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

By A. J. KENNY, S. G. GEORGE and S. G. R. APARICIO. (*Departments of Biochemistry and Pathology, University of Leeds*)

We have previously reported that a neutral peptidase capable of hydrolysing [^{125} I]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.

This work was supported by the Science Research Council.

Kenny, A. J., Wong-Leung, Y. L. & Ingram, J. (1968). *Biochem. J.* **110**, 6P.

Wong-Leung, Y. L., George, S. G., Aparicio, S. G. R. & Kenny, A. J. (1968). *Biochem. J.* **110**, 5P.

Yphantis, D. A. (1964). *Biochemistry*, **3**, 297.

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

By J. F. WOODLEY and A. J. KENNY. (*Department of Biochemistry, University of Leeds*)

During a study of peptidases of the intestinal brush border of the rat we observed that the hydrolysis of [^{125} I]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.

Surgical ligation of the bile duct, into which the pancreatic ducts drain (Colwell, 1950), was performed on two groups of rats. In one group the ligature was placed at the junction with the duodenum. In the other group it was above the points of entry of the pancreatic ducts. Seven days after ligation the peptidase in mucosal preparations from both operated groups had become insensitive to di-isopropyl phosphorofluoridate. In some experiments, but not in all, the low ligation had diminished the recovery of peptidase without affecting the recovery of alkaline phosphatase. Although the interpretation of these experiments is complicated by the unexpected similarity in the effects of low and high ligation of the bile duct and to some extent by variable recoveries of enzyme activity, we conclude that part of the peptidase activity in the brush border is attributable to pancreatic proteases, of which chymotrypsin and elastase are probably implicated.

J.F.W. holds a Science Research Council Research Studentship.

Colwell, A. J. (1950). *Amer. J. Physiol.* **164**, 812.

Jesuitova, N. N., De Laey, P. & Ugolev, A. M. (1964). *Biochim. biophys. Acta*, **86**, 205.

Ugolev, A. M. (1960). *Nature, Lond.*, **188**, 588.

Primary Phase of Rennin Action on Whole Milk

By G. SINKINSON and J. V. WHELOCK. (*School of Biological Sciences, University of Bradford, Bradford 7*)

The coagulation of milk by the enzyme rennin (EC 3.4.4.3) is considered to be a two-stage process (Cherbuliez & Baudet, 1950). During the primary phase κ -casein is hydrolysed by the enzyme (Waugh & Hippel, 1956; Wake, 1959) and then in the presence of Ca^{2+} the casein micelles are precipitated (Pyne, 1953). When the κ -casein is hydrolysed peptides and glycopeptides are released (Jollès, Alais & Jollès, 1961). These glycopeptides contain almost all of the sugars attached to the κ -casein and are soluble in 12% (w/v) trichloroacetic acid (Armstrong, Mackinlay, Hill & Wake, 1967). It is expected therefore that the peptides would be present in the 12%-(w/v)-trichloroacetic acid filtrate of whole milk after the action of rennin. The increase of nitrogen in the 12%-(w/v)-trichloroacetic acid filtrate has been used as a method of studying the primary phase of rennin action in whole milk (Alais, Mocquot, Nitschmann & Zahler, 1953). In an attempt to substantiate this approach we are investigating the carbohydrates that are attached to these glycopeptides.

The carbohydrates are split from the peptide and from each other by methanolysis under

anhydrous conditions (Clamp, Dawson & Hough, 1967). Trimethylsilyl derivatives are then prepared and estimated by g.l.c. by using an adaptation of the method of Clamp *et al.* (1967).

To date, we have identified *N*-acetylneuraminic acid, D-galactose and 2-acetamido-2-deoxy-D-galactose on the glycopeptides. These sugars are the same as those identified in κ -casein (Alais & Jollès, 1961) and for the first time demonstrate that the 2-amino-2-deoxy-D-galactose is present as the *N*-acetyl derivative. Other sugars appear to be present in trace amounts, but as yet these have not been identified conclusively.

As the sugars are released at about the same rate as the nitrogen, the results confirm that the primary phase in whole milk consists at least of the splitting of κ -casein with the release of glycopeptides soluble in 12% (w/v) trichloroacetic acid. Our results also suggest that the sugar moiety of κ -casein may be more complex than suspected previously.

We thank the Agricultural Research Council for a grant to purchase a gas-liquid chromatograph. G.S. holds a University of Bradford Studentship.

Alais, C. & Jollès, P. (1961). *Biochim. biophys. Acta*, **51**, 315.

Alais, C., Mocquot, G., Nitschmann, H. & Zahler, P. (1953).

Helv. chim. Acta, **36**, 1955.

Armstrong, C. E., Mackinlay, A. G., Hill, R. J. & Wake, R. G. (1967). *Biochim. biophys. Acta*, **140**, 123.

Cherbuliez, E. & Baudet, P. (1950). *Helv. chim. Acta*, **33**, 1673.

Clamp, J. R., Dawson, G. & Hough, L. (1967). *Biochim. biophys. Acta*, **148**, 342.

Jollès, P., Alais, C. & Jollès, J. (1961). *Biochim. biophys. Acta*, **51**, 309.

Pyne, G. T. (1953). *Chem. & Ind.* **72**, 302.

Wake, R. G. (1959). *Aust. J. biol. Sci.* **12**, 479.

Waugh, D. F. & Hippel, P. H. von (1956). *J. Amer. chem. Soc.* **78**, 4576.

Action of Rennin on Heat-Sterilized Milk

By E. J. HINDLE and J. V. WHELOCK. (*School of Biological Sciences, University of Bradford, Bradford 7*)

When milk is heated there is an increase in the rennin coagulation time (Powell, 1935), which is particularly marked in milk that has been subjected to the ultra-high-temperature heat-sterilization process (180° for 3 sec. preceded by holding at 80° for 7 min.). It has been established that the coagulation phase is retarded by prior heating because of a decrease in the concentration of free Ca^{2+} in the milk (Pyne, 1945). Kannan & Jenness (1961) have concluded that the primary phase may also be affected by heat treatment of the milk. We have used the experimental approach described in the preceding communication (Sinkinson &