A Comparison of the Properties of Pure Human Pepsins 1, 2, 3 and 5

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Pepsins 1, 2, 3 and 5 were isolated from the histamine-stimulated gastric juice of individual patients by the method of Etherington & Taylor (1971). Qualitative agar-gel electrophoresis confirmed the homogeneity of each isolated enzyme. The yields were small, ranging from 10 to 100 μ g.

Pepsin 1, the pepsin migrating most rapidly towards the anode (Etherington & Taylor, 1967), is secreted in increased amounts in patients with peptic ulcer (Taylor, 1970). Its pH-activity curve, determined with preparations from three individuals, showed a relatively sharp single maximum at pH 1.9 with bovine haemoglobin as substrate. Pepsin 2 gave a single maximum, but at pH 2.1, with each of two preparations. Pepsin 3, the principal human pepsin, showed a broader pH-activity curve with a plateau-type maximum in the pH range 2.4–2.8. Pepsin 5 showed a plateau of fairly constant activity over the pH range 2.0–3.6, but with the highest point occurring between pH 2.8 and 3.4.

The sites of action of all four pepsins on the B-chain of oxidized insulin were investigated at pH 1.7 and pH 3.5. At pH 1.7 pepsins 1 and 2 showed similar specificities. The major sites of action were at Phe-25-Tyr-26, Leu-15-Tyr-16, Tyr-16-Leu-17 and Leu-11-Val-12. These sites were generally similar to those of pepsin 3, but the specificity of pepsin 5 differed (Etherington & Taylor, 1971). At pH 3.5 the overall rate of peptide-bond cleavage for pepsins 1 and 2 was much diminished, as would be predicted from the pH-activity curves. For pepsins 3 and 5 the rates were relatively unaffected. The specificity of each of the four enzymes did not differ significantly at the two different pH values.

Molecular-weight determinations by sodium dodecyl sulphate-polyacrylamide-gel electrophoresis (Weber & Osborn, 1969; Dunker & Rueckert, 1969) gave values of 43 800 for pepsin 1, 39 950 for pepsin 2 and 37 150 for pepsin 3. Each value is the mean for three different preparations. A single observation for pepsin 5 gave a value of 34 600.

Pepsin 1 is more readily inactivated above pH 6.0 than are the other pepsins.

There is thus a relationship between the electrophoretic mobility of the pepsins, the position of the pH maxima and increasing molecular weight.

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Isoenzymes of Acid Phosphatase in Human Endometrium

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Many observers have noted a rise in acid phosphatase (EC 3.1.3.2) activity in human endometrium during the second half of the menstrual cycle. This increase is thought to reflect the influence of progesterone.

Fresh endometrial specimens, obtained from patients undergoing diagnostic curettage or from hysterectomy material, were used in a study of the activity and isoenzyme pattern of acid phosphatase throughout the menstrual cycle.

Endometrium was homogenized in ice-cold 0.25 M-sucrose solution. A portion of the homogenate was treated with Triton X-100 (0.1%) and allowed to stand for 15 min at 4°C. Then both samples were centrifuged at 900g for 10 min. Diluted samples of the resulting supernatant were used for assay of the total acid phosphatase activity with 4-methyl-umbelliferyl phosphate (0.2 mM) as substrate by the method of Robinson & Willcox (1969).

Polyacrylamide-gel electrophoresis at pH 9.5 was carried out essentially as described by Smith *et al.* (1968). Samples were applied in 40% sucrose solution. Acid phosphatase activity was detected by using an azo-dye coupling technique with α -naphthyl phosphate as substrate. In a few experiments 4-methyl-umbelliferyl phosphate was used and yielded similar results.

NaF (10 mM) and L-(+)-tartrate (25 mM), known inhibitors of certain acid phosphatases, were used in some experiments.

A soluble fraction was prepared in a few instances by centrifuging a 900g supernatant, from a 0.25 M-sucrose homogenate, at 105 000g for 1 h.

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin (Sigma Chemical Co., St. Louis, Mo., U.S.A.) as standard.

Variations in acid phosphatase activity from tissue to tissue were similar whether related to wet weight or mg of protein. NaF inhibition was 80–100% and that by L-(+)-tartrate was 30–50%.

Three distinct patterns emerged as a result of electrophoresis of 900g supernatants. (a) A single origin band: Triton X-100 (0.5%) effected the complete penetration of this band into the gel.

(b) Three bands, namely an origin band and two bands migrating towards the anode: Triton X-100 had the same effect on the origin band as in (a) but did not seem to affect the other two bands. (c) Two bands, namely an origin band and one anodic band: again Triton X-100 had a similar effect to that in (a) and (b). The bands were completely abolished by 10mM-NaF whether α -naphthyl phosphate or 4-methylumbelliferyl phosphate was used as substrate. Blood content of the tissues did not contribute significantly to the acid phosphatase pattern or activity.

Preliminary evidence suggests that extracts having a relatively high L(+)-tartrate inhibition (45–50%) also had strong anodic bands (i.e. those not mobilized by Triton X-100) that were completely abolished by this inhibitor. Electrophoresis of the 105000g pellet and supernatant suggested that the fastest anodic band was cytoplasmic rather than lysosomal or microsomal.

It is concluded that human endometrium may contain up to three isoenzymes of acid phosphatase. Work is in progress to relate the isoenzyme pattern and total activity with physiological and clinical states.

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The Order of Addition of Substrates to Yeast Hexokinase

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There is much controversy whether the addition of substrates to hexokinase is ordered or random, a very recent paper concluding that it is the latter (Rudolph & Fromm, 1971). We have therefore investigated this aspect of the reaction by flux techniques (Britton, 1966). This approach leads to independent tests that may be interpreted relatively unequivocally.

The ratio (flux of material from glucose 6-phosphate to ATP)/(flux from glucose 6-phosphate to glucose) when the ATP and glucose concentrations are altered in turn should vary characteristically according to the way in which ATP and glucose add to the enzyme. The ratio will not be affected by the glucose 6-phosphate concentration. Measurements were made at equilibrium, and therefore the con-

centrations of ATP and glucose 6-phosphate and those of glucose and glucose 6-phosphate were varied in pairs to keep the concentrations of the other substrates constant. In general for random addition the flux ratio plotted against ATP should yield a hyperbola with the ratio increasing from a value less than unity to a plateau greater than unity and a similar curve should be obtained when the reciprocal of the ratio is plotted against glucose. For ordered addition with glucose adding first the flux ratio should be independent of glucose concentration and should increase linearly from unity with ATP concentration. If part of the reaction should proceed via addition of ATP before glucose and if the step:

should be very fast the flux ratio will vary as described for ordered addition except that it will reach a maximum at high ATP concentrations. The contribution of the pathway can be calculated from this value.

Flux measurements were made with ^{32}P - and ^{14}C -labelled substrates at pH 6.5 in imidazole buffer; ionic strength (0.2) and the concentration of free Mg^{2+} (5.00mM) were kept constant. The flux ratio was independent of glucose concentration (1.50 ± 0.09 for 0.1–0.7mM-glucose), but it increased linearly from unity at zero ATP to 5 at 1.2mM-ATP. It is concluded that the addition of substrates to yeast hexokinase is essentially ordered. If any ATP should add first to the enzyme this pathway would seem to contribute considerably less than 10% to the overall reaction.

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Criteria of De-adherence of Hydrophobic Associations in Proteins

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Reversible and irreversible protein denaturations can take place during the processes of separation and isolation, and these can give rise to additional bands, sediments or peaks that could be mistaken for new compounds or 'artifacts'. Recognition of denatured states is therefore desirable, and certain physico-chemical properties such as hyperchromicity, lowering of optical rotation and corresponding changes in circular dichroism have been utilized in this connexion. These, however, can result primarily from changes that would involve both de-adherence of