

乙醛对人类神经 tau 磷酸化的影响*

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摘要 用乙醛对人类神经 tau 进行醛胺化, 通过 NCLK (neuronal cdc2-like protein kinase) 和 $[\gamma\text{-}^{32}\text{P}]$ ATP 对其磷酸化. 磷酸化的产物经胃蛋白酶降解及 HPLC (C-18) 分析降解片段, 发现醛胺化 tau 的降解物中有两个新的磷酸化肽段 (A4 和 A6).

关键词 tau, 磷酸化, 乙醛, 老年性痴呆

学科分类号 Q344

人类神经 tau 蛋白具有稳定细胞微管系统、调控神经细胞生长发育的功能^[1], 并在神经系统的形成和轴突的通讯传导中起着至关重要的作用^[2]. tau 是一种磷酸化蛋白, 其功能主要通过磷酸化调节. 超磷酸化可导致 tau 蛋白分子的聚集, 形成配对螺旋样纤维^[3], 这是老年性痴呆在病理学上的基本病变之一. 研究表明, NCLK 可以催化 tau 蛋白的磷酸化, 在体外能引起 tau 分子聚集^[4].

最近, Cullen 和 Halliday^[5]在慢性酒精中毒导致的痴呆病人脑中发现了配对螺旋样纤维的存在. 我们观察到乙醇和乙醛在体外较低浓度下能导致 tau 分子聚集^[6,7]. 乙醇在体内被乙醇脱氢酶转变为乙醛, 后者是毒性较强的分子, 导致大脑等器官的损伤. 因此我们研究了乙醛对 tau 蛋白磷酸化的影响.

1 材料与方法

1.1 实验材料

分离纯化基因重组人类神经 tau (htau-40) 所用 Q-Sepharose 及 SP-Sepharose 树脂购于 Pharmacia 公司. $[\gamma\text{-}^{32}\text{P}]$ ATP 和肝素分别购于 DuPont 和 Sigma 公司. 人类神经 tau 的全基因克隆由英国剑桥大学 Goedert 提供^[8], 所表达出的蛋白质与天然序列一致, 宿主菌为 *E. coli* BL21-DE3. 通过摇瓶培养、匀浆、离心 (12 000 r/min, 4°C, 10 min), 上清液过 Q-Sepharose、SP-Sepharose 及 Sephadex G50, 收集蛋白质. 其他试剂均为分析纯级.

1.2 tau 蛋白的醛胺化

取 0.5 mg tau, 溶于 0.1 mol/L 磷酸缓冲液

(pH 7.0), 加入 0.05% 乙醛, 25°C 过夜. 过 Sephadex G25 分子筛柱 (1 cm×30 cm), 收集蛋白质 (280 nm), 冷冻干燥备用.

1.3 tau 蛋白的磷酸化

NCLK 的分离纯化见参考文献 [9]. 取 0.5 mg tau 蛋白, 溶于 500 μl 50 mmol/L HEPES 缓冲液 (15 mmol/L MgCl_2 ; 1.0 mmol/L DTT; 1.0 mmol/L PMSF; 0.5 mmol/L $[\gamma\text{-}^{32}\text{P}]$ ATP; lupeptin 和 pepstatin 各 0.5 μl , pH 7.2), 加入 100 U NCLK, 30°C 保温过夜. 用 SDS-聚丙烯酰胺凝胶电泳 (PAGE) 检验磷酸化的效果^[10]. 按所需浓度将肝素 (200 mg/L) 加入反应体系. 10% 聚丙烯酰胺凝胶电泳后抽干用于放射自显影.

1.4 放射性计数

将磷酸化样品 (2 μl) 置于滤纸上用闪烁计数器进行放射性计数.

2 结果与讨论

2.1 人类神经 tau 蛋白的醛胺化

用不同浓度的乙醛与人类神经 tau-40 保温, 醛胺化 tau 在 SDS-PAGE 上的迁移率变慢 (图 1), 并且随乙醛浓度的增加而变得明显. tau 含有 44 个 Lys 残基, 其正电荷对于稳定分子的天然构象有重要作用^[11], 醛基能与 N 端 α -及 Lys ϵ -氨基反应,

*国家自然科学基金 (39970236) 及国家重点基础研究发展规划项目 (G1999054007).

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收稿日期: 2001-04-29, 接受日期: 2001-07-05

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Effect of Acetaldehyde on Phosphorylation of Human Neuronal tau*

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Abstract Human neuronal tau-40 was acetylated and then phosphorylated by neuronal cdc2-like kinase (NCLK) with [γ -³²P] ATP, and then the phosphorylated acetyl-tau (PAtau) was digested with pepsin. Compared with the phosphorylated tau (Ptau), two new fractions of radioactivity from PAtau were obtained as it was eluted by C-18 column on HPLC.

Key words Tau, phosphorylation, acetaldehyde, Alzheimer's disease

* This work was supported by grants from the National Natural Foundation of Sciences (39970236) and National Key Foundation of Development and Research (G1999054007).

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Received: April 29, 2001 Accepted: July 5, 2001

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Received: April 29, 2001 Accepted: July 5, 2001