Ionic strength dependent enzyme activities of elastase

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Summary. Peptidase and esterase activities of elastase were each altered separately by the ionic strength of the buffer used. The esterase activity is exerted in a lower ionic strength medium where the peptidase activity is not detected.

It is well known that elastase (pancreatopeptidase E [EC 3.4.21.11]) has 2 different hydrolytic activities; that is, cleavage of C–N bond (peptidase) and C–O bond (esterase). The present experiments have been performed to elucidate whether the active center involved in the 2 activities is the same, or whether there are 2 different ones. Some enzyme inhibitors inhibited both activities in a parallel manner, but it was interesting to find out that the 2 activities could be separated, depending on the ionic strength of the buffer solution used.

Experimental. Elastase: porcine pancreas, labeled as 64 units/mg (Worthington Biochem.). Only a single band was detected in this preparation as checked by polyacrylamide gel (15%) electrophoresis, pH 4.5[2], and the elctrophoretic pattern and the enzymatic activities of this single band were obviously different from those of α-chymotrypsin and trypsin. Substrate: l-Ala-l-Ala-l-AlaOMe (Ala_3OMe) (Sigma Chem. Co.). Assay: Analytical procedures for the simultaneous determination of esterase and peptidase activities of elastase have been developed in our lab; that is, elastase was incubated with the substrate in a borate buffer, 0.2 M, pH 8.0, at 37 °C for 10 min. After stopping the reaction, an aliquot of the reaction mixture was spotted on a cellulose TLC plate (0.1 mm in thickness, Merck) and TLC was developed with a mixture of benzyl alcohol: AcOH:H_2O (4:1.5:1) to separate Ala_3OMe (substrate, $R_f=0.83$), Ala_3 (product of esterase, $R_f=0.66$), Ala_2 (product of peptidase, $R_f=0.61$), Ala ($R_f=0.42$) and enzyme ($R_f=0$). Developed spots were visualized by dipping the TLC plate into a 0.01% fluorescamine (Fluram, Roche, Basel) solution in a mixture of actone:hexane (1:4), and quantitative determinations of the spots were performed with the aid of Shimazu Dual-wavelength TLC Scanner CS-900 with a TLC reflecting scanner adaptor using known amount of Ala_3 and Ala_2 as the standards, thus the results are expressed as the fluorescent intensity relative to the standards.

Results. The concentration of the borate buffer, pH 8.0, was changed within the range of $2\times10^{-1}\sim1.6\times10^{-3}$ M (Na borate-HCl, pH 8.0) and $4\times10^{-1}\sim1.6\times10^{-3}$ M (boric acid-NaOH, pH 8.0), and esterase and peptidase activities of elastase, trypsin, α-chymotrypsin and pronase were assayed. As shown in figures 1 and 2, esterase activity remains in buffers of lower ionic strength, and thus both esterase and peptidase activities could be separated at the lower region of ionic strength. Such a tendency was shown with pronase (figure 2), but complete separation of the esterase activity from peptidase was accomplished in elastase. In order to examine any effect of chloride ion, $10^{-3}\sim1$ M NaCl was added to the boric acid-NaOH buffer, and it was found that the elastase activities were not influenced by Cl^-. The same tendency was observed for elastase when the concentrations of phosphate buffer were changed. Such enzyme inhibitors as DFP (diisopropyl phosphorofluoridate) (for –OH), soybean inhibitor and ovoinhibitor were used to inhibit, if

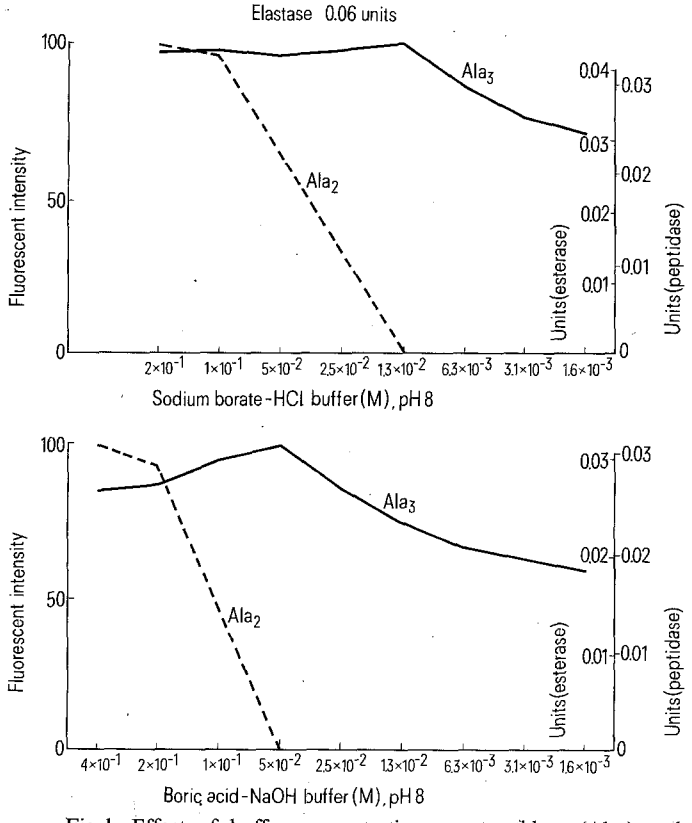

Fig. 1. Effect of buffer concentrations on peptidase (Ala_2) and esterase (Ala_3) activities.

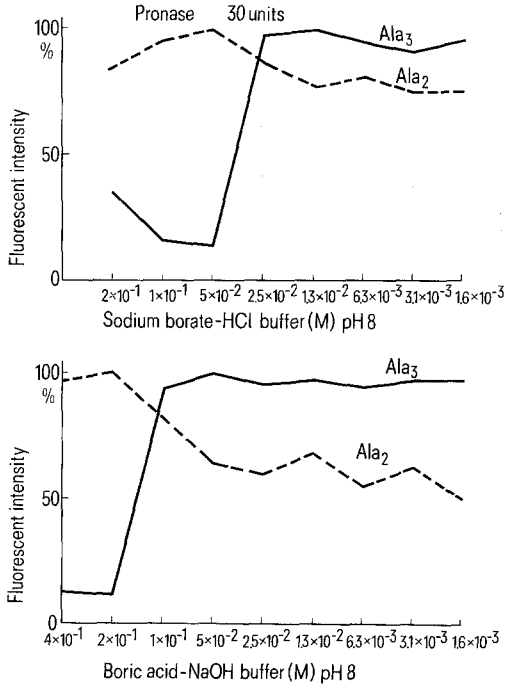

Fig. 2. Effect of buffer concentrations on pronase.

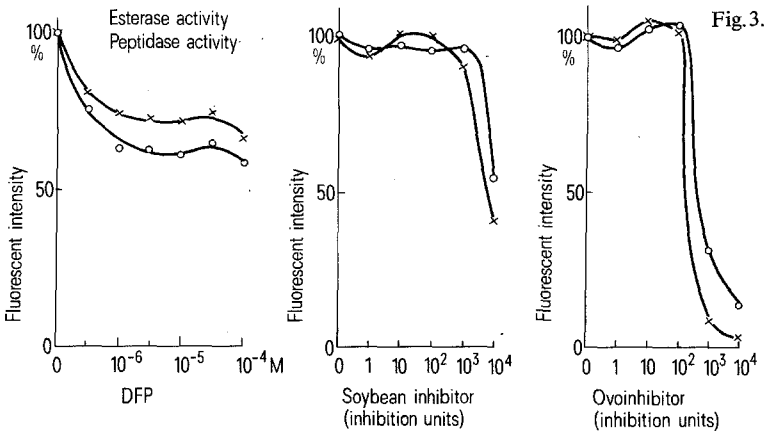

Fig. 3. Effect of addition of various inhibitors on elastase activity.

possible, only 1 activity of elastase, but both activities were inhibited simultaneously (figure 3). Amino acid residues involved in the active center of elastase may be the same for ester and peptide hydrolysis, but a slight conformational change might affect the nature of hydrolytic cleavage of C–N and C–O bonds. It might also be suggested that a more critical spatial conformation is required for the peptidase activity of elastase, and esterase activity may be exerted by a rather loose conformational relation of the various amino acid residues in the active center.

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2 R.A. Reisfield, U.J. Lewis and D.E. Williams, Nature *195*, 281 (1962).

Brain capillary guanosine triphosphatase: A distinction from adenosine triphosphatase[1]

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Summary. Differences in kinetic properties, pH response, sensitivity to ouabain, and disc-acrylamide electrophoresis resolution, are observed when GTP and ATP are used as the substrates for triphosphohydrolases in isolated rat brain microvessels. In brain parenchyma there are no such differences. It is concluded that substrate-specific GTPase exists in brain microvessels.

The essential role of adenosine triphosphatase (ATP phosphohydrolase, EC 3.6.1.3; ATPase) in the maintenance of the ionic distribution on the cell surface is well documented[3]. The importance of the high potassium and low sodium intracellular concentrations in nervous tissue can hardly be overemphasized, for this unequal distribution is the physicochemical basis for the formation of the membrane (synaptic) potential[4]; this potential and its changes are conditio sine qua non for brain function to exist.

It is considered that 2 compartments exist in brain: neurons and glia[5]. Recently, we postulated that the brain microvessels, representing the anatomical and functional basis of the blood-brain barrier[6], are a separate one, since microvessels have a specific enzymatic organization[7,8] indicative of a difference in metabolic fate of the substances in them; this is a characteristic of the compartment[5].

During our studies on enzymatic organization in isolated brain microvessels, noticeable ATPase and guanosine triphosphatase (GTPase) activities were found[7]. It is known that all the nucleotide triphosphates (i.e. ATP, ITP, GTP, CTP and UTP, in order of their supply ability) can be used as the energy fuel for the ionic pump[9,10].

Materials and methods. Adult male Wistar rats were sacrified by decapitation, the skulls were quickly removed, and the forebrains homogenized in 5 vol. of ice-cold homogenizing medium[11]. Capillary and parenchymal specimens were obtained as described by Djuričić and Mršulja[11]. Triphosphohydrolase activities were determined in the assay mixture, which contained the appropriate substrate (ATP or GTP) in the concentration indicated, 90 mM NaCl, 10 mM KCl, 3 mM $MgCl_2$, and N-2-hydroxyethyl-N-2-ethane sulfonic acid-NaOH (HEPES-NaOH) buffer (50 mM, pH 7.1). Phosphorus liberated after the proper incubation time at 37 °C was determined according to Lowry and Lopez[12]. The technique employed is not applicable for substrate concentrations lower than 0.1 mM, so K_i's were obtained from a Lineweaver-Burk plot rather than K_m's.

When the pH was varied, the same HEPES buffer at the pH indicated was used. The effects of ouabain were investi-

Rat brain microvessels and parenchymal ATPase and GTPase activities, the ratios (microvessels activity/parenchymal activity) and the half-maximal inhibitory constants (K_i) for ouabain

Triphosphohydrolase	Activity In microvessels	In parenchyma	Ratio	$K_{i_{ouabain}}$ (mM) In microvessels	In parenchyma
ATPase	196 ± 18	260 ± 17	0.75	0.068	0.062
GTPase	154 ± 12	170 ± 18	0.91	0.073	0.063

* The activities of triphosphohydrolases are expressed in nmoles of the liberated phosphorus min^{-1} mg $protein^{-1}$, 37 °C. Mean values ± SEM for 4–6 duplicate measurements are given. Concentrations of ATP (or GTP) were 3 mM.