The Localization of Peptidases in Microvilli from the Brush Border of the Proximal Renal Tubule of the Rabbit

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We have previously reported that a neutral peptidase capable of hydrolysing [¹²⁵I]iodinated insulin B chain (pH 7.5 peptidase) is, like arylamidase and alkaline phosphatase, localized in the microvilli of the proximal renal tubule. Moreover, electron micrographs of negatively stained preparations have revealed that the surface of the microvillus has a covering of discrete granules 80–100 Å in diameter (Wong-Leung, George, Aparicio & Kenny, 1968). It is also clear that the pH 7.5 peptidase is readily distinguishable from arylamidase in properties and substrate specificity (Kenny, Wong-Leung & Ingram, 1968). We now report some further experiments concerned with the localization of these enzymes in the microvillus and with the purification of the arylamidase.

Deoxycholate treatment of a light-microsomal fraction of the kidney cortex disrupted all the membranous elements and solubilized both the pH 7.5 peptidase and arylamidase to about the same extent. On the other hand, incubation of the fraction with papain brought about the selective release of arylamidase in a soluble form, while the pH 7.5 peptidase and alkaline phosphatase remained in the microsomal pellet. Electron micrographs of the pellets, negatively stained with phosphotungstate, were examined. After papain treatment the surface of many microvilli appeared smooth, in contrast with the control (which had been incubated without papain) in which the surface covering of particles was preserved.

The supernatant fraction obtained after papain treatment provided a useful starting point for the purification of arylamidase. Ammonium sulphate precipitation, DEAE-cellulose chromatography and column electrophoresis in a sucrose density gradient yielded a preparation with a specific activity of 57 units/mg. (1 unit $\equiv$ 1 μ mole of L-leucine *p*-nitroanilide hydrolysed/min. at 37° at pH 7.0) and which appeared to be homogeneous on disc electrophoresis. This preparation also hydrolysed L- α -amino acid β -naphthylamides, the relative rates for leucine, arginine and glutamic acid derivatives being 100:70:28. Iodinated insulin B chain was attacked about 1000 times more slowly than was L-leucine β -naphthylamide. Studies in the analytical ultracentrifuge using the sedimentation-equilibrium method of Yphantis (1964) gave a mol.wt. of $136\,000 \pm 13\,000$.

Our results indicate that alkaline phosphatase and the pH 7.5 peptidase are situated within the

microvillus and that arylamidase is located superficially. The results obtained from the electron microscope and the ultracentrifuge are consistent with the hypothesis that arylamidase is associated with the surface particles.

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The Presence of Pancreatic Proteases in Particulate Preparations of Rat Intestinal Mucosa

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During a study of peptidases of the intestinal brush border of the rat we observed that the hydrolysis of [¹²⁵I]iodinated insulin B-chain at pH 7.5 by a crude brush-border preparation was sensitive to inhibition by di-isopropyl phosphorofluoridate. This finding raised two possibilities: either the mucosa contained a serine peptidase of intestinal origin or it was contaminated with a serine peptidase derived from the pancreatic juice. The binding of pancreatic amylase to the mucosal surface of the small intestine has been reported (Ugolev, 1960; Jesuitova, De Laey & Ugolev, 1964). Our results suggest that pancreatic proteases may be bound in a similar manner.

Rats were anaesthetized and the intestinal lumen of each was irrigated with 0.9% NaCl. The small intestine was then excised, washed extensively and the mucosal lining extruded by pressure. The mucosa was homogenized in 0.33 M-sucrose and a microsomal fraction prepared. About 40% of the mucosal alkaline phosphatase was present in this fraction. Although it contained an active peptidase hydrolysing iodinated insulin B-chain, and would also attack elastin-Congo Red, it was almost inactive against synthetic substrates for chymotrypsin and trypsin. Peptidase activity was 50% inhibited by 0.05 mM-di-isopropyl phosphorofluoridate and about 60% inhibited by 1.4 mM-1-chloro-4-phenyl-3-toluene-*p*-sulphonamidobutan-2-one. Soya-bean trypsin inhibitor (1 mg./ml.) had no effect.

When the brush-border preparation was solubilized with 0.2% deoxycholate and passed through a column of CM-cellulose at pH 7.5, a portion of the peptidase was retained. This portion could be eluted by a 0.1–0.5 M-NaCl gradient. It proved to be sensitive to di-isopropyl phosphorofluoridate and 1-chloro-4-phenyl-3-toluene-*p*-sulphonamidobutan-2-one, in contrast with the peptidase, which was not retained by the column.