The Occurrence of a Trypsin Inhibitor in Brain

By A. S. BRECHER AND N. M. QUINN

*Department of Biochemistry, School of Medicine, George Washington University,
Washington, D.C., U.S.A.*

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1. The tryptic hydrolysis of benzoyl-DL-argininyl- β -naphthylamide is inhibited by homogenates of human and ox brain.
2. The inhibitor is associated primarily with the membranous portion of the particulate matter, and is not found in the 90000g supernatant fraction.
3. The reaction of the inhibitor with trypsin is rapid.
4. The inhibitor is heat-stable.

Inhibitors of tryptic activity have been reported from plant and animal sources. Soya bean (Kunitz, 1947) and lima bean (Tauber, Kershaw & Wright, 1949) are known to contain powerful trypsin inhibitors. Mammalian trypsin inhibitors have been observed in serum (Grob, 1943), plasma (Wu & Laskowski, 1960), pancreas (Kunitz & Northrop, 1936) and liver homogenates (Blackwood & Mandl, 1961).

We now report the occurrence of a heat-stable trypsin inhibitor in homogenates of human and ox brain and its localization in the particulate fraction of the cell.

MATERIALS AND METHODS

Whole human and ox brains were homogenized in 9 vol. of 0.32M-sucrose and fractionated according to the method of Marks & Lajtha (1963) to obtain crude nuclear (P_1), mitochondrial (P_2), microsomal (P_3), ribosomal (P_4),

membranous (S_5) and soluble (90000g supernatant, S_4) fractions. A 4% (w/v) casein solution was prepared according to the procedure of Moll, Sunden & Brown (1958) and diluted 1:3 with 0.05M-phosphate buffer, pH 7.5, to give 1% (w/v) casein. Twice-crystallized trypsin, salt-free, was purchased from Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A. To measure trypsin inhibition, 1 ml. of a trypsin solution (50 μ g./ml. in 1 mM-HCl) was mixed with 1 ml. of unbuffered homogenate (Blackwood & Mandl, 1961) or cell fraction diluted, when necessary, with 0.05M-phosphate buffer, pH 7.5, and incubated at room temperature for 1 hr. Mixtures containing phosphate buffer served as controls. The effect of casein was compared with that of the cell fractions to assess the role of a protein substrate on the tryptic hydrolysis of benzoyl-DL-argininyl- β -naphthylamide. Tryptic activity was then determined by further addition to the mixtures of 5 ml. of 1.5 mM-benzoyl-DL-argininyl- β -naphthylamide in 0.1M-phosphate buffer, pH 7.0, and 3 ml. of water (Blackwood & Mandl, 1961). After incubation at 37° for 1 hr., 2 ml. samples were inactivated with 1 ml. of 40% (w/v) trichloroacetic acid and the liberated β -naphthylamine was determined according to

Table 1. *Effect of human-brain cell fractions on the tryptic hydrolysis of benzoyl-DL-argininyl- β -naphthylamide*

Details are given in the text.

Additions	Tryptic activity		Concn. of of inhibitor (mg. of protein N/ml.)
	(μ moles of β -naphthylamine liberated)	(% of control activity)	
1 ml. of 0.05M-phosphate buffer, pH 7.5	255	100	—
1 ml. of homogenate	103	40.4	1.47
0.5 ml. of homogenate + 0.5 ml. of phosphate buffer	150	58.8	0.74
1 ml. of crude nuclear fraction (P_1)	203	79.6	0.30
1 ml. of mitochondrial fraction (P_2)	225	88.3	0.31
1 ml. of microsomal fraction (P_3)	60	23.5	0.33
0.5 ml. of microsomal fraction (P_3) + 0.5 ml. of phosphate buffer	115	45.2	0.17
1 ml. of 90000g supernatant fraction (S_4)	258	101.0	0.29
1.0 ml. of 1% casein	275	107.8	1.60

Fig. 1. Inhibition of the tryptic hydrolysis of benzoyl-DL-argininyl- β -naphthylamide by ox-brain cell fractions. Δ , Casein; $\bullet$, homogenate; $\ominus$, membranous fraction (S_5); $\times$, nuclear fraction (P_1); $\circ$, mitochondrial fraction (P_2). Details are given in the text. The 90000g supernatant fraction (S_4) (0.26 mg. of protein N/ml.) and the ribosomal fraction (P_4) (0.13 mg. of protein N/ml.) effected 5% and 11% inhibition of tryptic activity respectively.

the Goldberg & Rutenburg (1958) modification of the Bratton & Marshall (1939) reaction. Protein nitrogen was determined by the micro-Kjeldahl procedure.

RESULTS AND DISCUSSION

Table 1 and Fig. 1 show the respective effects of human-brain and ox-brain homogenates and cell fractions on the hydrolysis of benzoyl-DL-argininyl- β -naphthylamide by trypsin. Brain homogenates inhibit considerably the tryptic hydrolysis of benzoyl-DL-argininyl- β -naphthylamide. Though comparable inhibition is found in the crude nuclear and mitochondrial fractions, the greatest inhibition/mg. of protein N is detected in the microsomal (P_3) fraction of human-brain preparations and in the membranous (S_5) fraction of ox-brain preparations that was derived from the microsomal fraction by deoxycholate. Slight inhibitory activity is observed in the ox-brain ribosomal fraction (P_4). Anti-trypsin activity is not observed in the 90000g supernatant fraction (S_4) of human brain; the corresponding ox-brain fraction similarly contains negligible anti-trypsin activity. Casein does not inhibit the hydrolysis of benzoyl-DL-

Fig. 2. Effect of reaction time on the inhibition of tryptic activity by the ox-brain cell fractions. To 6 ml. of trypsin (50 μ g./ml. in 1mN-HCl) was added 6 ml. of ox-brain fraction S_5 in 0.32M-sucrose. Samples (2 ml.) were withdrawn after incubation at room temperature for 1, 15, 30, 60 and 90 min., and assayed for tryptic activity together with controls, as described in the text.

argininyl- β -naphthylamide by trypsin, thereby indicating that the inhibition effected by the homogenates or cell fractions is not the result of serving primarily or preferentially as trypsin substrates. The results in Fig. 2 indicate that the reaction of trypsin with the membranous-fraction inhibitor is rapid, since essentially a constant inhibition is evident from a 1-90 min. reaction time.

The anti-trypsin activity of the homogenate and membranous fractions is not lost on heating in a boiling-water bath for 10 min. The level of inhibition is comparable with that reported with liver homogenates (Blackwood & Mandl, 1961).

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