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Chaperone-Like Manner of Human Neuronal Tau Towards Lactate Dehydrogenase

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In our experiments, inactivation of lactate dehydrogenase (LDH, EC1.1.1.27) in the presence of human microtubule-associated tau is observably suppressed during thermal and guanidine hydrochloride (GdnHCl) denaturation. Kinetic studies show tau can prevent LDH from self-aggregation monitored by light scattering during thermal denaturation. On the other hand, neuronal tau promotes reactivation of LDH and suppresses self-aggregation of non-native LDH when GdnHCl solution is diluted. Furthermore, the reactivation yield of LDH decreases significantly with delayed addition of tau. All experiments were completed in the reducing buffer with 1 mM DTT to avoid between tau and LDH forming the covalent bonds during unfolding and refolding. Thus, Tau prevents proteins from misfolding and aggregating into insoluble, nonfunctional inclusions and assists them to refold to reach the stable native state by binding to the exposed hydrophobic patches on proteins instead of by forming or breaking covalent bonds. Additionally, tau remarkably enhances reactivation of GDH (glutamic dehydrogenase, EC 1.4.1.3), another carbohydrate metabolic enzyme, also showing a chaperone-like manner. It suggests that neuronal tau non-specifically functions a chaperone-like protein towards the enzymes of carbohydrate metabolism.

KEY WORDS: Alzheimer's disease; carbohydrate metabolism; chaperone; lactate dehydrogenase; neuronal tau.

INTRODUCTION

The mechanisms, by which protein molecules fold and attain their biologically active conformations, are of significantly fundamental interest (1). The investigation of the role of molecular chaperones in the processes has been a critical contribution to cell biology (2). Protein folding and refolding usually requires the participation and assistance of molecular chaperones (3–5). On the other hand, proteins may suffer from misfolding and aggregation in response to various stresses or insults. The recent researches show that aberrations of protein folding are responsible for a number of human nervous diseases (5). In particular, the propensity for a group of otherwise unrelated, soluble, globular proteins to aggregate and deposit extracellularly as amyloid fibrils is important because amyloidosis is a feature of neurodegeneration, for instance Alzheimer's disease (AD) (6). The AD is pathologically and diagnostically characterized by neurofibrillary tangles (NFTS) composed of tau and senile plaques of amyloid-beta (Aβ). In AD

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Abbreviations: AD, Alzheimer's disease; DTT, dithiothreitol; EDTA, ethylene diamine tetraacetic acid; GdnHCl, guanidine hydrochloride; GDH, glutamic dehydrogenase; HTAU, human neuronal tau; LDH, lactate dehydrogenase.

patient's brain, several chaperones show aberrant expression patterns including heat shock protein (HSP) 60, HSP 70 RY, heat shock cognate (HSC) 71, alpha crystallin B chain, glucose-regulated protein (GRP) 75, and GRP 94, and these patterns are compatible with neuropathological and biochemical abnormalities of patients (7–16).

Recently, a variety of results suggest that abnormalities of cerebral metabolism contribute significantly to the pathophysiology of AD (17–19). Several groups found that the activities of some metabolic enzymes, such as pyruvate kinase, lactate dehydrogenase (LDH), hexokinase, cholinesterase, acetylcholinesterase, (Na^+,K^+)-ATPase, malate dehydrogenase, acid phosphatase and glucose 6-phosphate dehydrogenase, manifest abnormalities in different degrees in AD brains (20). Among them, the expression level and the activity of LDH increase significantly in the frontal and temporal cortex, the thalamus ventrolateralis, the nucleus caudatus and the nucleus basalis of Meynert of AD brains (21), suggesting that LDH is a sensitive enzyme to neurodegeneration.

Neuronal tau, one of the major neuronal microtubule-associated proteins, promotes the assembly and stabilization of microtubules (22–24), playing important roles in neuronal morphogenesis and axonal transport (25, 26). Under some conditions, tau aggregates and forms straight or paired helical filaments (PHFs) that are the major components of NFTS, a totally inactivated form in neuropathological characteristic of AD (26–28). In this lab, it has been found that tau interacts with D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH), in a chaperone-like manner (29). In addition, tau is able to stabilize DNA double helices resulting in decrease of the melting temperature, to prevent DNA from denaturation and accelerate the renaturation (30–33). These effects of tau may be due to its flexibility and worm-like conformation, in which hydrophobic region is exposed and interacts with other proteins (34–36). Tau is such a flexible protein that it can fold and refold readily by itself, exhibiting resistance to denaturation by heat and denaturants. It is supposed that this feature makes it possible to keep its biological activity under an extreme condition and to assist some proteins folding and refolding in a chaperone-like manner. This paper is concerned with tau acting as a chaperone towards LDH, which may be related to the cellular energy metabolism concerned.

EXPERIMENTAL PROCEDURE

Materials. The clone of human neuronal tau-40 (htau-40) was a gift from Dr. Goedert (University of Cambridge, Cambridge, U.K.). Sephadex G-50, Q-Sepharose and SP-Sepharose were from Pharmacia. Rabbit muscle LDH, bovine liver glutamic dehydrogenase (GDH), Guanidine hydrochloride (GdnHCl), dithiothreitol (DTT), EDTA and insulin B chain came from Sigma. NADPH and NADH were from Roche. Other reagents used were analytical grade without further purification. Rabbit muscle LDH and bovine liver GDH showed a single band on SDS-PAGE, respectively. The specific activities of LDH and GDH were approx. 800 and 335 units, respectively. The concentration of LDH was determined spectrophotometrically, by using an A_{280} of 1.40 cm^{-1} for 1 mg/ml solution (37). The activity assay of LDH was carried out in 0.1 M phosphate buffer (pH 7.4) with a final volume of 1.0 ml, containing 0.72 mM pyruvate, 0.2 mM NADH, 1 mM DTT and 1 mM EDTA by monitoring the absorbance at 334 nm at 25°C in 60 s (37,38). The phosphate buffer (0.1 M, pH 7.4) was used throughout the experiments, except where stated otherwise. Absorbance was measured on a Hitachi VIS/UV 2010 spectrophotometer. Both light scattering and intrinsic fluorescence were measured on a Hitachi F-4500 fluorescence spectrophotometer.

Expression and purification of tau. Tau protein was expressed in *E. coli* and purified as described by Paudel (39). Tau was further purified with a Sephadex G-50 column to remove small molecules and it showed a single band on SDS-PAGE after purification. The concentration of tau was determined spectrophotometrically, by using an A_{280} of 0.27 cm^{-1} for 1 mg/ml solution.

Effect of tau on LDH denaturation by GdnHCl. LDH (0.1 μM) was incubated with tau at equal molar ratio in the phosphate buffer containing GdnHCl at desired concentrations and 1 mM DTT at 25°C for 16 h until the denaturation of the enzyme reached equilibrium. Intrinsic fluorescence was measured at 25°C (Ex = 292 nm, Em = 344 nm). An aliquot of 50 μl was taken for activity assay.

Kinetic measurements of fluorescence and activity during LDH denaturation by 0.5 M GdnHCl. Conditions were mentioned as above. Denaturation of LDH by 0.5 M GdnHCl (25°C) in the presence of tau was followed by measurements of intrinsic fluorescence and enzymic activity at different time intervals until no further changes could be observed.

Effect of tau on LDH during thermal denaturation. LDH (0.1 μM) in the presence of tau (molar ratio of tau/LDH, 4/1) was incubated in the phosphate buffer containing 1 mM DTT at the desired temperatures for 10 min (40), and then an aliquot of 50 μl was taken for activity assay.

Effect of tau on LDH aggregation monitored by light scattering. LDH (0.05 μM) was incubated with tau at different molar ratios (tau/LDH) at 80°C in the phosphate buffer (40,41) containing 1 mM DTT and 90° light scattering (Ex = Em = 500 nm, 25°C) was measured.

Effect of tau on reactivation of GdnHCl-denatured LDH. According to *Ma* and *Tsou* (38), LDH (3 μM) was incubated in 3.0 M GdnHCl at 4°C for 16 h to reach the complete denaturation, and then the denatured enzyme was reactivated by dilution (25-fold) with the phosphate buffer containing tau of different concentrations and 1 mM DTT (40–42). The mixture was kept at 4°C for 1 h after dilution, followed by measurement of the activity (an aliquot of 50 μl) at different time intervals at 25°C.

Effect of delayed addition of tau on reactivation of denatured LDH. Referred to GAPDH reactivation as described previously (29,43), effect of delayed addition of tau on reactivation of LDH was detected as follows: LDH (3 μM) was incubated in 3.0 M GdnHCl at 4°C for 16 h to reach the complete denaturation, and then the mixture was diluted (25-fold) by the phosphate buffer at 4°C containing 1 mM DTT. Tau (1.0 μM) was added to the reactivation mixture at different time intervals after dilution. The reactivation mixture was first kept at 4°C for 1 h, and then was incubated at 25°C for further 30 min before an aliquot of 50 μl was taken for activity assay at 25°C.

Effect of tau on reactivation of GdnHCl-denatured GDH. On the basis of the methods as described by *West* et al. (44) and *Bell* et al. (45), GDH (3 μM) was incubated in 2.0 M GdnHCl at 25°C for 1 h, and then the denaturated mixture was diluted in 50-fold by the phosphate buffer containing tau of different concentrations and 1 mM DTT. The diluted mixture was kept at 4°C for 1 h, and then at 25°C for 6 h, followed by measurement of activity (an aliquot of 50 μl) at 25°C. The concentration of GDH was determined spectrophotometrically, by using an A_{280} of 0.93 cm^{-1} for 1 mg/ml solution (46). Assay of GDH was performed by recording the change in A_{340} due to the production of NADH at 25°C (44–46). The standard assay mixture contained 6 mM α-ketoglutarate, 50 mM ammonium chloride (NH_4Cl), 100 μM NADPH, 1 mM DTT and 1 smM EDTA in the phosphate buffer.

RESULTS

Effect of tau on LDH denaturation by GdnHCl. Rabbit muscle LDH contains six Trp residues (47–49). In order to eliminate the Tyr disturbance, we detected intrinsic fluorescence of Trp residues of LDH at 292 nm, instead of 275 nm (41). Furthermore, neuronal tau (26) does not contain any Trp residue and its intrinsic fluorescence comes mostly from Tyr residues. The maximal emission of Trp is at 330 nm and Tyr at 305 nm. The emission intensity of Trp is much stronger than that of Tyr. The intrinsic fluorescence intensity of tau (2.0 μM) at 344 nm by excitation at 292 nm is only 0.5% of LDH (0.1 μM) under the experimental conditions (data not shown). In addition, the absorbance of tau (2 μM) at 334 nm is less than 0.04. Thus, the absorbance and fluorescence intensity of tau are in background.

Concentrated GdnHCl commonly leads to the exposure of hydrophobic regions of proteins, which can be detected through changes in intrinsic fluorescence. As shown in (Fig. 1A), activity of LDH in the presence of tau is slightly enhanced when GdnHCl concentration is less than 0.25 M. However, an inactivation is remarkable when the denaturant concentration is more than 0.25 M, accompanied with a distinct decrease in the intrinsic fluorescence intensity (Fig. 1B). It indicates that LDH exposes its hydrophobic regions in the presence of GdnHCl (< 0.25 M), and forms a kind of properly loose conformation, which observably benefits the exertion of enzymic activity. However, conformation of LDH becomes looser and inactivation occurs when the GdnHCl concentration is higher than 0.25 M. Inactivation reaches the completion in the ~0.8 M GdnHCl solution. LDH alone, as control, is slightly more susceptible to disturbance by the denaturant than LDH in the presence of tau (Fig. 1). That is to say, tau may bind to the exposed hydrophobic regions of LDH during unfolding, detectably preventing inactivation and unfolding of LDH.

Kinetic measurements of fluorescence and activity during LDH denaturation by 0.5 M GdnHCl. Neuronal tau represses inactivating kinetics of LDH in the presence of 0.5 M GdnHCl (Fig. 2A). The inactivation is a biphasic process: a fast and a slow phase. The first order rate constants of the fast and slow phases of inactivation for tau-incubated LDH are less than those of LDH alone (Table I). Simultaneously, the time course of decrease in the emission intensity of LDH also displays two phases (Fig. 2B). The first order rate constant of the fast phase for intrinsic fluorescence of LDH alone is approx. two times as much as that of tau-incubated enzyme. It is known that little changes in intrinsic fluorescence intensity of neuronal tau could be detected during GdnHCl denaturation as described previously (50,51). It suggests that tau binds to the exposed hydrophobic regions of LDH during unfolding in the reduced buffer with DTT, protecting LDH from self-aggregation of the exposed hydrophobic regions.

Effect of tau on LDH during thermal denaturation. The results mentioned above are involved in the denaturant, GdnHCl, a strong polar molecule (52) which may give rise to charge interaction with protein. Consequently, hydrophobic kernels of the protein may approach to the exterior of the molecule during conformational changes. Denaturant may change the microenvironment of aromatic residues and disturb tau interacting with LDH. Therefore, we investigated the effects of tau on both activity and conformation of LDH during thermal denaturation. Similar results are obtained when LDH is thermally denatured (Fig. 3). Inactivation of LDH is markedly observed when temperature is higher than 50°C. However, residual activity of the enzyme in the presence of tau is about 86% at 50°C, while that of LDH alone is about 66%. It suggests tau interacts with

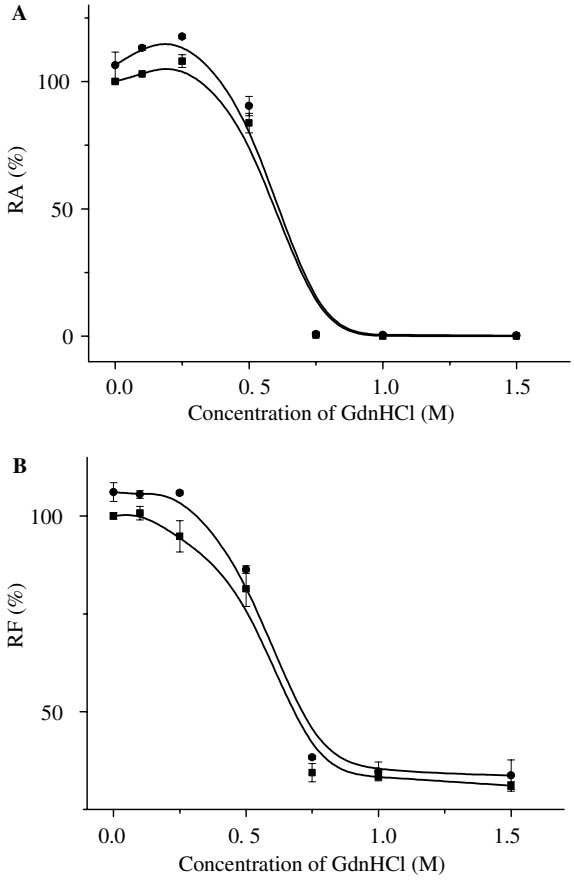

Fig. 1. Changes in activity and conformation of LDH in the presence of tau in GdnHCl solutions of different concentrations. LDH (0.1 μM) mixed with tau at equal molar ratio was incubated in the phosphate buffer containing GdnHCl at desired concentrations and 1 mM DTT at 25°C for 16 h, and then enzymic activity at 334 nm and intrinsic fluorescence at 344 nm ($E_x = 292$ nm) were measured at room temperature. RA and RF represents the relative residual activity and the relative residual intrinsic fluorescence, respectively. LDH alone (■), under the same conditions, was used as control. (A) Residual activity of LDH in the presence of tau (●) in GdnHCl solutions at different concentrations. (B) Intrinsic fluorescence intensity of LDH in the presence of tau (●) in GdnHCl solutions.

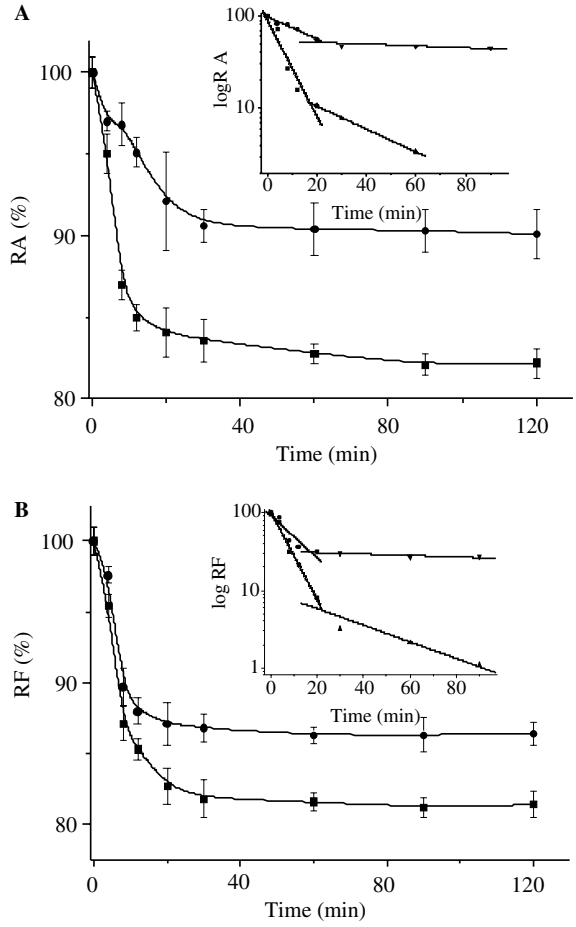

Fig. 2. Kinetics of intrinsic fluorescence and Inactivation of LDH in the presence of tau in 0.5 M GdnHCl solution. Conditions were as in Fig. 1. LDH in the presence of tau was added to 0.5 M GdnHCl solution, followed by measurement of residual activity and intrinsic fluorescence at 25°C at different time intervals. LDH alone (■), under the same conditions, was used as control. (A) Kinetics in inactivation of LDH in the presence of tau (●). The inset is the same data plotted semi-logarithmically, according to *Tsou* (53). (B) Kinetics in intrinsic fluorescence of LDH in the presence of tau (●). The inset is the same data plotted semi-logarithmically, according to *Tsou* (53).

LDH and represses the inactivation as temperature is below 60°C.

Effect of tau on LDH aggregation monitored by light scattering. We detect protein aggregation by light-scattering method since protein polymerization leads to a colloidal solution and increases in light scattering (29,43). According to Rudolph et al. (40), 80°C is a commonly used temperature to study non-native LDH aggregation. Figure 4A shows that the light scattering intensity of LDH solution rapidly increases with time and approaches to a constant value within 2 min. Addition of tau markedly reduces the intensity of light scattering LDH. The time course of the increase in light scattering of LDH is a biphasic process: a relaxation phase at the initial stage followed by a fast phase (Table I). The first-order rate constant of LDH alone is approx. two times as much as that of tau-incubated LDH. Furthermore, the maximum intensity of light scattering of tau-incubated LDH is smaller than that of LDH alone. It suggests that neuronal tau associates with LDH, suppressing the kinetics of LDH aggregation, which leads to lower the maximum scattering intensity.

Table I. The First-order Rate Constants of Activity, Conformation and Aggregation Changes During Unfolding of GdnHCl- and Thermal-denatured LDH in the Presence of TAU

	Activity [a]		Intrinsic fluorescence [a]		Light scattering [b]	
	Fast phase(s^{-1})	Slow phase(s^{-1})	Fast phase(s^{-1})	Slow phase(s^{-1})	Relaxation (s)	Fast phase(s^{-1})
LDH alone	51.9 ± 8.95	12.4 ± 0.19	56.6 ± 4.22	10.8 ± 2.69	23 ± 3	28.7 ± 0.52
LDH + tau-40	12.0 ± 1.19	0.96 ± 0.60	27.4 ± 6.38	0.91 ± 0.29	48 ± 4	16.6 ± 1.56

All constants are in 10^3. a. Denaturation in 0.5 M GdnHCl solution. b. Denaturation at 80°C.

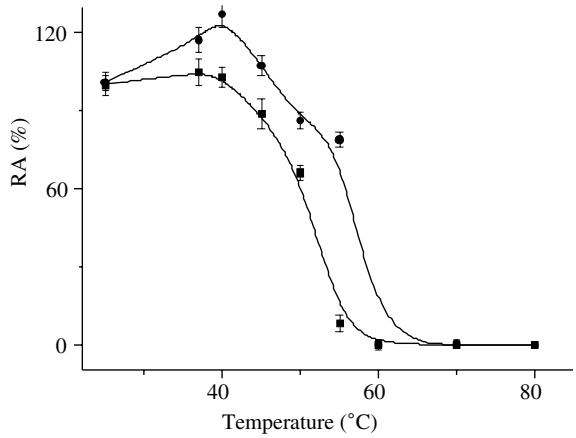

Fig. 3. Changes in residual activity of LDH in the presence of tau during thermal denaturation. LDH (0.1 μM) in the presence of tau (●) (molar ratio, protein: LDH = 4:1) was incubated in the 0.1 M phosphate buffer (pH 7.4) containing 1 mM DTT at the desired temperatures for 10 min, followed by measurement of residual activity at 25°C. LDH alone (■), under the same conditions, was used as control. RA represents the relative residual activity.

Figure 4B shows the changes in light scattering of LDH in the presence of neuronal tau at different concentrations at 80°C. Aggregation of LDH is markedly suppressed with increasing of tau concentration. That is to say, during thermal denaturation, the interaction of tau with the exposed hydrophobic regions of LDH reduces the possibility of self-aggregation. The relationship between the light scattering intensity and the molar ratio of tau/LDH ($0 <$ the ratio ≤ 4) is approximately linear. A little of suppression can be detected when the molar ratio further increases (the ratio > 4). The interaction of tau with LDH has almost reached the saturation when the molar ratio (tau/LDH) is approx. 4. It appears that four molecules of tau bind to one molecule of LDH tetramer by an equal possibility to interact with the four subunits under the thermal conditions.

Effect of tau on reactivation of LDH. Dilution of GdnHCl-denatured LDH (0.1 μM) solution in the presence of tau improves the reactivation yield (Fig. 5A). The reactivation approaches to a constant value in 20 min after tau is added. Furthermore, the yield of reactivation increases when tau concentration increases, though the relationship is not linear (Fig. 5B). It supports that tau is involved in a chaperone-like manner toward LDH. On the other hand, the first-order rate constants (fast and slow phases) of enzymic self-reactivation are slightly higher, but the self-reactivation yield is only 25% (Table II). For tau-incubated LDH, the reactivation yield is higher although the first-order rate constants of the two phases are relatively lower. For a putative explanation, it takes time for tau to associate with LDH and to induce the enzyme molecules to convert into the native conformation.

Effect of delayed addition of tau on LDH reactivation. The reactivation yield of LDH decreases significantly with delayed addition of tau (Fig. 6). Neuronal tau becomes not available to reactivate LDH when it is added over 40 min after dilution. The result shows that tau can promote the refolding of denatured LDH to the active state by the interaction with non-native LDH, but not the enzyme that has been self-aggregated. It supports strongly again that neuronal tau acts in a chaperone-like manner towards LDH *in vitro*.

Effect of human neuronal tau on reactivation of GdnHCl-denatured GDH. In order to investigate whether tau specifically acts in a chaperone-like function towards LDH, we detected the effect of tau on reactivation of GdnHCl-denatured GDH. According to the result (Fig. 7), tau is also able to promote the reactivation yield of GdnHCl-denatured GDH from 21% to 45% with increasing in tau concentration. GDH alone as control, the yield of self-reactivation is only 21%. It appears that tau acts in chaperone-like manner towards GDH. It is possible for human neuronal tau to function in a chaperone manner towards enzymes of carbohydrate metabolism non-specifically.

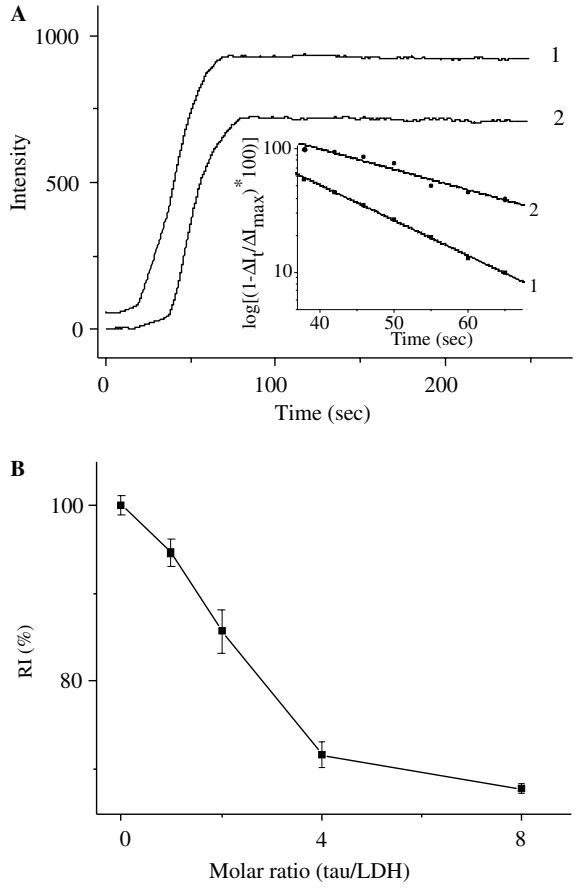

Fig. 4. Light scattering change of LDH in the presence of tau during thermal denaturation. LDH (50 nM) in the presence of tau in the 0.1 M phosphate buffer (pH 7.4) containing 1 mM DTT was incubated at 80°C for 100 s, followed by monitoring the light scattering at 25°C at 500 nm. (A) Dynamic light scattering of LDH alone (50 nM, curve 1) and LDH in the presence of tau (200 nM, curve 2), respectively. $\triangle I_t$ is the light scattering change at any time and $\triangle I_{max}$ is the light scattering change when the reaction reached the completion. The inset is the same data plotted semi-logarithmically in $[(1\text{-}\triangle I_t/\triangle I_{max})*100]$. (B) The maximum light scattering intensities of LDH in the presence of tau at the different molar ratio. The maximum of light scattering of LDH alone, under the same conditions, was as 100%. RI represents the relative intensity of light scattering.

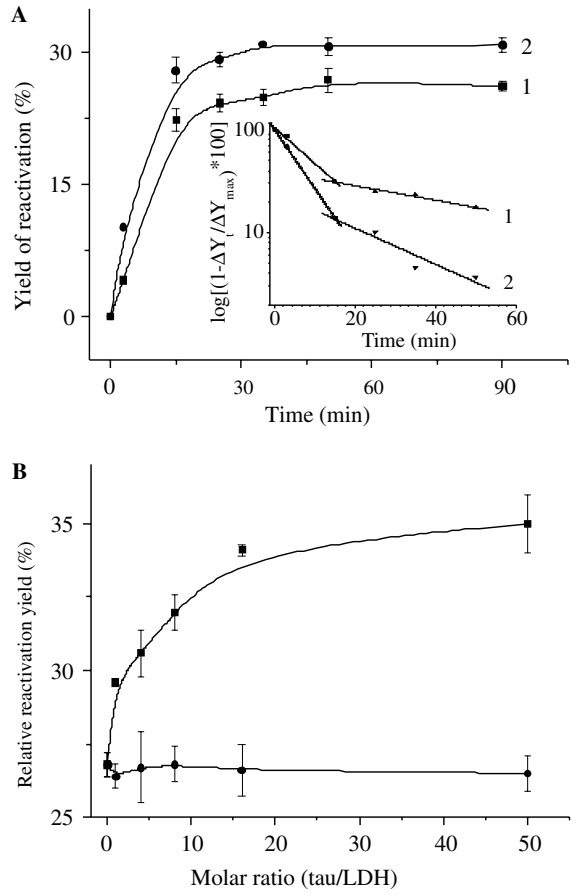

Fig. 5. Effect of tau on reactivation of LDH. LDH (final concentration, 3 μM) was denatured by 3.0 M GdnHCl for 16 h at 4°C, reactivated by diluting (25-fold) the denaturant solution with the 0.1 M phosphate buffer (pH 7.4) containing different molar ratios (tau/LDH) and 1 mM DTT at 4°C for one hour, followed by measurement of reactivation yield at 334 nm at 25°C. (A) The time courses of reactivation of LDH in the presence of tau (curve 2) (molar ratio of tau/LDH, 4:1). LDH alone (curve 1), under the same conditions, was used as control. $\triangle Y_t$ is the change of reactivation at any time and $\triangle Y_{max}$ is the change of reactivation when the reactivation in the presence of tau reaches the completion. The inset is the same data plotted semi-logarithmically in $[(1\text{-}\triangle Y_t/\triangle Y_{max})*100]$. (B) Effects of tau (■) and insulin B chain (●) of different concentrations on reactivation of GdnHCl-denatured LDH, respectively. The activity of native LDH alone, under the same conditions, was used as 100%.

DISCUSSION

Ellis (54) and Jakob et al. (55) have described the characteristics of molecular chaperone exhibiting *in vitro*: (i) the ability to protect protein from aggregation during protein unfolding under stress conditions; (ii) the ability to suppress aggregation during protein refolding from a denatured state; and (iii) the recovery of biological activity. Chaperones prevent proteins from misfolding and aggregating into insoluble, non-functional inclusions and assist them to refold to reach the stable native state by binding to the exposed hydrophobic patches on proteins instead of by forming new or breaking old covalent bonds. As the results in the text, neuronal tau binds to the denatured LDH in the reduced buffer with superfluous DTT, suppressing LDH inactivation and promoting the enzyme reactivation, which is consistent with the characteristics of a chaperone. It

Table II. The First-order Rate Constants of LDH Reactivation in the Presence of TAU

	Fast phase (s^{-1})	Slow phase (s^{-1})
LDH alone	57.3 ± 0.62	17.6 ± 3.8
LSDH + tau-40	34.7 ± 2.90	7.02 ± 0.77

All constants are in 10^3.

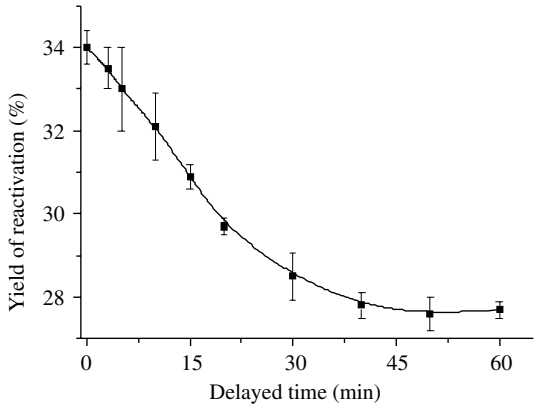

Fig. 6. Effect of delayed addition of tau on reactivation of LDH. Conditions were as in Fig. 5. Add tau (1.0 μM) at different time intervals after dilution the denatured enzyme solutions by the 0.1 M phosphate buffer (pH 7.4) containing 1 mM DTT. The reactivation mixture was first kept at 4°C for one hour, and then for a further 30-min period at 25°C before an aliquot of 50 μl was taken for activity assay.

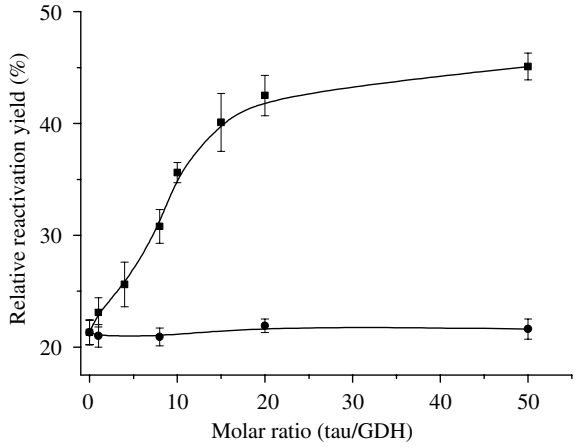

Fig. 7. Effect of human neuronal tau on reactivation of GdnHCl-denatured GDH. GDH (3 μM) was incubated in 2.0 M GdnHCl at 25°C for 1 h, and then 50-fold dilute the denaturation mixture by the phosphate buffer containing tau (■) at different concentrations and 1 mM DTT. The mixture was kept at 4°C for 1 h after dilution, and then kept at 25°C for 6 h followed by measurement of the activity (an aliquot of 50 μl) at 25°C. The activity of native GDH alone, under the same conditions, was used as 100%. Insulin B chain (●), under the same conditions, was used as control.

suggests tau has chaperone-like function towards LDH *in vitro*.

Tau slightly represses inactivation of LDH in GdnHCl solutions and enhances reactivation when the denaturant is diluted. It is supposed that tau could not be completely inactivated under the denatured conditions and should own the biological function when neuronal tau exerts the protective effect in a chaperone-like manner towards LDH. There are several observations supporting the idea. First, tau is resistant to both heat and acid treatment, still keeping its ability to promote microtubule assembly (34,35,55). Secondly, intrinsic fluorescence of tau at 305 nm (from Tyr residues) does not change markedly when GdnHCl concentration is less than 1.0 M (29). Third, it is negligible that changes in the intensity of light scattering of tau alone at 80°C for 10 min. Furthermore, according to the review of Dyson et al. (56), there are now numerous examples of proteins that are unstructured or only partially structured under physiological conditions and yet are nevertheless functional, such as chaperone function. Indeed, such intrinsically unstructured proteins appear to own a unique region of 'charge-hydrophobicity space' (58) and is inherent flexibility, which allows their local and global structure to be modified in response to different molecular targets. Tau is an intrinsically unstructured protein under physiological conditions owning worm-like conformation and a little secondary structure (59, 60). According to Uversky et al. (58), tau contains +2 charges with a hydrophobicity of 175.8 in total (mean, 0.4). The non-specifical chaperone effects of tau on both LDH and GDH may be just due to its flexible charge-hydrophobicity region, which is exposed to interact with other proteins. It is possible for human neuronal tau to function as a chaperone protein towards enzymes of carbohydrate metabolism non-specifically.

A number of available studies on various components of cerebral glucose metabolism *in vitro* have reviewed a reduced activity of different enzymes of glucose and energy metabolism consistent with the notion that the impaired glucose utilization in AD might influence the neurodegenerative loss of neurons and synaptic connections or a potential down regulation of gene expression for oxidative phosphorylation within neuronal mitochondria (17–19). Furthermore, activity and expression of LDH

increases significantly in several parts of brain, such as frontal and temporal cortex, the nucleus caudatus, thalamus ventrolateralis and the nucleus basalis of Meynert of AD brains (20,21). LDH, as an important enzyme in carbohydrate metabolism, catalyzes pyruvate to produce lactate (61). In AD patients, brain glucose metabolic ratio, especially oxidative phosphorylation decreases whereas the brain lactate metabolic ratio increases. According to the investigation of possible synaptic dysfunction in AD by Sakurai et al. the effects of exogenous glucose deprivation and replacement of glucose with lactate on the synaptic transmission, synaptic plasticity, and the morphological integrity of hippocampal neurons were determined. Their results indicate that lactate can sustain the neural transmission and support the morphological integrity of hippocampal neurons, but fail to induce long-term potentiation, which could at least in part, cause the memory impairment in AD (62). Additionally, lactate resulting from astrocytic degradation of glucose or glycogen may serve as a supplementary energy source for activated neurons (63). A number of mechanisms have been described that activated neurons are allowed to recruit additional fuel from astrocytes for energy production if the supply of glucose is insufficient (64,65). The result strongly supports the viewpoint of Bigl et al. that increased LDH activity observed in brains of AD patients may contribute to an increased lactate production in activated astrocytes and reflect an adaptive process to the reduced neuronal glucose consumption (20). Recently, tau protein is detected in neuronal cell bodies, proximal dendrites, gray matter neuropil, axons, glial cells in normal human hippocampus and neocortex (66). Tapiola et al. (67) measured tau concentrations in cerebrospinal fluid (CSF) samples taken during the lifetime of 43 patients with AD. The CSF tau values show a positive correlation with neocortical NFT scores ($r = 0.44$, $p < 0.005$), while glial fibrillary acid protein immunoreactivity does not correlate with CSF tau. Their study reveals a high variation in CSF tau values in patients with neuropathologically confirmed AD (range 194–1539 pg/ml) and indicates that high CSF tau values in the late phase of AD predict severe neurodegeneration as evidenced by increased NFT score. In addition, tau immunoreactivity has been observed in astrocytes in AD patients (68,69). Experiments on muscle model also show that expression of tau increases in astrocytes of transgenic mice under some conditions (70). Our experiments *in vitro* show that tau represses inactivation of LDH in GdnHCl solutions and enhances reactivation towards LDH non-specifically. It indicates tau might be involved in neuronal metabolic pathways. However, whether tau interacts with the energetic enzymes through a chaperone-like manner *in vivo* needs further investigating.

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