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Brain 14-3-3 protein is an activator protein that activates tryptophan 5-monooxygenase and tyrosine 3-monooxygenase in the presence of Ca^{2+},calmodulin-dependent protein kinase II

Tohru Ichimura, Toshiaki Isobe, Tsueno Okuyama, Takashi Yamauchi[+] and Hitoshi Fujisawa[+]

Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, Setagaya, Tokyo 158 and [+]*Department of Biochemistry, Asahikawa Medical College, Asahikawa 078-11, Japan*

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We have found that the 14-3-3 protein, an acidic neuronal protein, is substantially identical to the 'activator' protein [(1981) J. Biol. Chem. 256, 5404–5409] that activates tryptophan 5-monooxygenase and tyrosine 3-monooxygenase in the presence of Ca^{2+},calmodulin dependent protein kinase II. This finding is based on the remarkable similarity of both these proteins in physicochemical, biochemical and immunochemical properties, as well as on detection for the 14-3-3 protein of an activator activity towards tryptophan 5-monooxygenase. The result suggests that the 14-3-3 protein plays a role in the regulation of serotonin and noradrenaline biosynthesis in brain.

14-3-3 protein; Activator protein; Tryptophan 5-monooxygenase; Tyrosine 3-monooxygenase; Ca^{2+}/calmodulin-dependent protein kinase; (Brain)

1. INTRODUCTION

The 14-3-3 protein is a soluble acidic protein originally designated by Moore and Perez [1]. This protein is distributed abundantly in brain tissue (about 1% of brain soluble proteins), while it is also found in other tissues such as testis and intestine at lower concentrations [2]. Within brain tissue, the protein is localized specifically in neurons [3], and is subject to axonal transport in the retinal ganglion cells [4]. Biochemical studies showed that the 14-3-3 protein had a molecular mass of 50 kDa [4] or 67 kDa [3] consisting of two dissimilar 25 kDa subunits [5] or 29 and 32 kDa subunits [3]. It has been shown that the 14-3-3 protein isolated from human [3] and bovine [5] brain is heterogeneous in containing two closely related molecular species having different subunit composition [3]. However, the functional significance of this neuronal protein is unknown.

As part of our program of systematic studies on acidic brain proteins [6–10], we purified the 14-3-3 protein from bovine brain and characterized it by amino acid analysis and peptide mapping [11]. In the course of this study we noticed that the amino acid composition of the 14-3-3 protein is remarkably similar to the published composition of the activator protein [12] that has previously been isolated as a protein factor which activates tryptophan 5-monooxygenase and tyrosine 3-monooxygenase in the presence of Ca^{2+}, calmodulin-dependent protein kinase II (kinase II). Further biochemical and immunochemical studies have provided evidence that this neuronal protein is in fact identical to the activator protein, sug-

Correspondence address: T. Isobe, Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, Fukasawa 2-1-1, Setagaya, Tokyo 158, Japan

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gesting a role for the 14-3-3 protein in the regulation of catecholamine and indolamine synthesis in neurons and other non-neuronal cells.

2. MATERIALS AND METHODS

The bovine brain 14-3-3 protein was prepared by a procedure similar to that described for the purification of neuron specific enolase [9]. In a final purification step on DEAE-Sephadex A-50, the 14-3-3 protein was eluted following the enolase as noted in previous papers [3,5]. An antiserum against the 14-3-3 protein was produced in BALB/c mice by serial injections of the purified antigen (50 μg/50 μl) mixed with an equal volume of Freund's complete adjuvant. A preliminary report on the purification and characterization of purified 14-3-3 protein has been presented [11], details will be published elsewhere. Rat brain activator protein was purified as in [12]. Tryptophan 5-monooxygenase (EC 1.14.16.4) was prepared from rat brain stem as in [12]. Kinase II was purified from rat cerebral cortex as in [13].

Activator protein was assayed on the basis of its ability to activate tryptophan 5-monooxygenase in the presence of excess amounts of kinase II [12]. SDS-polyacrylamide (10%) gel electrophoresis in micro slab gels and subsequent immunoblot analysis were carried out as in [14]. The amino acid composition was determined as in [8] after hydrolysis of protein samples with 6 M HCl in evacuated, sealed tubes.

3. RESULTS

The 14-3-3 protein, isolated from bovine brain, showed molecular characteristics very similar to those described in previous papers [3–5]; i.e. an acidic isoelectric point (4.7–5.3), a molecular mass estimated in the absence of denaturating agents (55 kDa), subunit composition (two distinct polypeptides in SDS-polyacrylamide gel electrophoresis; see fig.1), amino acid composition (table 1), and a blocked N-terminus. The rather wide range of p*I* values of the 14-3-3 protein may reflect the reported heterogeneity of this protein [3]. The rat brain activator protein, on the other hand, was characterized in [12]. It was shown to be an acidic, dimeric protein (70 kDa) composed of two 35 kDa subunits.

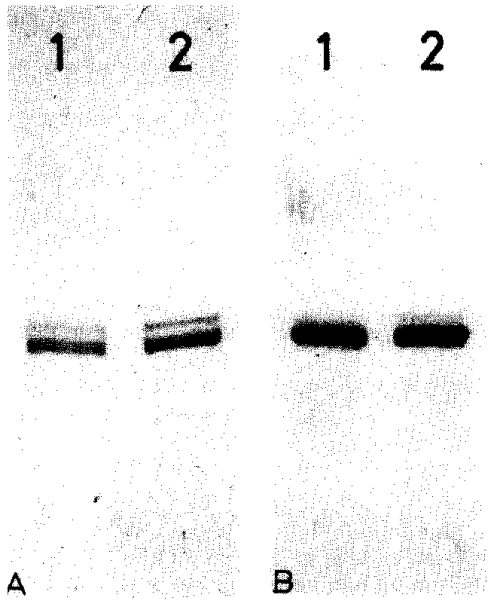


Fig.1. (A) SDS-polyacrylamide gel electrophoresis and (B) immunoblot analysis of the bovine brain 14-3-3 (lane 1) and rat brain activator (lane 2) proteins. (A) Coomassie blue staining and (B) immunoperoxidase staining using a mouse antiserum against bovine brain 14-3-3 protein.

We first noticed a possible relationship between the 14-3-3 and activator proteins in the similarity of their amino acid compositions. As shown in table 1, the composition of the bovine brain 14-3-3 protein determined in this study or of the human equivalent presented by Boston et al. [2] exhibited very good coincidence (correlation coefficient; 0.97) with the published composition of the activator protein from rat brain [12]. The acidic and dimeric nature of the 14-3-3 protein, the molecular size of subunits, as well as its high content in brain tissue, also fit the reported characteristics of the activator protein.

For a more direct comparison of both these proteins we purified the activator protein from rat brain as described [12] and analyzed the 14-3-3 and activator proteins in parallel by SDS-polyacrylamide gel electrophoresis. As shown in fig.1a, these proteins gave rise to a very similar electrophoresis pattern; there are two distinct polypeptides in which the major faster migrating polypeptide accounted for approx. 90% of the total

Table 1

Amino acid composition of the 14-3-3 and activator proteins

Amino acid	14-3-3		Rat activator
	Bovine	Human [3]	[12]
Lys	8.1	9.3	7.7
His	0.9	0.8	0.8
Arg	4.9	4.8	5.0
Asp	11.9	11.9	12.0
Thr	4.3	4.4	4.0
Ser	6.3	6.4	6.7
Glu	18.2	18.3	16.5
Pro	2.2	1.9	2.3
Gly	4.6	4.2	4.4
Ala	10.1	9.8	10.0
Cys	n.d.	n.d.	1.4
Val	5.4	3.8	5.1
Met	2.8	2.9	2.8
Ile	3.8	4.1	4.1
Leu	9.7	10.3	10.1
Tyr	4.6	4.6	4.6
Phe	2.4	2.3	2.4
Trp	n.d.	n.d.	0.8

Results expressed as mol%. n.d., not determined

protein (estimated from the Coomassie blue staining). This is comparable to the observation of Boston et al. [3] that the human brain 14-3-3 protein contained subunits of two different molecular mass class, 29 kDa and 32 kDa, at a relative content of 9:1. In addition, these polypeptides of the activator protein reacted with an antiserum against the 14-3-3 protein in a subsequent immunoblot analysis on a nitrocellulose sheet (fig.1b). Thus, the 14-3-3 protein shares various physicochemical and immunochemical properties in common with the activator protein.

We have finally examined whether the 14-3-3 protein has an activator activity towards tryptophan 5-monooxygenase. As noted in [15], tryptophan 5-monooxygenase was activated by kinase II only in the presence of the activator protein (fig.2). The concentration of activator protein required for half-maximal activation of the monooxygenase was about 1 μg/ml. The bovine brain 14-3-3 protein added into the assay mixture in place of the activator protein was also effective in

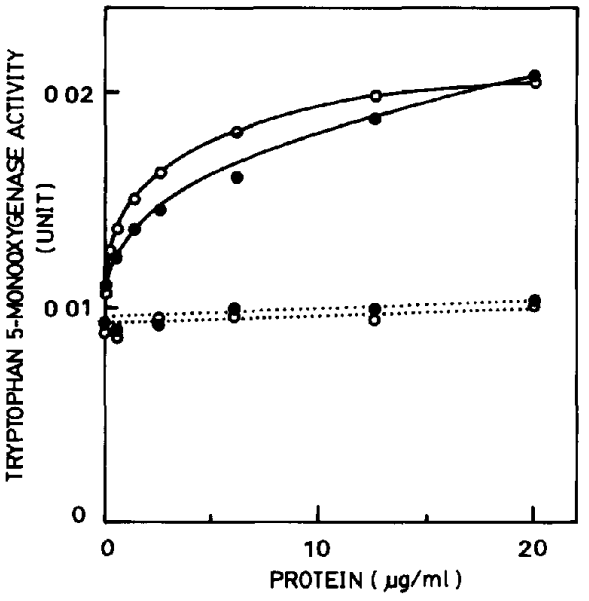


Fig.2. Effect of varying the concentration of the rat brain activator (○) and bovine brain 14-3-3 (●) proteins on activity of tryptophan 5-monooxygenase. The activity of tryptophan 5-monooxygenase (1.4 μg of protein) was assayed with different amounts of 14-3-3 or activator protein under conditions described in [12]. Control experiments (···) were performed with a complete assay system without kinase II. One unit of tryptophan 5-monooxygenase is defined as the amount which catalyzes the formation of 1 nmol 5-hydroxytryptophan/min at 30°C.

the kinase dependent activation of tryptophan 5-monooxygenase (fig.2). The concentration of the 14-3-3 protein required for the half-maximal activation was about 2.5 μg/ml.

4. DISCUSSION

Since the first description of the 14-3-3 protein in 1967, the biological significance of this neuronal protein has been the subject of investigation. For instance, Boston et al. [3] screened the 14-3-3 protein for various enzymatic activities and made attempts to correlate with brain cytoskeletal elements, both with negative results. We determined a portion of the amino acid sequence, and the sequence was subjected to computer-assisted analysis for the search of homologies to other known proteins using the PIR-NBRF database. However no significant homology was detectable

through the computer programs we used. We anticipated, therefore, that the 14-3-3 protein would be unique in its amino acid sequence and also in biological function.

In this report we have shown that the bovine brain 14-3-3 protein activates tryptophan 5-monooxygenase in the presence of kinase II. This fact, together with a remarkable similarity in physicochemical parameters and a common immunochemical reactivity, indicates that the 14-3-3 protein is identical to the protein activator an essential factor for the kinase-dependent activation of tryptophan 5-monooxygenase and tyrosine 3-monooxygenase [12,13]. The bovine brain 14-3-3 protein and rat brain activator exhibited slightly different specific activities in activating tryptophan 5-monooxygenase (fig.2), we assume that this may be a species-specific event raised by our experiment in which the rat assay system was employed for the bovine protein, although further experiments are needed to clarify this point.

Tryptophan 5-monooxygenase and tyrosine 3-monooxygenase are rate-limiting enzymes in indolamine and catecholamine biosynthesis, respectively, and are known to be the targets of various regulatory factors including kinase II or/and cAMP-dependent protein kinase. In the tyrosine 3-monooxygenase-cAMP-dependent kinase system, the activation of monooxygenase occurs directly by the phosphorylation without additional protein factors [15]. It is interesting, therefore, that kinase II requires the 14-3-3 or activator protein for the activation of these monooxygenases not only with respect to its biological significance but also with respect to its molecular mechanism of action. Yamauchi and Fujisawa [15] have reported that the kinase II dependent activation of the monooxygenases involves two step processes, phosphorylation of the monooxygenases and activation of the phosphorylated monooxygenases, and that the latter process requires the activator protein.

It should also be noted that the 14-3-3 activator is present in brain tissue in extraordinarily high concentration (13.3 μg/mg soluble protein [2]). Although brain has the highest concentration, the radioimmunoassay [2] and the activity measurement [12] have detected lower levels of this protein in almost all tissues including such tissues where the activities of the monooxygenase are not detectable at a significant level. Thus, the cellular distribution of the 14-3-3 protein does not appear to parallel the distribution of the monooxygenases. These observations, together with an apparent heterogeneity of this protein, suggest that the 14-3-3 protein may be involved not only in the regulation of serotonin and noradrenaline biosynthesis but also in other biological processes in neurons and other non-neuronal cells.

ACKNOWLEDGEMENTS

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