Purification of Ribonuclease Inhibitor from Pig Cerebral Cortex

It is now well known from the experiments of HYDÉN and his group[1] that RNA may play important roles in the brain function. From this laboratory, several papers[2-4] on the brain RNA were previously published, for example, on ribonuclease (RNase) of the rat brain tissue and the incorporation of C^{14}-orotic acid into RNA of rabbit brain in vivo and in vitro.

In the course of the studies on rat brain RNase, it was found that the supernatant fraction of the rat brain tissue inhibited the RNase activity of brain microsome and mitochondria, and this inhibitor was purified about 80 fold. However, further purification was not carried out. Furthermore it was found that the polysomes from rat brain were stabilized by the addition of RNase inhibitor fraction from pig brain tissue[5]. The present report describes the evidence of RNase inhibitor in the pig brain tissue supernatant and purification of this inhibitor. RNase inhibitor was discovered in rat liver supernatant by ROTH[6] and purified by SHORTMAN[7,8].

RNA was purified from yeast RNA of the Nutritional Biochemical Corporation by a modification of the phenol method. Pancreatic crystalline RNase was Sigma product.

The methods for assay of RNase and RNase inhibitor were as follows: the reaction mixtures contained in a total volume of 0.8 ml: 0.03 *M* veronal buffer (pH 7.8) 0.3 ml, RNase solution (0.2 μg, 0.1 μg, or 0.05 μg) 0.1 ml, 1% RNA solution 0.2 ml, inhibitor solution (or brain supernatant) 0.1 ml, and redistilled water 0.1 ml. After buzzing, the tubes were incubated in a water bath of 37 °C for 30 min. The reaction was stopped by the addition of 0.8 ml of acid ethanol (1 *N* HCl in 76% ethanol). After the tubes were kept in the cold room for 1 h, they were centrifuged at 3000 rpm for 10 min. An aliquot (0.2 ml) of each clear supernatant was diluted 20 fold in water and then the absorbance at 260 nm was determined. 1 unit of RNase inhibitor was expressed according to ROTH.

Purification of the RNase-inhibitor of the pig cerebral cortex

Fractions	Total volume (ml)	Total units	Protein (mg)	Specific activity (units/mg protein)	Purification
(1) Homogenate				0.027	1
(2) Supernatant	80	104	880.0	0.11	4
(3) 3 most active fractions from DEAE-cellulose chromatography	12	72	85.0	0.86	32
(4) 3 most active fractions from hydroxylapatite chromatography	9	63	3.9	16.2	610
(5) 3 most active fractions from Sephadex G-100 gel filtration	7.5	59	0.8	74.0	2810
(6) The most active fraction from Sephadex G-100 gel filtration	2.5	42	0.25	170.0	6400

The purification of the RNase inhibitor from pig brain was as follows: pig brains were quickly brought in an ice jar from the slaughter house and cerebral cortex separated. The cerebral cortex was homogenized in 2 volumes of 0.25 *M* sucrose solution for about 3 min and then this homogenate was centrifuged at 105,000 *g*, for 90 min to yield the brain supernatant fraction. This supernatant fraction contained the RNase inhibitor. Further purification procedures of RNase inhibitor by DEAE cellulose column chromatography and hydroxylapatite column chromatography were similar to those of SHORTMAN. As the third step of purification, a Sephadex G-100 gel

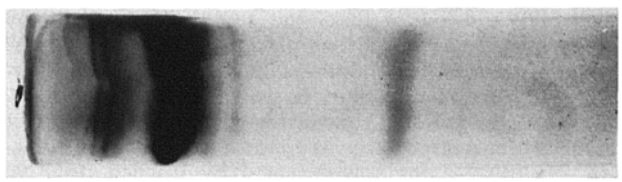

Fig. 1. Disc electrophoretic pattern of the purified RNase inhibitor (90 μg protein) from pig cerebral cortex.

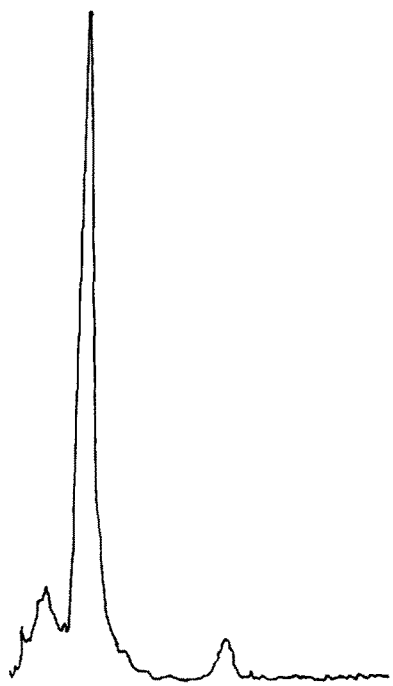

Fig. 2. Densitometory (Joyce double beam microdensitometer) of the electrophoretic pattern in Figure 1.

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filtration was carried out. The 3 fractions from the hydroxylapatite column having the highest inhibitor activity were combined, and EDTA was added to give a final concentration of 0.001 M. This fraction was concentrated to about 1.5 ml in Visking tube using Sephadex G-25, and fed into the Sephadex G-100 column (1 · 60 cm). The column was developed with the phosphate buffer (NaCl 0.15 M, EDTA 0.001 M, and potassium phosphate 0.01 M pH 6.4). The rate of elution was about 15 ml/h. The elution was collected in 3 ml fraction and assayed.

An analysis of the results of the purification procedures of RNase inhibitor from pig cerebral cortex is shown in the Table. Specific activity of the most active fraction from Sephadex G-100 gel filtration was purified about 6500 fold and purer than the sample from liver by SHORTMAN. Some properties of the most active RNase inhibitor fraction are as follows. This fraction was colourless and clear. The protein concentration was about 100 μg/1.0 ml, and RNA concentration was lower than 1 μg/ml. No carbohydrate was found by the Molisch reaction. No hexosamine was detected by Ehrlich test. No heparin was detected by the toluidin blue test. Inhibitor was labil for heating and not dialyzable. The UV-absorption spectrum of this active inhibitor fraction showed maximum absorption peak at 280 nm. Figure 1 shows Disc electrophoretic pattern of the most purified RNase inhibitor fraction. Densitometric estimation of the electrophoretic pattern taken with Joyce double-beam recording microdensitometer is also shown (Figure 2). The major band represented about 80–85% of total protein. The preincubation of this inhibitor with streptomyces protease and trypsin reduced the activity of inhibitor. No loss of activity was found by freezing (– 20 °C) of this purified inhibitor for more than 2 months. Also no loss of activity was observed by storing for 2 weeks at 0–4 °C. The low concentration of PCMB ($1 \cdot 10^{-6} M$) inactivated the inhibitor and was largely reversed by cysteine ($1 \cdot 10^{-3} M$) suggesting the role of SH group in RNase inhibitor. This RNase inhibitor did not have any effect on the RNase T_1[9]. Such properties suggested that this RNase inhibitor might be an acidic protein having the molecular weight of about 60,000, in consideration of the elution rate from Sephadex G-100.

The possible physiological function of this RNase inhibitor in the protein biosynthesis of the brain tissue was reported in a separate paper[5]. The authors intend to try further purification. A detailed account will be published later[10].

Zusammenfassung. Der natürliche Ribonuclease-Hemmstoff aus Schweinsgrosshirnrindengewebe konnte mit DEAE-Cellulose-Chromatographie, Hydroxylapatit-Chromatographie und Sephadex G-100 Gel-Filtration ca. 6500fach gereinigt werden. Der gereinigte RNase-Hemmstoff zeigte eine Hauptbande (etwa 80–85% des Totaleiweisses) und einige kleinere Bande bei der Polyacrylamid-Gel-Electrophorese. Untersuchungen über die Natur dieses Hemmstoffs werden weitergeführt.

Y. TAKAHASHI, K. MASE and Y. SUZUKI

Department of Neurochemistry, Brain Research Institute and Department of Neurology and Psychiatry, Niigata University School of Medicine, Niigata (Japan), 6th February 1967.

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[10] Authors would like to thank Dr. H. SUGANO (Department of Chemistry, Niigata University School of Science) and Dr. K. TANIMURA (Brain Research Institute, Niigata University School of Medicine) for valuable help and also thank Dr. T. KURIHARA (Department of Chemistry, Tokyo University School of Science) for the gift of RNase T_1.

Effects of β-Internal (P^{32}) Irradiation on the 5-HT Content of CNS Levels[1]

It is known that the CNS is a highly radiosensitive tissue, as proved not only from the histopathological and neurophysiological but also neurochemical standpoint. As to the last, one of the most important aspects is the one concerning the central neurotransmitters, e.g. ACh (EGAÑA[2,3]), GABA, L-noradrenalin and 5-HT. In recent years several authors have reported effects of irradiation on the 5-HT content of the brain: ERSHOFF et al.[4] do not find significant 5-HT variations after X- and γ-whole body exposure as compared to untreated pair-fed control; however, the values of these groups are lower than those of untreated ad libitum fed controls. RANSON et al.[5] communicate a significant descent of 5-HT of rat hypothalamus after X-irradiation. SPECK[6] verifies a decrease of brain 5-HT, following high dose exposure with a subsequent recovery at 48 h. RANDIĆ et al.[7,8] found that a 900–4000 r whole body irradiation produces so significant changes; higher doses increase 5-HT concentration of rat brain. All the aforementioned results refer to whole brain (RENSON et al.[5] excepted) and use whole body exposure. We are not acquainted with publications on results of β-internal irradiation and the effects at diverse CNS levels. In addition, the β-internal exposure constitutes a distinct radio-energy absorption compared with the X- or γ-whole body one. So the analysis of the exposure effects at different CNS levels allows us to examine this problem in more specific brain areas in which 5-HT has a particular neurochemical significance, e.g. hypothalamus, mesencephalon etc.

[1] This work has been carried out under a grant from the International Atomic Energy Agency, Vienna; contract No. 95 RB/1-RB/2.

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