Unsaturated Fatty Acids Induce Cytotoxic Aggregate Formation of Amyotrophic Lateral Sclerosis-linked Superoxide Dismutase 1 Mutants*

Yeon-Jeong Kim‡, Reiko Nakatomi§, Takumi Akagi§, Tsutomu Hashikawa§, and Ryosuke Takahashi‡

From the Laboratories for Motor System Neurodegeneration and Neural Architecture, RIKEN Brain Science Institute, Saitama 351-0198, Japan and Department of Neurology, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan

Formation of misfolded protein aggregates is a remarkable hallmark of various neurodegenerative diseases including Alzheimer disease, Parkinson disease, Huntington disease, prion encephalopathies, and amyotrophic lateral sclerosis (ALS). Superoxide dismutase 1 (SOD1) immunoreactive inclusions have been found in the spinal cord of ALS animal models and patients, implicating the close involvement of SOD1 aggregates in ALS pathogenesis. Here we examined the molecular mechanism of aggregate formation of ALS-related SOD1 mutants in vitro. We found that long-chain unsaturated fatty acids (FAs) promoted aggregate formation of SOD1 mutants in both dose- and time-dependent manners. Metal-deficient SOD1s, wild-type, and mutants were highly oligomerized compared with holo-SOD1s by incubation in the presence of unsaturated FAs. Oligomerization of SOD1 is closely associated with its structural instability. Heat-treated holo-SOD1 mutants were readily oligomerized by the addition of unsaturated FAs, whereas wild-type SOD1 was not. The monounsaturated FA, oleic acid, directly bound to SOD1 and was characterized by a solid-phase FA binding assay using oleate-Sepharose. The FA binding characteristics were closely correlated with the oligomerization propensity of SOD1 proteins, which indicates that FA binding may change SOD1 conformation in a way that favors the formation of aggregates. High molecular mass aggregates of SOD1 induced by FAs have a granular morphology and show significant cytotoxicity. These findings suggest that SOD1 mutants gain FA binding abilities based on their structural instability and form cytotoxic granular aggregates.

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† To whom correspondence should be addressed: Dept. of Neurology, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. Tel.: 81-75-751-3770; Fax: 81-75-761-9780; E-mail: ryosuke@kuhp.kyoto-u.ac.jp.

‡ The abbreviations used are: ALS, amyotrophic lateral sclerosis; FALS, familial amyotrophic lateral sclerosis; SOD1, superoxide dismutase 1; FA, fatty acid; AA, arachidonic acid; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium.

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Amyotrophic lateral sclerosis (ALS)† is a progressive and fatal neurodegenerative disorder that mainly affects motor neurons in the brain stem and spinal cord. Approximately 10% of ALS patients are familial cases, with autosomal dominant inheritance. More than 90 different mutations in the gene coding for superoxide dismutase 1 (SOD1) have been identified in about 20% of familial ALS (FALS) families (1, 2). Although the molecular mechanisms of selective motor neuron degeneration by SOD1 mutants in FALS remain largely unknown, common pathological features of conformational diseases, as evidenced by SOD1 immunoreactive inclusions, are found in the spinal cord of ALS patients and in the SOD1 mutant FALS mouse model (3–8). The characteristics of FALS resemble those of many other neurodegenerative diseases in which a causative protein undergoes a conformational rearrangement, which endows it with a tendency to aggregate and form deposits within affected tissues.

SOD1 is a 32-kDa homodimeric enzyme that decreases the intracellular concentration of superoxide radicals by catalyzing their dismutation to O2 and H2O2. ALS-linked mutations of SOD1 are distributed throughout the primary and tertiary structures, and most mutations appear unrelated to the dismutase activity. Many biochemical and biophysical studies have reported that SOD1 mutants are structurally unstable compared with wild-type forms (10–13). These observations suggest that the mutations primarily affect the structural stability of SOD1 rather than the enzyme activity.

The half-life of SOD1 mutants is shorter than that of wild-type forms in cultured cells (14). SOD1 mutants form a complex with Hsp70 and CHIP, which promotes degradation of SOD1 through the ubiquitin-proteasome system (15). Hsp70 directly binds metal-deficient wild-type SOD1 as well as SOD1 mutants, suggesting that destabilized SOD1 is targeted by the molecular chaperone system (15, 16). These observations imply that structural stability of SOD1 may also be strongly involved in refolding by the chaperone or in degradation of SOD1 by the ubiquitin-proteasome system. On the other hand, aggregates of mutant SOD1 are observed to have aggresome-like morphology when cells are treated with a proteasome inhibitor (14). This aggresome-like morphology resembles pathological inclusions of neurodegenerative diseases in affected tissues. These findings suggest that in disease states, misfolded proteins overwhelm the protein handling systems, including chaperones and proteasomes. Therefore, the formation of cellular inclusions may be required for other factors to act as modulators to promote protein aggregates. In fact, lipid molecules, including unsaturated fatty acids (FAs), phosphatidylinerine, and phosphatidylserine, promote amyloidogenesis of amyloid β-peptides, tau (17), and α-synuclein (18, 19) in vitro. These molecules are biologically significant as mediators for signal-
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Experimental Procedures

Expression, Purification, and Characterization of Recombinant SOD1 Proteins—pDNA3-SOD1 (20) was digested with EcoRV and XhoI and subcloned into blunt-neol and XhoI sites of pET-15(b) (Novagen) to express recombinant SOD1. Expressions were performed in Escherichia coli (E. coli) strain BL21(DE3)pLysS by adding 1 mM isopropyl-1-thio-beta-D-galactopyranoside, 0.1 mM CuCl2, and 0.1 mM ZnCl2 until cells were grown to 0.6 absorbance unit at 600 nm, and then bacterial cells were further cultured at 23 °C for 6 h. Cells were pelleted and resuspended in TNE buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.1 mM EDTA) supplemented with protease inhibitor mixture (Roche Applied Science). Cells were then disrupted by sonication. Insoluble materials were removed by centrifugation at 10,000 × g for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification. Purification was performed according to Hayward et al. (11), with minor modifications. Briefly, ammonium sulfate powder was added to the supernatant for 30 min. Supernatant was collected for further purification.
Apo-enzymes demonstrated higher oligomerization propensity than holo-enzymes depending on AA concentration (Fig. 2B). Thus, AA efficiently promoted oligomerization of SOD1s.

Next, we performed a time-course analysis of SOD1 oligomerization in the presence of AA. Metal-deficient G93A and A4V were oligomerized in a time-dependent manner (Fig. 2C). Maximum oligomerization was reached within 60 min of incubation in the presence of AA (Fig. 2C).

We then examined the effect that various FAs, including stearic acid, oleic acid, linoleic acid, and AA, have on SOD1 oligomerization. Unsaturated FAs, including oleic acid, linoleic acid, and AA, promoted SOD1 oligomerization (Fig. 3). However, saturated FAs and stearic acid had little effect on SOD1 oligomerization (Fig. 3). SOD1 oligomerization induced by FAs required at least monounsaturated FAs. This result may reflect the difference of solubility between unsaturated and saturated FAs in the buffer.

We verified the formation of SOD1 oligomers using a 10–40% glycerol density gradient centrifugation because presumable artifacts after detection of SOD1 oligomers using non-reducing SDS-PAGE may have remained. After fractionation, we could not observe high molecular mass SOD1 oligomers from the incubated sample in the absence of AA; fractions were <67 kDa and potentially represented monomer and dimer states (Fig. 4A, top panel). In contrast, we detected high molecular mass oligomers in fractions of >440 kDa from the incubated sample in the presence of AA (Fig. 4A, bottom panel). Under these conditions, SOD1 with molecular mass of <67 kDa was dramatically decreased compared with the sample incu-
densitometric analysis from Western blotting images to estimate boiling of the SDS-PAGE loading buffer. We next performed panel bated at 37 °C for 90 min in the absence or presence of 100 μM AA and then further incubated at 37 °C for 1 h. SDS-PAGE was performed under non-reducing conditions. Proteins were detected by Western blotting as described under “Experimental Procedures.” Arrows indicate the position of stacking gels.

**Fig. 4.** Glycerol density gradient centrifugation and densitometric analysis of SOD1 oligomers. A, apo-A4V (2.5 μM) was incubated at 37 °C for 90 min in the absence or presence of 100 μM AA before loading on the glycerol cushion. After centrifugation, fractions were collected from the bottom of the tubes and then subjected to SDS-PAGE under non-reducing conditions. SOD1 proteins were detected by Western blotting. B, for densitometric analysis, we measured mean density per lane after background subtraction. Total mean density was similar under each condition, by calculating the mean density of visible lanes (lanes 1–8 for oligomers and lanes 21–25 for dimer or monomer). Arrows indicate the position of stacking gels.

bated in the absence of AA (Fig. 4A, bottom panel). Although oligomers of >440 kDa were fractionated by the glycerol density gradient centrifugation, these were detected as monomer, dimer, and smeared high molecular mass bands that reached stacking gels under non-reducing SDS-PAGE (Fig. 4A, bottom panel). This indicates oligomers are partly disrupted during the boiling of the SDS-PAGE loading buffer. We next performed densitometric analysis from Western blotting images to estimate the amount of oligomerized SOD1 (Fig. 4B). The resulting image analysis found that immunoreactivity for oligomers was ~80% of the total immunoreactivity.

**Structural Instability of SOD1 Is Correlated to Oligomerization Propensity and FA Binding**—We showed the FA-induced oligomerization propensity of apo-SOD1s was higher than that of holo-SOD1. This implies that protein stability might be strongly associated with FA-induced oligomerization propensity. Among the holo-enzymes, wild-type and G93A were not oligomerized under our experimental conditions (Fig. 2B, top panel). To examine the correlation between oligomerization propensity and protein stability of holo-enzymes, holo-SOD1 was heated and then oligomerized by AA. In the absence of AA, only heat-treated A4V was oligomerized (Fig. 5, left panel). In the presence of AA, heat-treated G93A and A4V were highly aggregated, but under the same conditions, wild-type SOD1 was not (Fig. 5, right panel). Oligomerization was observed above 58 °C for G93A and above 48 °C for A4V (Fig. 5, right panel). In the previous study, A4V was more unstable than G93A for heat treatment analyzed by differential scanning calorimetry (12). This result suggests that structural instability is strongly correlated with oligomerization propensity induced by FAs.

Although we showed that FAs promoted SOD1 oligomerization, the mechanism is not perfectly understood. Similarly, unsaturated FAs oligomerize α-synuclein and tau. In the case of α-synuclein and tau, FAs were bound to proteins, which suggested that oligomerization mechanisms underlie the FA binding characteristics of protein. To examine whether SOD1 binds to FAs, we carried out a solid-phase oleic acid binding assay. Among the holo-enzymes, very small amounts of holo-A4V were bound to the oleate-Sepharose column, whereas wild-type and G93A were not (Fig. 6A). All of the apo-enzymes were bound to oleate-Sepharose, regardless of their mutations (Fig. 6A). In contrast, bound proteins were not observed in mock-Sepharose (Fig. 6A). Nearly all of the input amounts of metal-deficient proteins were bound, which was estimated by 50% input. This finding suggests that metal-deficient SOD1 proteins strongly bind to FAs. We next examined whether heat-treated holo-enzymes bind to FAs. Apo-enzymes were used as control binding. Heat-treated SOD1 mutant (G93A) at 58 °C and 68 °C was bound to FAs, whereas wild-type was not (Fig. 6B). The results of the FA binding assay were strongly correlated with the oligomerization propensity of SOD1. These findings suggest that FA binding alters the conformation of SOD1 to form oligomers.

**FA-induced SOD1 Aggregates Result in Granular Morphology and Are Cytotoxic**—We analyzed the ultrastructure of SOD1 aggregates by electron microscope. SOD1 proteins (~40 μM) were incubated in the presence of 100 μM AA at 37 °C for 24 h. Holo-enzymes were heated at 50 °C for 30 min before incubation in the presence of AA. After incubation, granular aggregates were observed in all of apo-enzymes and heat-treated SOD1 mutants (Fig. 7A). In contrast, no visible materials were found in wild-type holo-SOD1s, even though they were heat-treated (Fig. 7A). The morphology of the aggregates was round or amorphous large granules composed of clustered small granules (Fig. 7A). We could not observe any visible protein aggregates in the samples incubated without AA, except in apo-A4V, which revealed a fibril structure (data not shown).

We next examined the effect of FA-induced aggregates on cell
viability of differentiated neuro2a cells. Aggregates of SOD1s were formed using the same methods as described for observation under an electron microscope. Aliquots incubated in the presence or absence of AA were diluted in the culture medium, which was directly added to differentiated neuro2a cells. After incubation for 18 h, toxicity was assessed with MTS reduction (Fig. 7B) and trypan blue staining (Fig. 7C). The presence of the granular aggregates formed by AA from Apo-SOD1s and heat-treated SOD1 mutants significantly reduced cell viability (Fig. 7, B and C). In contrast, no significant decrease of viability was detected when the cells were exposed either to incubated proteins in the absence of AA or to the buffer solutions used to form the aggregates in the absence of added protein (Fig. 7, B and C). These findings suggest that FA-induced SOD1 aggregates were highly toxic to the cells.

**DISCUSSION**

Numerous neurodegenerative diseases are accompanied by highly insoluble inclusions of protein aggregates within characteristic neuronal populations. In the case of FALS, the prototypical Lewy body-like hyaline inclusions, composed largely of granule-coated fibrils of SOD1-insoluble filaments, have been detected in the spinal cord of FALS patients with SOD1 gene mutations (5, 28). Although there has been controversy about whether such inclusions are a cause or a consequence of the neuronal degeneration, accumulating evidence suggests that aggregates formed via misfolded proteins, especially soluble oligomeric assemblies, may cause cell injury (29–31). Moreover, cytotoxicity of protein aggregates may have common features because granular aggregates form non-pathological proteins that can also be toxic (26). These findings suggest the avoidance of protein aggregation may be crucial for therapy of oligomerization of SOD1 Mutants by Fatty Acids.
conformational diseases including FALS.

In the present study, we demonstrated that unsaturated FAs promoted SOD1 oligomerization at physiological pH. SOD1 oligomers were detected by SDS-PAGE under non-reducing conditions. Although immunoreactivity for SOD1 oligomers was decreased in SDS-PAGE under reducing conditions, SOD1 oligomers were considerably SDS-resistant under non-reducing conditions. Based on this method, we found that apo-SOD1 proteins were highly oligomerized by AA compared with holo-SOD1 proteins in time-dependent and FA concentration-dependent manners (Fig. 2, B and C). Metal-deficient SOD1s may be representative of misfolding intermediates for their oligomeric assemblies because they are oligomerized independent of their mutations. These findings suggest that metal-deficient SOD1 proteins have a high oligomerization propensity, which is consistent with previous studies (9, 10, 13, 32). Moreover, heating of holo-SOD1 mutants increased the tendency to form oligomer complexes, especially in the presence of AA; however, the wild-type holo-SOD1 did not form oligomers, even after heating to 68 °C and exposure to AA (Fig. 5). This finding suggests that mutations of SOD1 primarily affect their conformation. Our time-course analysis of oligomerization demonstrates that FAs induced the oligomerization process fairly rapidly. We could detect oligomers within 1 h of incubation in the presence of AA (Fig. 1C). Glycerol density gradient centrifugation analysis showed that oligomer species were roughly 80% of the total SOD1 after a 90-min incubation in the presence of AA (Fig. 4). The conversion efficiency and the speed of oligomer formation may be considered as supportive evidence that these reactions occur in vivo.

Aggregations of misfolded proteins are primarily affected by their mutations, especially in inherited conformational diseases. Mutant proteins in conformational diseases have a common characteristic of easily unfolding in a physiological condition and favoring aggregate formation. Protein aggregation has also been shown to be modulated by several factors, including protein concentration, pH, and interactions with other elements such as lipid molecules. It has been reported that FAs stimulated the polymerization of amyloid β-peptides, tau (17, 33), and α-synuclein (18, 19) in vitro. These studies suggest that FAs play a pivotal role as nucleates in the self-assembly of misfolded proteins. Although the precise mechanism of how lipid molecules accelerate protein aggregation has not been elucidated, it has been proposed that lipid-bound proteins change their conformation or anionic surfaces, presenting as micelles or vesicles, which can serve to nucleate aggregate formation (18, 34, 35). We confirmed that apo-SOD1s or heat-treated holo-SOD1 mutants were bound to oleic acid (Fig. 6). The FA binding properties of SOD1s were strongly correlated to their conformational instability. These results are consistent with the notion that misfolding intermediates of SOD1 caused by mutations or metal loss may be facilitated by FAs to form oligomeric structures. Another possible mechanism is protein oxidation by FAs. Oxidation also enhances misfolding and aggregation of SOD1 (32). In particular, FAs can lead to the production of radicals because they are easily peroxidized by auto-oxidation to generate peroxy radicals. However, we could not inhibit SOD1 oligomerization using even a considerable amount of radical scavenger (data not shown). Moreover, oxidized derivatives of FAs also induced SOD1 oligomerization to a similar extent with fresh FAs (data not shown). This finding suggests that oxidation or oxidative damage of SOD1 does not directly drive SOD1 oligomerization. Rather, it is most likely to be associated with a SOD1-denaturing event.

Recently, several studies for in vitro aggregation of SOD1 have been published. Aggregation of SOD1 can be induced by metal-catalyzed oxidation (32), trifluoroethanol, or heat treatment (10), which induces oxidative modification or protein destabilization. This indicates that structurally unstable SOD1 has an influence on its aggregate formation in vitro. Crystallographic studies suggest that metal-deficient SOD1 forms an amyloid-like assembly caused by non-native conformational changes and permits dimer interaction (36, 37). This amyloid-like structure was represented by prolonged incubation of SOD1 at acidic pH (9). In the present study, ultrastructural analysis showed that the FA-inducing aggregates had round or amorphous morphology with clustered tiny spherical aggregates (Fig. 7A). They resemble pre-fibrillar aggregates of the N-terminal domain of Escherichia coli HypF protein or aggregates of the Src homology 3 domain of cytosolic phosphatidylinositol 3-kinase as reported by Stefani and co-workers (26). They demonstrated that granular aggregates of proteins, even non-pathological proteins, are cytotoxic when applied externally (26). Our data also demonstrate that granular aggregates of SOD1s reveal significant cytotoxicity (Fig. 7, B and C). Although the cytotoxic mechanism of the aggregates is not completely understood, it has been proposed that such pre-fibrillar intermediates may lead to cytotoxicity by permeabilization of the membrane bilayer (38, 39).

The present findings may provide considerable pathological implications for FALS. Lipid molecules such as FAs may be positive modulators for misfolded protein aggregations. Most misfolded proteins including SOD1 mutants are rapidly degraded by the ubiquitin-proteasome system. Unsaturated FAs may promote misfolded protein aggregations before they are degraded. In addition, cytotoxic aggregate formation of SOD1 may require FAs because granular aggregates structures were markedly observed in SOD1s incubated with AA. Although it is not clear whether the cytotoxic aggregates of SOD1s are generated intracellularly, we have provided a protein aggregation model system to help understand the pathological significance of FAs as a positive modulator for the aggregate formation in FALS. We believe that our system will contribute to efficient drug screening for inhibitors of SOD1 aggregation.

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REFERENCES
1. Deng, H. X., Hentati, A., Tainer, J. A., Iqbal, Z., Cayabyab, A., Hung, W. Y., Getzoff, E. D., Hu, P., Herzfeldt, B., Roos, R. P., Warner, C., Deng, G., Segrer, E., Smyth, C. L., H. K., Ahmed, A., Roses, A. D., Hallewell, R., Pericak-VANCE, M. A., and Siddique, T. (1993) Science 261, 1047–1051.
2. Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O’Regan, J. P., Deng, H. X., Rahmani, Z., Kranzus, A., McMicken-Yasek, D., Cayabyab, A., Goston, S., Tanzi, R., Halperin, J. J., Herzfeldt, B., Van den Berg, R., Hung, W., Bird, T., Deng, G., Mulder, D. W., Smith, C., Laing, N. G., Soriano, E., Pericak-VANCE, M. A., Haines, J. E., Rouleau, G. A., Gusella, J. M., Horvitz, H. R., and Brown, B. H. (1999) Nature 362, 59–62.
3. Shibata, N., Asayama, K., Hirano, A., and Kobayashi, M. (1996) Dev. Neurosci. 18, 492–498.
4. Brujin, L. I., Becher, M. W., Lee, M. K., Anderson, K. L., Jenkins, N. A., Copeland, N. G., Sinodis, S. S., Rothschild, J. D., Borchelt, D. R., Price, D. L., and Cleveland, D. W. (1997) Neuron 18, 327–338.
5. Kata, S., Hayashi, H., Nakashima, K., Namba, H., Kato, M., Hirano, A., Nakano, I., Asayama, K., and Obama, E. (1997) Am. J. Pathol. 151, 611–620.
6. Shibata, N., Hirano, A., Kobayashi, M., D’Aleno, M. C., Gurney, M. E., Komori, T., Umahara, T., and Asayama, K. (1998) Acta Neurologica Japonica 98, 135–142.
7. Watanabe, M., Dykes-Hoberg, M., Calotta, V. C., Price, D. L., Wang, P. C., and Rothstein, J. D. (2001) Neurobiol. Dis. 8, 933–941.
8. Wang, J., Xu, G., Gonzales, V., Coupland, M., Frumholt, D., Copeland, N. G., Jenkins, N. A., and Borchelt, D. R. (2002) Neurobiol. Dis. 10, 128–138.
9. DiDonato, M., Craig, L., Huff, M. E., Thayer, M. M., Cardoso, R. M., Kassmann, C. J., Lo, T. P., Bruns, C. K., Powers, K. T., Kelly, J. W., Getzoff, E. D., and Tainer, J. A. (2003) J. Mol. Biol. 332, 601–615.
10. Stathopulos, P. R., Rumfeldt, J. A., Scholz, G. A., Irani, R. A., Frey, H. E., Hallewell, R. A., Lepock, J. R., and Meiering, E. M. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 7021–7026.
11. Hayward, I. J., Rodriguez, J. A., Kim, J. W., Tiwari, A., Goto, J. S., Cabelli, D. E., Valentine, J. S., and Brown, R. H. (2002) J. Biol. Chem. 277, 15923–15931.
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12. Rodriguez, J. A., Valentine, J. S., Eggers, D. K., Roe, J. A., Tiwari, A., Brown, R. H., Jr., and Hayward, L. J. (2002) J. Biol. Chem. 277, 15902–15907
13. Lindberg, M. J., Tibell, L., and Oliveberg, M. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 16607–16612
14. Johnston, J. A., Dalton, M. J., Gurney, M. E., and Kopito, R. R. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12571–12576
15. Urushitani, M., Kurisu, J., Tateno, M., Hatakeyama, S., Nakayama, K., Kato, S., and Takahashi, R. (2004) J. Neurochem. 90, 231–244
16. Shinder, G. A., Lacourse, M. C., Minotti, S., and Durham, H. D. (2001) J. Biol. Chem. 276, 12791–12796
17. Wilson, D. M., and Binder, L. I. (1997) Am. J. Pathol. 150, 2181–2195
18. Sharon, R., Goldberg, M. S., Bar-Josef, I., Betensky, R. A., Shen, J., and Selkoe, D. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 9110–9115
19. Sharon, R., Bar-Joseph, I., Frisch, M. P., Walsh, D. M., Hamilton, J. A., and Selkoe, D. J. (2003) Neuron 37, 583–595
20. Urushitani, M., Kurisu, J., Tsukita, K., and Takahashi, R. (2002) J. Neurochem. 83, 1030–1042
21. Fried, R. (1975) Biochimie (Paris) 57, 657–660
22. Crow, J. P., Sampson, J. B., Zhuang, Y., Thompson, J. A., and Beckman, J. S. (1997) J. Neurochem. 69, 1938–1944
23. Boissinot, M., Karnas, S., Lepock, J. R., Cabelli, D. E., Tainer, J. A., Getzoff, E. D., and Hallewell, R. A. (1997) EMBO J. 16, 2171–2178
24. Bartnikas, T. B., and Gitlin, J. D. (2003) J. Biol. Chem. 278, 33602–33608
25. Peters, T., Jr., Taniuchi, H., and Anfinsen, C. B., Jr. (1973) J. Biol. Chem. 248, 2447–2451
26. Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zardo, J., Todde, N., Ramponi, G., Dobson, C. M., and Stefani, M. (2002) Nature 416, 507–511
27. Kayed, R., Head, E., Thompson, J. L., McIntire, T. M., Milton, S. C., Cotman, C. W., and Glade, C. G. (2003) Science 300, 486–489
28. Kato, S., Saito, M., Hirano, A., and Ohama, E. (1999) Histol. Histopathol. 14, 973–989
29. Walsh, D. M., Hartley, D. M., Kusumoto, Y., Fezoui, Y., Condron, M. M., Lomakin, A., Benedek, G. B., Selkoe, D. J., and Teplow, D. B. (1999) J. Biol. Chem. 274, 25945–25952
30. Hartley, D. M., Walsh, D. M., Ye, C. P., Diehl, T., Vasquez, S., Vassilev, P. M., Teplow, D. B., and Selkoe, D. J. (1999) J. Neurosci. 19, 8876–8884
31. Tateno, M., Sadakata, H., Tanaka, M., Itohara, S., Shin, R. M., Miura, M., Masuda, M., Aosaki, T., Urushitani, M., Misawa, H., and Takahashi, R. (2004) Hum. Mol. Genet. 13, 2183–2196
32. Rakshit, R., Cunningham, P., Furtas-Matei, A., Dahan, S., Qi, X. F., Crow, J. P., Cashman, N. R., Kondevjevskia, L. H., and Chakrabarty, A. (2002) J. Biol. Chem. 277, 47551–47556
33. Gamblin, T. C., King, M. E., Kuret, J., Berry, R. W., and Binder, L. I. (2000) Biochemistry 39, 14203–14210
34. Necula, M., Chirita, C. N., and Kuret, J. (2003) J. Biol. Chem. 278, 46674–46680
35. Chirita, C. N., Necula, M., and Kuret, J. (2003) J. Biol. Chem. 278, 25644–25650
36. Elam, J. S., Taylor, A. B., Strange, R., Antonyuk, S., Doucette, P. A., Rodriguez, J. A., Hasnain, S. S., Hayward, L. J., Valentine, J. S., Yeates, T. O., and Hart, P. J. (2003) Nat. Struct. Biol. 10, 461–467
37. Strange, R. W., Antonyuk, S., Hough, M. A., Doucette, P. A., Rodriguez, J. A., Hart, P. J., Hayward, L. J., Valentine, J. S., and Hasnain, S. S. (2003) J. Mol. Biol. 328, 877–891
38. Caughey, B., and Lansbury, P. T. (2003) Annu. Rev. Neurosci. 26, 267–298
39. Kayed, R., Sokolov, Y., Edmonds, B., McIntire, T. M., Milton, S. C., Hall, J. E., and Glade, C. G. (2004) J. Biol. Chem. 279, 46363–46366