75. Age-Related Protein detected in Human Brain by Isoelectric Focusing

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The ageing of the nervous system has recently become a subject of interest that needs to be elucidated. To accomplish our purpose, it was thought advisable to investigate molecular changes of the ageing brain, especially the neuron which are unique and distinguishable from dividing somatic cells. But, little information has been obtained on biomolecular changes, especially brain-specific protein and nucleic acid alterations accompanied with the ageing of human brains.¹⁾

During the course of studying ageing process,^{2),3)} we found out by isoelectric focusing an age-related protein in the water-soluble fraction which was extracted from frontal lobes of human brains. This paper reports the mode of age-dependent alteration and preliminarily some properties of this protein.

Materials and methods. *Preparation of water-soluble fractions from brain and other organs.* Human brains and other organs were obtained from autopsy within a 24 hr post-mortem and stored at -80°C prior to preparation. All preparation procedures were performed at 4°C . Pieces of frontal lobe of brain and several organs were washed with 0.85% NaCl solution to remove excess blood and then homogenized by an Ultra-turrax (IKA-Werk) in 0.1 M potassium-phosphate buffer pH 7.1, including 1 mM EDTA and 35% saturated ammonium sulfate. The homogenates were centrifuged at 15,000 g for 20 min. The resulting supernatants were brought with ammonium sulfate to 85% saturation and left overnight. The precipitate was collected by centrifugation at 15,000 g for 20 min and dissolved in 0.1 M potassium-phosphate buffer pH 7.1, including 1 mM EDTA and 0.05 M NaCl. The resulting solutions were dialyzed against the same buffer, followed by distilled water and then were lyophilized. Obtained materials were dissolved in distilled water at a concentration of 2% for electrophoresis.

Electrophoresis of water-soluble materials. Isoelectric focusing of obtained materials was performed mainly according to the previous report.⁴⁾ A mixture of 240 μl Pharmalyte 2.5–5.0 (Pharmacia) and 40 μl Pharmalyte 3.0–10.0 (Pharmacia) was used as carrier ampholyte instead of LKB Ampholine. As electrode solution, 0.1 M H_2SO_4 and 0.5 M NaOH were used for the anode and the cathode, respectively. Five μl of samples were applied with LKB sample pieces at 1.5 cm from the anode on the gel. After electrophoresis, proteins and carbohydrates of glycoproteins were stained with 0.1% Coomassie brilliant blue R250 (Bio-Rad) and Schiff's reagent, respectively.

Enzyme treatment. Neuraminidase from *Vibrio cholerae* was purchased from BDH, α -chymotrypsin and trypsin from bovine pancreas, and pronase were purchased from Sigma and Kaken, respectively. Reaction mixtures were incubated at 25°C overnight. Other reaction conditions were described in foot-notes of Table II.

Results and discussion. *Electrophoresis of water-soluble fractions in human*

Age (years)	0	8	30	40	54	60	72	74
Sex	F	M	F	M	M	M	F	M
Sample no.	1	2	3	5	10	11	13	14

Fig. 1. Detection of age-related protein in water-soluble fractions extracted from human brains with different age and sex by Coomassie blue staining following isoelectric focusing (M, male; F, female). The arrow indicates the location of age-related protein.

brains. Water-soluble proteins extracted from the frontal lobe of 15 human subjects between 7 months and 74 years of age were analyzed by polyacrylamide gel isoelectric focusing (Fig. 1). As shown in Fig. 1, a unique band was observed in the region of the gel corresponding to a pH of approximately 3.0 by Coomassie blue staining after electrophoresis. Materials obtained from the subjects between 7 months and 40 years of age gave the band with strong intensity independently from sex of subjects, whereas in samples from the subjects of older than 41 years of age the corresponding band could not be observed, or even if presented, it was very faint. Therefore, irrespective of sex, the protein corresponding to this band on the gel existed until approximately 40 years of age, but above about 41 years did not, or if presented the contents were considered to remarkably decrease. As shown in Table I, the existence of this protein shows age-dependent and sex-independent alteration. The protein band is also independent from at least blood groups ABO, MN, and secretor-status. Previously, we found out an age-related protein (Ugl-Y) in urine samples of boys and girls.^{2),3)} But, considering the mode of age-dependent alteration and its electrophoretic properties, the age-related protein found in human brain may

Table I. Disappearance of the age-related protein in human brain accompanied with increase of age

Sample no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age (years)	0*	8	30	36	40	40	41	41	47	54	60	65	72	74	74
Sex**	F	M	F	M	M	F	F	F	M	M	M	M	F	M	F
Appearance***	++	+	++	++	+	+	±	±	—	±	—	±	—	—	—

* 7 months. ** F, female; M, male. *** Intensity of band on the gel, which corresponds to the age-related protein, detected by Coomassie blue staining: ++, strong; +, very faint; —, not detectable.

not coincide with Ugl-Y. Highly acidic proteins were purified from human brains,⁵⁾ but these and the age-related protein reported here differed from each other in the pI values.

Comparison of the age-related brain protein with the extracts from other organs. In order to clarify the distribution of the age-related protein, the similar water-soluble fractions which were prepared from six organs, heart, lung, liver, pancreas, spleen, and kidney derived from a female subject of 30 years of age (sample no. 3 in Table I), were analyzed by the electrophoresis. As a result, in the case of each organ except pancreas, we could not detect the band on the gel that corresponds to the age-related protein in brain. And it was not found in serum, saliva and urine samples collected from some individuals with different sex and age. In the pancreas extract a very faint band was seen, and its pI value was extremely close to the age-related brain protein. But we could not conclude that the band observed in pancreas corresponds to that of the same protein as the age-related one in brain. Although the content of age-related protein in each organ was not determined quantitatively in our experiment, it is obvious that the protein is much more present in brain than in other organs from the staining intensity of the band corresponding to it.

Effect of various enzymes and heat treatment. In order to know some chemical properties of this age-related protein, effect of various enzymes and heat treatment on its electrophoretic patterns were examined (Table II). Neuraminidase and trypsin gave no effect, whereas the corresponding band on the

Table II. Effect of various enzymes* and heat treatment on electrophoretic patterns of the age-related protein in human brain

Treatment of	Effect**
Enzymes	
α -Chymotrypsin	+
Pronase	+
Trypsin	—
Neuraminidase	—
Heat	
55°C, 5 min	—
100°C, 5 min	—

* Reaction conditions were as follows: α -chymotrypsin (final concentration, 5 mg/ml), 0.05M K-phosphate buffer pH 8.0; pronase (5 mg/ml), 0.05M Tris-HCl buffer pH 7.5 with 5 mM CaCl_2 ; trypsin (5 mg/ml), 0.05M Tris-HCl buffer pH 8.0 with 10 mM CaCl_2 ; neuraminidase (125 units/ml), 0.1M Na-acetate buffer pH 5.0 with 7 mM CaCl_2 and 0.15M NaCl. ** +, effective on the electrophoretic pattern; —, not effective.

gel disappeared after treatment with α -chymotrypsin and pronase. Also, when it was stained for carbohydrates in glycoproteins with periodic acid-Schiff (PAS) procedure, this band was PAS-negative. So, this protein may be not glycoprotein. And, heat treatment at 55°C and 100°C for 5 min did not cause disappearance of the corresponding band, so it may be considered that the protein is thermostable.

Summary. By isoelectric focusing technique, we found out the age-related protein in human brain which is present in the individuals of younger than approximately 40 years of age. But the protein disappears or remarkably decreases in its content above 40 years of age.

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