SEROTONIN-SENSITIVE ARYL ACYLAMIDASE ACTIVITY OF ACETYLCHOLINESTERASE

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1. Introduction

An aryl acylamidase (EC 3.5.1.13) activity that is inhibited by low concentrations of serotonin has been found in rat brain [1]. This enzyme activity is also inhibited by drugs which are structurally and pharmacologically related to serotonin [2]. It was suggested that this enzyme could serve as a model for studying the interactions of serotonin and drugs with the receptors [1,2]. However, the enzyme has not yet been purified and characterized. This paper reports evidence that acetylcholinesterase (EC 3.1.1.7) molecule has this aryl acylamidase activity.

2. Methods

Partially purified acetylcholinesterase from *Electrophorus electricus* was obtained from P-L Biochemicals, Inc., USA. The enzyme was further purified according to the method described by Leuzinger [3]. Brain extract was prepared as described in the previous paper [1].

Aryl acylamidase activity was assayed with a slight modification of the method of Hoagland and Graf [4]. The incubation mixture contained *o*-nitroacetanilide (15 μmol) as substrate and the enzyme sample in 3 ml of 0.01 M potassium phosphate buffer, pH 7.4. Incubation was carried out at 37°C for 30–120 min and the amount of nitroaniline liberated was determined by measuring absorbance at 430 nm. One unit of enzyme liberated 1 μmol of nitroaniline per min under this condition.

Acetylcholinesterase activity was assayed according to the method of Ellman et al. [5]. The incubation mixture contained acetylthiocholine (0.3 μmol) and the enzyme sample in 3 ml of 0.01 M potassium phosphate buffer, pH 7.4. Incubation was carried out at 37°C for 10 min and the amount of thiocholine liberated was determined with 5,5′-dithiobis(2-nitrobenzoate) (DTNB). One unit of enzyme hydrolyzed 1 μmol of acetylthiocholine per min under this condition.

3. Results and discussion

An extract from mammalian brain contains an aryl acylamidase activity that is inhibited by low concentrations of serotonin [1]. This enzyme activity was found to be highly sensitive to anticholinesterase reagents such as eserine and di-isopropyl phosphorofluoridate (DFP) (data not shown). Therefore, it seemed of interest to examine the relation between this enzyme activity and acetylcholinesterase. Since a very high concentration of acetylcholinesterase is found in electric organs of *E. electricus*, acetylcholinesterase from this source was tested.

A pig brain extract had acetylcholinesterase activity of 0.36 U/mg protein and aryl acylamidase activity of 0.0016 U/mg protein. The ratio of acetylcholinesterase to aryl acylamidase activity was about 225. A partially purified acetylcholinesterase preparation from *E. electricus*, whose specific activity was 1330 U/mg protein, had aryl acylamidase activity of 7.9 U/mg protein. The ratio of acetylcholinesterase activity to aryl acylamidase activity was about 170. The specific activity of purified acetylcholinesterase was more than 6000 U/mg protein and it gave

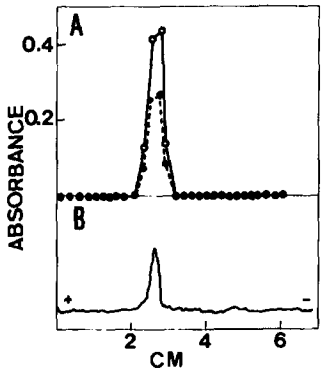

Fig.1. Polyacrylamide gel electrophoretic pattern of purified acetyl-acetylcholinesterase. Polyacrylamide gel electrophoresis, at pH 8.3, was performed according to the method of Orstein and Davis [7]. After electrophoresed at 3 mA per tube for 4 h at 4°C, the gel was cut into 2 mm thick slices. Each slice was extracted with 2 ml of 0.02 M potassium phosphate buffer, pH 7.4, at 4°C for 24 h. An aliquot (5 μl) of the extact was incubated with acetylthiocholine (0.1 mM) and DTNB (0.3 mM) in a total volume of 3 ml at 30°C for 5 min and absorbance at 412 nm was measured (o——o). Another aliquot (250 μl) was incubated with nitroacetanilide (5 mM) at 37°C for 120 min and absorbance at 430 nm was measured (•- - -•). Another gel was stained with 1% amido black and scanned with an Atago KEMIC gel scanner (B).

a single band on polyacrylamide gel electrophoresis (fig.1). The ratio of acetylcholinesterase activity to aryl acylamidase activity was unchanged during the purification. When subjected to polyacrylamide gel electrophoresis, both the enzyme activities migrated with the same mobilities (fig.1).

The aryl acylamidase activity of *E. electricus* acetylcholinesterase preparations was also highly sensitive to the anticholinesterase reagents, eserine and DFP (table 1).

These results suggest that acetylcholinesterase molecule does catalyze the hydrolysis of the aryl acylamide. It may not be surprising that acetylcholinesterase has an aryl acylamidase activity, since it has been reported that the enzyme has an amidase activity and catalyzes the hydrolysis of 2-acetaminoethyl-trimethylammonium-iodide [6]. However, it is of special interest that the aryl acylamidase activity was specifically inhibited by serotonin (table 1). The inhibition was non-competitive and K_i value was about 7×10^{-6} M. In contrast, acetylcholinesterase activity was inhibited by serotonin only slightly. The biological significance and the action mechanism of this serotonin-sensitive aryl acylamidase activity of acetylcholinesterase molecule remain to be elucidated.

Table 1
Effects of various inhibitors on enzyme activities

Substance	Concentration (M)	Per cent inhibition	
		Acetylcholinesterase	Aryl acylamidase
Eserine	10^{-8}	20	44
Eserine	10^{-7}	73	88
Eserine	10^{-6}	93	95
DFP	10^{-6}	18	36
DFP	10^{-5}	76	90
DFP	10^{-4}	93	95
Serotonin	10^{-6}	< 3	15
Serotonin	10^{-5}	< 3	67
Serotonin	10^{-4}	20	91
Noradrenaline	10^{-4}	< 3	< 3
Tyramine	10^{-4}	< 3	< 3
Histamine	10^{-4}	< 3	< 3
N-Acetylserotonin	10^{-4}	< 3	< 3

Acetylcholinesterase activity was measured using 10 mU of enzyme. Aryl acylamidase activity was measured using 3 mU of enzyme. Other conditions were described in the text.

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