nation may be that centrifugation of blood on a Ficoll-Isopaque gradient mainly separates lymphocytes and monocytes (mononuclear cells).

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Comparison of X-prolyl dipeptidyl-aminopeptidase activity in human cerebrospinal fluid with that in serum

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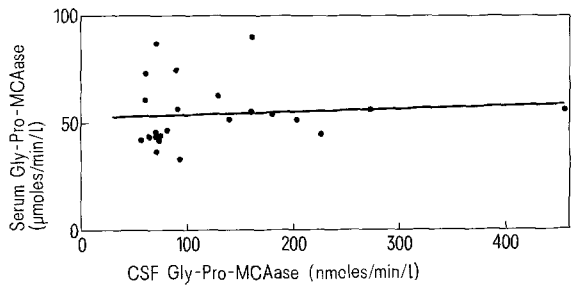
Summary. X-Prolyl dipeptidyl-aminopeptidase activities in cerebrospinal fluid and serum from the same patients without neurological diseases, undergoing surgery under lumbar anesthesia, were assayed fluorometrically with a newly synthesized fluorogenic substrate, 7-glycylproline-4-methylcoumarinamide; the values were 129.1±19.5 nmoles/min/l and 54.17±3.11 μmoles/min/l (mean±SEM, n=23), respectively, and there was no correlation between both activities (r=0.0894).

X-Prolyl dipeptidyl-aminopeptidase[2] cleaves N-terminal X-proline from peptides. The enzyme was purified from porcine and lamb kidney[3-6] and human submaxillary gland[7]. The N-terminal amino acid (X) can be an amino acid with a free amino terminal such as Gly, Ala, Arg, Lys, Glu and Asp, and glycine gives the highest activity[8]. The presence of Pro in the penultimate position is substantial, but Ala and Hyp can give a weak activity[9]. The physiological role of this enzyme is not clear, but since the enzyme preferentially hydrolyzes Gly-Pro sequence which is rich in the collagen molecule, it may work on the degradation of peptides with the N-terminal Gly-Pro sequence derived from collagen in the connective tissue.

We found the enzyme in human serum[10] and saliva[11]. We have recently synthesized a new fluorogenic substrate, 7-(Gly-Pro)-4-methylcoumarinamide for the enzyme[12], and developed a highly sensitive fluorescence assay for X-prolyl dipeptidyl-aminopeptidase. By using this method, the enzyme was found also in human cerebrospinal fluid[12]. Preliminary data showed that the enzyme activity in cerebrospinal fluid was only about 0.2% of the serum enzyme activity. Since the cerebrospinal fluid protein concentration is about 0.3% of serum protein, the enzyme activity of cerebrospinal fluid could be directly related to the serum protein content of the spinal fluid. To examine this question, we have measured the enzyme activity in cerebrospinal fluid and serum simultaneously from the same patients.

Cerebrospinal fluid was obtained from patients at Fujita-Gakuen University School of Medicine Hospital by lumbar puncture. The patients were undergoing surgery under lumbar anesthesia. No patient suffering from central or peripheral neurological diseases was included, and the general physical and nutritional states of the patients were within normal range. Care was taken to avoid any contamination of blood into cerebrospinal fluid. The first 5 ml was removed for chemical and cytological examinations, and the next 5 ml was used for the assay of X-prolyl dipeptidyl-aminopeptidase activity. The samples of cerebrospinal fluid were all clear, and no red cells were detected. Blood samples were obtained by venipuncture and serum was removed. X-Prolyl dipeptidyl-aminopeptidase incubation mixture (total volume 100 μl) contained 40 μl of 0.15 M glycine-NaOH buffer (pH 8.7), 25 μl of 2 mM 7-(Gly-Pro)-4-methylcoumarinamide tosylate, and 35 μl of cerebrospinal fluid or 35 μl of diluted serum containing 1 μl of serum. The control tube contained no enzyme. All the tubes were incubated at 37 °C for 30 min, and the reaction was stopped by adding 1.0 ml of 1 M sodium acetate buffer, pH 4.2. The same amount of enzyme was added to the control, after stopping the reaction. The fluorescence intensity was read at 460 nm with excitation at 380 nm, using a Shimadzu RF-500 spectrofluorometer, and 7-amino-4-methylcoumarin liberated by the enzyme reaction was measured.

X-Prolyl dipeptidyl-aminopeptidase activity in cerebrospinal fluid and serum (mean±SEM, n=23) were 129.1±19.5 nmoles/min/l and 54.17±3.11 μmoles/min/l, respectively. Thus, the enzyme activity in cerebrospinal fluid was 0.24% of the serum enzyme activity. As shown in the figure, a correlation coefficient between the activity in cerebrospinal fluid and that in serum was 0.089, and there was no significant correlation between the activity in cerebrospinal fluid and that in serum. The result suggests that the enzyme in cerebrospinal fluid could be derived from the brain and not from the blood. It should be noted in this connection



Correlation between X-prolyl dipeptidyl-aminopeptidase activity in human cerebrospinal fluid (CSF) with that in serum. 7-(Gly-Pro)-4-methylcoumarinamide was used as substrate, and 7-(Gly-Pro)-4-methylcoumarinamidase (Gly-Pro-MCAase) activity was expressed as 7-amino-4-methylcoumarin liberated (nmoles or μmoles)/min per l of cerebrospinal fluid or serum.

that we have found X-prolyl dipeptidyl-aminopeptidase activity in human brain[13]. We have not yet compared molecular properties of the enzymes isolated from cerebrospinal fluid and from serum, but we have preliminary data indicating that the molecular properties of the brain enzyme are different from those of the serum enzyme (unpublished results). We have also found that the homogeneous enzyme from human submaxillary gland hydrolyzes N-terminal dipeptide Arg^1-Pro^2 and subsequent dipeptide Lys^3-Pro^4 from substance P, a putative neurotransmitter[14]. The physiological and pathological significance of this enzyme in cerebrospinal fluid remains for further investigation.

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The use of cell free extracts derived from fungal protoplasts in the study of aflatoxin biosynthesis

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Summary. A supernatant fraction derived from protoplasts of *Aspergillus flavus* was shown to be capable of converting both sterigmatocystin and versiconal hemiacetal acetate to aflatoxin B_1. Versicolorin A was not converted under the same conditions.

The isolation and characterization of the individual enzymes involved in the biosynthesis of aflatoxins is a difficult undertaking as the liberation of these labile enzymes by mechanical means can result in their denaturization[1,2]. In order to overcome this difficulty the technique of digesting the cell wall has been employed, using lytic enzymes derived from *Trichoderma viride*, resulting in the formation of fungal protoplasts[3]. These protoplasts have already been shown to be capable of synthesizing aflatoxins[4], hence the results reported here describe the isolation of a cell-free extract from lysed *Aspergillus flavus* protoplasts capable of converting ^{14}C versiconal hemiacetal acetate to aflatoxin B_1.

Materials and methods. Protoplasts were isolated from 3- and 4-day-old *Aspergillus flavus* mycelium as previously described[4] and collected by centrifugation at 500×g for 10 min. The pellet was shaken with 5 ml 0.1 M phosphate buffer, pH 8.0 and frozen for 30 min at 0 °C. The resulting slurry was then thawed and centrifuged (10,000×g for 30 min) to yield a supernatant fraction which was utilized as the cell-free extract, and a residue fraction. Protein was estimated using the Biuret method (1 ml of extract). The remaining extract (4 ml) was added to a cofactor medium[6] (1 ml) to give a final concentration of FAD (10^{-6} M), EDTA (10^{-3} M), methionine (10^{-3} M), dithiothreitol (10^{-3} M), NADPH (1 µmole), NADH (1 µmole) and the labelled substrate dissolved in NN-dimethylformamide. Labelled substrates were prepared after the method of Yao and Hsieh[7]. The 'cell-free' extract was incubated in a standard Warburg flask at 30 °C and shaken constantly. At

Conversion of added compounds to aflatoxin B_1 by a supernatant fraction isolated from lysed protoplasts of *Aspergillus flavus**

	Substrate added**	Incubation period (h)	Aflatoxin B_1 formed µCi recovered	Specific activity (mCi/mole)	Percent conversion***
A	(G)^{14}C Versiconal hemiacetal acetate	1	0.0000072	0.9	3.6
		18	0.0000192	2.0	9.6
B	(G)^{14}C Versiconal hemiacetal acetate	18	0.0000002	0.02	0.1
C	(G)^{14}C Versiconal hemiacetal acetate	1	0.0000153	1.9	7.7
	(G)^{3}H Sterigmatocystin	1	0.000056	ND	28.0
	(G)^{14}C Versicolorin A	1	zero	–	–
		18	zero	–	–

* All results are an average of essentially reproducible duplicate experiments. ** 0.0002 µCi of sp. act. 3.8 mCi/mole were added in each experiment. *** $\frac{\text{µCi product formed}}{\text{µCi precursor added}} \times 100$. A, Fraction derived from protoplasts of 3-day-old mycelium; B, residue fraction derived from protoplasts of 3-day-old mycelium; C, fraction derived from protoplasts of 4-day-old mycelium. ND, not determined.