A CALMODULIN-DEPENDENT PROTEIN KINASE THAT IS INVOLVED IN THE ACTIVATION OF TRYPTOPHAN 5-MONOOXYGENASE IS SPECIFICALLY DISTRIBUTED IN BRAIN TISSUES

Takashi YAMAUCHI and Hitoshi FUJISAWA
Department of Biochemistry, Asahikawa Medical College, Asahikawa 078-11, Japan

Received 13 May 1981

1. Introduction

Calmodulin is involved in regulating a variety of cellular enzyme systems as an important mediator of Ca^{2+} functions in eukaryotes [1,2]. The functioning of the nervous system is closely related to the metabolism of Ca^{2+}. In [3], 3 distinct calmodulin-dependent protein kinases were demonstrated in rat brain cytosol. Two of them appeared to be similar to phosphorylase kinase and myosin light chain kinase from various tissues, respectively, but the other, kinase II, which was involved in the activation of tryptophan 5-monooxygenase, was a new calmodulin-dependent protein kinase. Here, kinase II is demonstrated to be specifically distributed in brain tissues.

2. Materials and methods

[γ-^{32}P]ATP (2000 Ci/mmol) was purchased from Radiochemical Centre, Amersham. Calmodulin-dependent protein kinase (kinase II) was prepared from rat cerebral cortex extracts as in [3]. Tryptophan 5-monooxygenase was prepared from rat brainstem extracts as in [3]. The light chain from chicken gizzard myosin was prepared essentially as in [4]. Rat brain calmodulin was prepared as in [5].

Tissues from adult male Wistar rats were homogenized in 4 vol. 0.32 M sucrose containing 10 mM Tris–HCl (pH 7.6), 0.01 mM EDTA, 1 mM DTT, and 1 mM phenylmethylsulfonyl fluoride with a Potter-Elvehjem homogenizer. The supernatant fluids which were obtained from the homogenates by centrifugation at 105 000 × g for 1 h were passed through a Bio-Gel P-10 column in order to remove low M_r substances and were used as the source of the soluble enzymes.

Subcellular fractionation of rat cerebral cortex were done as in [6]. Freshly dissected cerebral cortex from rat brain (~2 g) was homogenized in 18 ml of 0.32 M sucrose and the homogenate was subjected to subcellular fractionation.

Kinase II was assayed on the basis of its ability to activate tryptophan 5-monooxygenase [7]. The standard incubation mixture contained 50 mM Hepes buffer (pH 7.0), 1 mM ATP, 5 mM $Mg(CH_3COO)_2$, 0.12 mM $CaCl_2$, 0.1 mM EGTA, 40 mM NaF, 5 μg calmodulin, 0.4 mM tryptophan, 0.3 mM 6-MPH_4, 0.05 mM $Fe(NH_4)_2(SO_4)_2$, 2 mM DTT, 50 μg catalase, ~0.05 unit tryptophan 5-monooxygenase, and a suitable amount of the protein kinase in 0.4 ml final vol. After incubation at 30°C for 20 min with shaking, 5-hydroxytryptophan was determined fluorometrically. The activation of tryptophan 5-monooxygenase showed a linear dependence on added kinase II up to 1.8-fold activation (fig.1). Therefore, kinase II was assayed under these conditions. One unit of activity is defined as 1 incremental nmol 5-hydroxytryptophan produced under standard conditions over controls without ATP.

Myosin light chain kinase was assayed essentially as in [8] using light chain from chicken gizzard myosin as a substrate. Tryptophan 5-monooxygenase was assayed essentially as in [9]. Tyrosine 3-monooxygenase was assayed fluorometrically [10]. Monoamine

Abbreviations: DTT, dithiothreitol; 6-MPH_4, 2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

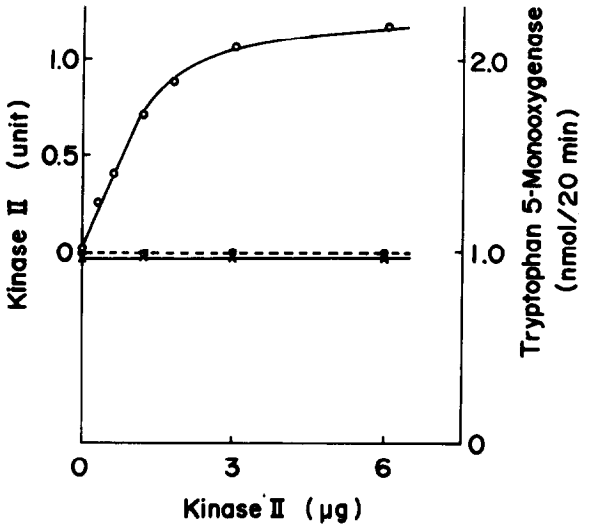

Fig.1. Effect of varying the concentration of kinase II on the activation of tryptophan 5-monooxygenase. Tryptophan 5-monooxygenase (227 μg protein) was incubated with different amounts of kinase II under the standard assay condition (○), or in the absence of calmodulin (●) or ATP (×). After incubation for 20 min, 5-hydroxytryptophan was determined as in section 2.

oxidase activity was measured using benzylamine as a substrate [11]. Acetylcholine esterase was assayed as in [12]. 2′,3′-Cyclic nucleotide phosphohydrolase, a marker enzyme of myelin fraction, was assayed spectrophotometrically as in [13]. Lactate dehydrogenase was assayed as in [14]. DNA was determined as in [15]. Protein was determined as in [16] with bovine serum albumin as a standard.

3. Results and discussion

Table 1 shows the distribution of kinase II, compared with those of myosin light chain kinase, tryptophan 5-monooxygenase, and tyrosine 3-monooxygenase, in cytosol fractions of a number of rat tissues. In contrast to widespread occurrence of myosin light chain kinase in various tissues, kinase II appeared to be almost specifically distributed in brain tissues. Kinase II was demonstrated to be involved in the activation of tryptophan 5-monooxygenase [3,7] and tyrosine 3-monooxygenase [17,18]. While only brainstem in nervous system showed the high activities of both monooxygenases, the other parts of brain including cerebral cortex and cerebellum also showed the high level of kinase II, indicating that kinase II may play roles not only in the regulation of serotonin or catecholamine biosynthesis but also in other functions in nervous system, in accord with the observation [3] that kinase II showed a broad substrate specificity with respect to endogenous protein substrates in brain cytosol.

Table 2 shows the subcellular distribution of kinase II in rat cerebral cortex. The soluble fraction showed the highest relative specific activity of kinase II and contained ~43% of the total activity. On the other hand, the microsomal fraction also showed a relatively high specific activity and the crude mitochondrial fraction contained almost 30% of the total activity. Osmotic shock treatment of both crude mitochondrial and microsomal fractions did not release significant kinase II activity, indicating that

Table 1
Activities of kinase II, myosin light chain kinase, tryptophan 5-monooxygenase and tyrosine 3-monooxygenase in various tissues of rat

Tissues	Kinase II (units/mg protein)	Myosin light chain kinase	Tryptophan 5-monooxygenase	Tyrosine 3-monooxygenase
			(nmol . mg protein^{-1} . min^{-1})	
Cerebral cortex	10.8	0.59	0.008	0.014
Brain-stem	9.8	0.49	0.077	0.050
Cerebellum	7.4	1.13	0	0.002
Adrenal gland	0.4 ± 0.4	0.34	0.011	1.57
Lung	0.4 ± 0.3	0.38	0	0.001
Kidney	0.3 ± 0.2	0.03	0.011	0.001
Testis	0.3 ± 0.3	0.09	0	0
Spleen	0	1.35	0	0.008
Skeletal muscle	0	0.66	0	0
Liver	0	0.10	0.015	0.001
Heart	0	0.01	0	0.002

Table 2
Distribution of kinase II in subcellular fractions of rat cerebral cortex

Fraction	Protein (mg)	Kinase II	DNA	Monoamine oxidase	Acetyl-choline esterase	2',3'-cyclic nucleotide phospho-hydrolase	Lactate dehydro-genase
				(Relative specific activity)			
Nuclei	28	0.60	3.68	1.57	1.00	1.04	0.29
Crude mitochondrial	103	0.56	0.79	1.35	1.23	1.16	0.33
Microsomal	24	1.37	0.67	0.83	1.66	1.45	0.25
Soluble	46	1.89	0	0	0.26	0.38	3.30

Kinase II was assayed under the standard conditions in the presence of tryptophan 5-monooxygenase (327 μg protein), except that 0.2 mM ouabain was added to the mixture. Markers of subcellular fractions were determined as in section 2

kinase II of both fractions might be associated with the membrane fraction. Thus, kinase II appeared to be present in both cytosol and membrane fractions in brain tissues. When further subfractionation of crude mitochondrial fraction was done as in [6], 13% of the activity of kinase II was observed in myelin fraction, 14% in synaptosomal fraction, and 3% in mitochondrial fraction.

A phospholipid-sensitive Ca^{2+}-dependent protein kinase was reported in [19] and the phosphorylation of cytosol proteins in brain tissues through the action of the kinase in [20]. Kinase II absolutely required calmodulin for the activation of tryptophan 5-monooxygenase and calmodulin could not be replaced by phosphatidylserine, indicating that both kinases should be distinguished.

Acknowledgements

This work has been supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture of Japan and by grants from the Naito Foundation Research Grant and the Chiyoda-Seimei Foundation for 1980.

References

[1] Cheung, W. Y. (1980) Science 207, 19–27.
[2] Means, A. R. and Dedman, J. R. (1980) Nature 285, 73–77.
[3] Yamauchi, T. and Fujisawa, H. (1980) FEBS Lett. 116, 141–144.
[4] Perrie, W. T. and Perry, S. V. (1970) Biochem. J. 119, 31–38.
[5] Wang, J. H. and Desai, R. (1977) J. Biol. Chem. 252, 4175–4184.
[6] Whittaker, V. P. and Barker, L. A. (1972) Methods Neurochem. 2, 1–52.
[7] Yamauchi, T. and Fujisawa, H. (1979) Biochem. Biophys. Res. Commun. 90, 28–35.
[8] Hathaway, D. R. and Adelstein, R. S. (1979) Proc. Natl. Acad. Sci. USA 76, 1653–1657.
[9] Friedman, P. A., Kappelman, A. H. and Kaufman, S. (1972) J. Biol. Chem. 247, 4165–4173.
[10] Yamauchi, T. and Fujisawa, H. (1978) Anal. Biochem. 89, 143–150.
[11] McEwen, C. M. jr (1971) Methods Enzymol. 17B, 686–692.
[12] Ellman, G. L., Courtney, K. D., Andres, V. jr and Featherstone, R. M. (1961) Biochem. Pharmacol. 7, 88–95.
[13] Kurihara, T. and Takahashi, Y. (1973) J. Neurochem. 20, 719–727.
[14] Pesce, A., MaKay, R. H., Stolzenbach, F., Cahn, R. D. and Kaplan, N. O. (1964) J. Biol. Chem. 239, 1753–1761.
[15] Schneider, W. C. (1957) Methods Enzymol. 3, 680–684.
[16] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[17] Yamauchi, T. and Fujisawa, H. (1980) Biochem. Intl. 1, 98–104.
[18] Yamauchi, T., Nakata, H. and Fujisawa, H. (1981) J. Biol. Chem. in press.
[19] Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T. and Nishizuka, Y. (1979) J. Biol. Chem. 254, 3692–3695.
[20] Wrenn, R. W., Katoh, N., Wise, B. C. and Kuo, J. F. (1980) J. Biol. Chem. 255, 12042–12046.