BI 20

PEPSINOGEN-LIKE IMMUNOREACTIVITY IN THE STOMACH OF ASCIDIANS.

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To study the origin of vertebrate pepsinogens, we examined the stomachs of ascidians with indirect immunofluorescence employing the anti-embryonic and anti-adult chicken pepsinogen antisera (anti-ECPg and anti-ACPg). The epithelial cells lining the stomach villi of 5 species examined contained substance immunoreactive to the anti-ECPg. Positive cells located mainly in mid to distal part of the villi. In some species like Styela plicata, the top of the villi was negative to the anti-ECPg but was stained with anti-ACPg. Immunoblotting with anti-ECPg showed that the molecular weight of immunoreactive material extracted from the stomach of S. plicata was 57-60 kD, slightly higher than that of embryonic chicken pepsinogen. Since crude extract of S. plicata stomach showed very low activity of acid protease against hemoglobin or albumin, the substances reactive to the antisera in ascidian stomach may be different from the digestive enzyme in functions. The understanding of their functions may throw light on the origin of vertebrate pepsinogens.

BI 21

BIOCHEMICAL STUDY OF ACID PROTEASES IN VERTEBRATE STOMACHS.

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The presence of substances immunoreactive to the anti-adult chicken pepsinogen antiserum was studied in the stomachs of vertebrates with indirect immunofluorescence and biochemical methods. Indirect immunofluorescence showed that the stomach glandular cells of all vertebrates examined contained the immunoreactive substances but mucous cells did not react to the antiserum. Crude extracts of stomachs obtained from some representatives of each class showed acid protease activity at pH2.0, of which 70 to 100% were sensitive to pepstatin. Moreover, zymograms and immunoblots after polyacrylamide gel electrophoresis revealed that most of the bands of immunoreactive substances coincided with those of acid protease activity. These results suggest that the immunoreactive substances in the vertebrate stomachs are pepsinogens and that these pepsinogens have been well conserved concerning their immunological properties during vertebrate evolution.

BI 22

PROPERTIES OF THE CARBOXYL PROTEASE IN RAT NEUTROPHILS.

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An acid protease which does not cross-react with anti-rat liver cathepsin D (CD) antibody was partially purified from the extract of rat neutrophils. The enzyme resembled rat liver CD in respect of the pH dependence for activity, the preference for substrates and the resistance to various metal ions, but was more resistant to urea denaturation than CD. Pepstatin completely inhibited the neutrophil enzyme as well as CD, at a concentration of 0.29 μM, whereas phenylmethylsulfonyl fluoride or iodoacetamide had no effect on the enzymes. The molecular weights of the neutrophil enzyme and liver CD were 49,000 and 41,000, respectively, as determined by gel filtration on Toyopearl HW 55. The neutrophil acid protease also differed from liver CD in the specificity of action against the oxidized B-chain of insulin: Liver CD cleaved Phe^{1}-Val^{2}, Ala^{14}-Leu^{15}, Leu^{15}-Tyr^{16}, Tyr^{16}-Leu^{17}, Phe^{24}-Phe^{25} and Phe^{25}-Tyr^{26} bonds, while the neutrophil enzyme cleaved Glu^{13}-Ala^{14}, Leu^{17}-Val^{18} and Gly^{23}-Phe^{24} bonds in addition to the bonds cleaved by CD, except for Phe^{1}-Val^{2}. The results indicate that the properties of the neutrophil carboxyl protease are apparently different from those of cathepsin E reported earlier, suggesting the existence of another carboxyl protease in rat neutrophils.

BI 23

CHANGES IN PROTEIN KINASE ACTIVITIES IN RAT LIVER NUCLEI DURING REGENERATION

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Phosporylation of histone H1 is prerequisit to the DNA synthesis of the regenerating rat liver and occurs between 21 and 24 h after partial hepatecomy. To know the enzyme(s) responsible for the phosphorylation, changes in activities of cAMP-depandent and independent protein kinases in nuclei were investigated. The independent kinase increased from 18h to 21h. However, the same change occurred also in the nuclei, where phosphorylaiton of H1 was inhibited by 4.8 Gy of X-irradiation. The dependent kinase activity in nuclei decreased gradually from 15 h to 24h. Similar changes also occurred in the irradiated nuclei. The nuclei was fractionated by extraction with 0.3 M NaCl. The unextractable part was dispersed by DNase I digestion. The DNase treatment solubilized only ca 5 % of the total enzymes. About 35% of the independent enzyme(s) was extracted by NaCl treatment and the value did not change during regeneration and X-irradiation had no effect on the distribution. On the other hand, from 18h to 24h when the activity decreasing, the dependent kinase seemed to become more extractable with NaCl and irradiation seemed to inhibit the process. Since the dependent kinase activity is low (about 1/4 of the independent kinase), more work is required to confirm it.