well as the conservation among IDE homologs suggest that the substrate-free, closed state of IDE is stable and may therefore represent the predominant resting state of IDE. Consistent with this idea, we found that the incorporation of mutations that destabilize the interface between IDE-N and IDE-C profoundly increase the Vₘₐₓ of IDE-mediated Aβ degradation without altering the Kₘₚ. Furthermore, such a mutant is more effective in degrading Aβ than wild-type enzyme without the enhanced degradation of soluble APP.

Johnson *et al.* (31) proposed that the binding of substrate to AtPreP induces a switch from the open conformation to the closed state so that catalysis can occur. However, this model may need to be amended. Using the structure of AtPreP, we found that the interaction between PreP-N and PreP-C is also extensive. Accordingly, substrate-free AtPreP in its closed conformation should also be reasonably stable. Thus, the conformational switch between the unbound, closed state to the open state of AtPreP could also be a key step controlling its function.

Both IDE and PreP serve as a mini-protease to entrap the short peptides such as Aβ into the ~13,000 Å³ chamber for their degradation (4, 7, 31, 45). However, our structural comparison of IDE and AtPreP reveals several key differences. We observe major differences in the charge distribution at the inner chamber of C-terminal domains of these two enzymes as well as the presence of a conserved exosite for IDE in its substrate binding chamber (Fig. 2, B and C). These differences are likely to contribute significantly on the substrate selectivity of these two enzyme and would provide the molecular basis in why only IDE can effectively degrade intact insulin, whereas PreP can only cleave insulin B chain (1, 45). We also found that the linkers that join N and C domains of IDE and AtPreP are diverse both in their structure and localization (Fig. 2A). These differ-
ences may render these enzymes to have different types of regulation in controlling the conformational switch between the closed and open states.

Less is known about the control of the conformational switch between the closed and open states of IDE in regulating the catalytic activity of this enzyme. ATP is known to allosterically enhance the ability of IDE to degrade short peptide substrates (23). Here we report that the activating effect of ATP is reduced in IDE mutants containing mutations that destabilize the closed state. Moreover, we provide evidence that ATP induces substantial intramolecular conformational changes within IDE. Together, these findings are consistent with the idea that ATP and other nucleotides facilitate the transition from the closed state to the open state. The attempt to decipher the ATP binding site using our IDE crystallization condition was unsuccessful.4 Although we could co-crystallize IDE with the high affinity ATP analog, TNP-ATP, and obtain a 2.8 Å resolution data, we did not find electron density corresponding to the TNP-ATP (52). To make the issue more complicated, the purified IDE has been recently shown to have the intrinsic ATPase activity (53). The interplays between the ATP-induced conformational switch and ATP hydrolysis in controlling IDE activity await future biochemical and structural studies.

The catalytic rate of IDE may also be controlled by additional cellular factors that could lock IDE in its inactive conformation or block access to the active site. We showed that the internal chamber of catalytically inactive IDE could specifically bind copurified peptides that were resistant to extensive purification steps. This raises the possibility that the catalytic chamber of catalytically inactive IDE could specifically bind co-chamber of catalytically inactive IDE could specifically bind co-

ACKNOWLEDGMENTS—We acknowledge Raymond Bourdeau, Julia Warren, and Ray Hulse for their critical reading of the manuscript and thank Samir Maji and Elena Solomaha for assistance with circular dichroism measurements and data analysis. Use of the Advanced Photon Source was supported by the United States Department of Energy, Office of Basic Energy Sciences, Contract W-31-109-ENG-38.

REFERENCES

1. Duckworth, W. C., Bennett, R. G., and Hamel, F. G. (1998) Endocr. Rev. 19, 608–624
2. Kurochkin, I. V. (2001) Trends Biochem. Sci. 26, 421–425

4 E. Malito, H. Im, M. Manolopoulou, and W. J. Tang, unpublished observations.

3. Leissring, M. A., and Selkoe, D. J. (2006) Nature 443, 761–762
4. Shen, Y., Joachimiak, A., Rosner, M. R., and Tang, W. J. (2006) Nature 443, 870–874
5. Hersh, L. B. (2006) Cell. Mol. Life Sci. 63, 2432–2434
6. Mursky, I. A., and Broth-Kahn, R. H. (1949) Arch. Biochem. 20, 1–9
7. Kurochkin, I. V., and Goto, S. (1994) FEBS Lett. 345, 33–37
8. Farris, W., Mansourian, S., Chang, Y., Lindsley, L., Eckman, E. A., Frosch, M. P., Eckman, C. B., Tanzi, R. E., Selkoe, D. J., and Guenette, S. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 4162–4167
9. Farris, W., Mansourian, S., Leissring, M. A., Eckman, E. A., Bertram, L., Eckman, C. B., Tanzi, R. E., and Selkoe, D. J. (2004) Am. J. Pathol. 164, 1425–1434
10. Miller, B. C., Eckman, E. A., Sambamurti, K., Dobbs, N., Chow, K. M., Eckman, C. B., Hersh, L. B., and Thiele, D. L. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 6221–6226
11. Leissring, M. A., Farris, W., Chang, A. Y., Walsh, D. M., Wu, X., Sun, X., Frosch, M. P., and Selkoe, D. J. (2003) Neurosci. 103, 1087–1093
12. Tanzi, R. E., and Bertram, L. (2005) Cell 120, 545–555
13. Qi, W. Q., and Feldstein, M. F. (2006) Neurobiol. Aging 27, 190–198
14. Kim, M., Hersh, L. B., Leissring, M. A., Ingelsson, M., Matsu, T., Farris, W., Lu, A., Hyman, B. T., Selkoe, D. J., Bertram, L., and Tanzi, R. E. (2007) J. Biol. Chem. 282, 7825–7832
15. Gu, H. F., Efendic, S., Nordman, S., Ostenson, C. G., Brismar, K., Brookes, A. J., and Prince, J. A. (2004) Diabetes 53, 2137–2142
16. Karamohamed, S., Demissie, S., Volcjak, J., Liu, C., Heard-Costa, N., Liu, J., Shoemaker, C. M., Panhuysen, C. I., Meigs, J. B., Wilson, P., Atwood, L. D., Cupples, L. A., and Herbert, A. (2003) Diabetes 52, 1562–1567
17. Florez, J. C., Wilshire, S., Agapakis, C. M., Burt, N. P., de Bakker, P. I., Almgren, P., Bengtsson Bostrom, K., Tuomi, T., Gaudet, D., Daly, M. J., Hirschhorn, J. N., McCarthy, M. I., Altshuler, D., and Groop, L. (2006) Diabetes 55, 128–135
18. Groves, C. J., Wilshire, S., Smedley, D., Owen, K. R., Frayling, T. M., Walker, M., Hitman, G. A., Levy, J. C., O’Rahilly, S., Menzel, S., Hattersley, A. T., and McCarthy, M. I. (2003) Diabetes 52, 1300–1305
19. Lynch, J. A., George, A. M., Eisenhauer, P. B., Conn, K., Gao, W., Carreras, I., Wells, J. M., McKee, A., Ullman, M. D., and Fine, E. R. (2006) J. Neurosci. Res. 83, 1262–1270
20. Bondy, C. A., Zhou, J., Chin, E., Reinhardt, R. D., Ding, L., and Roth, R. A. (1994) J. Clin. Investig. 93, 966–973
21. Bertram, L., Blacker, D., Mullin, K., Keeney, D., Jones, J., Basu, S., Yhu, S., McInnis, M. G., Go, R. C., Vekrellis, K., Selkoe, D. J., Saunders, A. J., and Frosch, M. P., and Selkoe, D. J. (2003) Science 290, 2302–2303
22. Song, E. S., Juliano, M. A., Juliano, L., and Hersh, L. B. (2003) J. Biol. Chem. 278, 49789–49794
23. Song, E. S., Juliano, M. A., Juliano, L., Fried, M. G., Wagner, S. L., and Hersh, L. B. (2004) J. Biol. Chem. 279, 54216–54220
24. Bennett, R. G., Hamel, F. G., and Duckworth, W. C. (2003) Diabetes 52, 2315–2320
25. Shimall, H., Song, E. S., and Hersh, L. B. (2005) Biochemistry 44, 15345–15350
26. Ogawa, W., Shi, K., Yonezawa, K., Baba, S., and Yokono, K. (1992) J. Biol. Chem. 267, 1310–1316
27. Saric, T., Muller, D., Seitz, H. J., and Pavelic, K. (2003) Mol. Cell. Endocrinol. 204, 1–11
28. Camberos, M. C., Perez, A. A., Udrisar, D. P., Wanderley, M. I., and Cresto, J. C. (2001) Exp. Biol. Med. 226, 334–341
29. Hamel, F. G., Upward, J. L., and Bennett, R. G. (2003) Endocrinology 144, 2404–2408
30. Li, P., Kuo, W.-L., Yousef, M., Rosner, M. R., and Tang, W.-J. (2006) Biochem. Biophys. Research Commun. 334, 1032–1037
31. Johnson, K. A., Bhushan, S., Stahl, S., Hallberg, B. M., Frohn, A., Glaser, E., and Eneqvist, T. (2006) EMBO J. 25, 1977–1986
32. Maskos, K. (2004) in Handbook of Metalloproteins (Messerschmidt, A., Dode, W., and Cygler, M., eds) Vol. 3, pp. 190–198, John Wiley & Sons, Inc., New York
33. Farris, W., Leissring, M. A., Hemming, M. L., Chang, A. Y., and Selkoe, D. J. (2005) Biochemistry 44, 6513–6525
34. Orwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
35. Collaborative Computational Project Number 4 (1994) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **50**, 760–767
36. McCoy, A. J., Grosse-Kunstleve, R. W., Storoni, L. C., and Read, R. J. (2005) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **61**, 458–464
37. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **54**, 905–921
38. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **53**, 240–255
39. Emsley, P., and Cowtan, K. (2004) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **60**, 2126–2132
40. Leissring, M. A., Lu, A., Condron, M. M., Teplow, D. B., Stein, R. L., Farris, W., and Selkoe, D. J. (2003) *J. Biol. Chem.* **278**, 37314–37320
41. Sciarretta, K. L., Gordon, D. J., Petkova, A. T., Tycko, R., and Meredith, S. C. (2005) *Biochemistry* **44**, 6003–6014
42. Kim, S. H., Ikeuchi, T., Yu, C., and Sisodia, S. S. (2003) *J. Biol. Chem.* **278**, 33992–34002
43. Kim, S. H., Yin, Y. I., Li, Y. M., and Sisodia, S. S. (2004) *J. Biol. Chem.* **279**, 48615–48619
44. Taylor, A. B., Smith, B. S., Kitada, S., Kojima, K., Miyaura, H., Otwinowski, Z., Ito, A., and Deisenhofer, J. (2001) *Structure* **9**, 615–625
45. Falkevall, A., Alikhani, N., Bhushan, S., Pavlov, P. F., Busch, K., Johnson, K. A., Eneqvist, T., Tjernberg, L., Ankarcrorna, M., and Glaser, E. (2006) *J. Biol. Chem.* **281**, 29096–29104
46. Lawrence, M. C., and Colman, P. M. (1993) *J. Mol. Biol.* **234**, 946–950
47. Baker, N. A., Sept, D., Joseph, S., Holst, M. J., and McCammon, J. A. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98**, 10037–10041
48. Landau, M., Mayrose, I., Rosenberg, Y., Glaser, F., Martz, E., Pupko, T., and Ben-Tal, N. (2005) *Nucleic Acids Res.* **33**, W299–W302
49. Sisodia, S. S. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 6075–6079
50. Song, E. S., Daily, A., Fried, M. G., Juliano, M. A., Juliano, L., and Hersh, L. B. (2005) *J. Biol. Chem.* **280**, 17701–17706
51. Rawlings, N. D., Tolle, D. P., and Barrett, A. J. (2004) *Nucleic Acids Res.* **32**, D160–D164
52. Yao, H., and Hersh, L. B. (2006) *Arch. Biochem. Biophys.* **451**, 175–181
53. Del Carmen Camberos, M., and Cresto, J. C. (2007) *Exp. Biol. Med.* **232**, 281–292
54. Potterton, E., McNicholas, S., Krissinel, E., Cowtan, K., and Noble, M. (2002) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **58**, 1955–1957