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Abstract

Filamentous inclusions composed of the microtubule-associated protein tau are a defining characteristic of a large number of neurodegenerative diseases. Here we show that tau degradation in stably transfected and non-transfected SH-SY5Y cells is blocked by the irreversible proteasome inhibitor lactacystin. Further, we find that in vitro, natively unfolded tau can be directly processed by the 20S proteasome without a requirement for ubiquitylation, and that a highly reproducible pattern of degradation intermediates is readily detectable during this process. Analysis of these intermediates shows that 20S proteasomal processing of tau is bi-directional, proceeding from both N- and C-termini, and that populations of relatively stable intermediates arise probably because of less efficient digestion of the C-terminal repeat region. Our results are consistent with an in vivo role for the proteasome in tau degradation and support the existence of ubiquitin-independent pathways for the proteasomal degradation of unfolded proteins.

Keywords: Alzheimer's disease, paired helical filament, proteasome, tau, ubiquitin.

J. Neurochem. (2002) 83, 176–185.

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In vitro ubiquitin-independent degradation of unfolded tau by the 20S proteasome

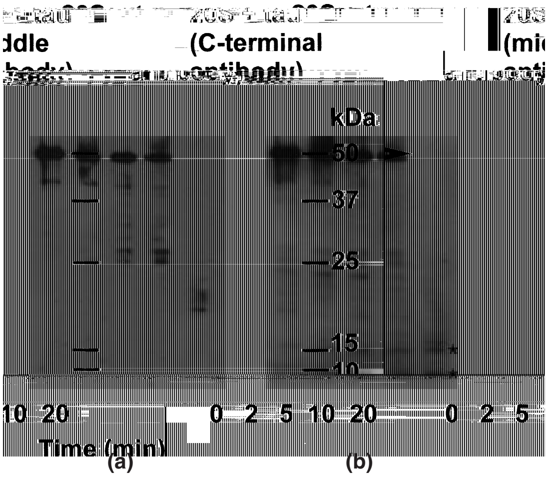

Fig. 7 In vitro 20S proteasomal degradation of tau43 gives rise to N-terminally truncated intermediates. Tau43 (275 nM) was incubated with 20S proteasome (14 nM) for the indicated times and tau degradation was detected by Western blotting with (a) TAU-5 antibody. The blot was then stripped and re-probed with (b) BR134 antibody. The arrowed band represents residual full-length tau The asterisks highlight species of apparent molecular mass 15.2 and 10.3 kDa, which contain only the C-terminal tau epitope and therefore represent N-terminally truncated tau.

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Discussion

Degradation of cellular tau by the proteasome

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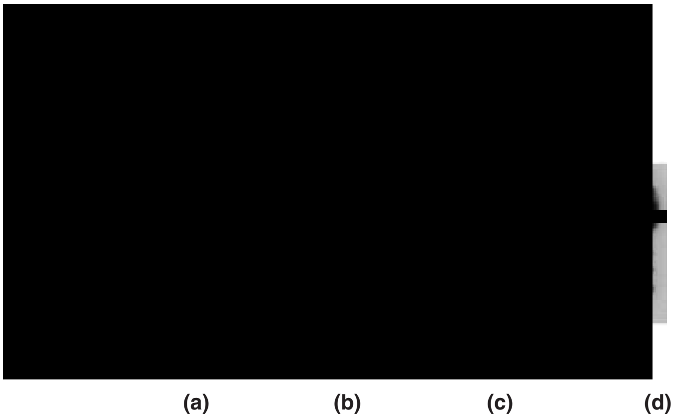
Fig. 8 Bi-directional in vitro 20S proteasomal degradation of tau46. Tau46 (275 nM) was incubated with 20S proteasome (14 nM) for the indicated times and tau degradation was detected by Western blotting, probing replicate membranes with (a) 5A6 (b) BR134 and (c) TAU-5 antibodies. C-terminally and N-terminally truncated intermediates are noted in panels (a) and (b), respectively. Boxed species in panel (c) are enlarged in (d) and represent proteasomal degradation products separated by 1Ð2 kDa lacking both N- and C-termini.



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Unfolded tau can be directly degraded by the 20S proteasome *in vitro* in a ubiquitin-independent manner

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Mechanism of tau degradation by the 20S proteasome *in vitro*

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