Dipeptidyl Peptidase IV, a Kidney Microvillus Serine Proteinase: Evidence for its Large Subunit Molecular Weight and Endopeptidase Activity

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Dipeptidyl peptidase IV was first described by Hopsu-Havu & Glenner (1966) and purified from kidney by Hopsu-Havu et al. (1968). Enzymes of this class (for a review, see McDonald et al., 1971) hydrolyse dipeptides from the N-terminus of susceptible substrates. Barth et al. (1974) demonstrated that dipeptidyl peptidase IV exhibits two specificities, involving substrates of the types X-Pro-Y and X-Ala-Y, and that it is sensitive to inhibition by di-isopropyl phosphorofluoridate. The enzyme is one of a group of membrane-bound peptidases located in the microvilli of the proximal convoluted tubule (Booth & Kenny, 1974).

Starting with pig kidney cortex, dipeptidyl peptidase IV was solubilized by autolysis at pH 3.8, and purified 300-fold by chromatography on columns of CM-cellulose, DEAE-cellulose, Sepharose 6B and hydroxyapatite, each step being monitored by an assay using Gly-Pro-2-naphthylamide as substrate. The preparation moved as a single band on polyacrylamide-gel electrophoresis and appeared to be homogeneous in the analytical ultracentrifuge. When either the microvillus membrane (Booth & Kenny, 1976) or a portion of the solubilized extract at an early stage in the purification was labelled with di-isopropyl [32P]phosphorofluoridate, the only labelled protein was found to co-chromatograph with the active enzyme.

Molecular-weight determination

A value of 130000 for the apparent subunit mol.wt. was obtained by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (Booth & Kenny, 1976). It was also possible to use the reaction of the enzyme with di-isopropyl [32P]phosphorofluoridate as a method for determining the active-site-subunit mol.wt. This was done in two experiments in which the specific radioactivity of the labelled enzyme was compared with the specific radioactivity of the reagent and with the specific radioactivity of two other serine proteinases, trypsin and chymotrypsin, labelled concurrently with dipeptidyl peptidase IV. The mol.wt. estimates were in the range 102000–164000 (mean 138 125).

Gel filtration on columns containing Sepharose 6B, calibrated with suitable marker proteins, gave a mean value for mol.wt. of 297000 (four experiments). Sedimentation-equilibrium experiments in the analytical ultracentrifuge gave an apparent mol.wt. of 240000. These results indicate that the purified enzyme is a dimer comprising two active subunits, each of mol.wt. about 130000. Electron micrographs of the purified enzyme negatively stained with uranyl acetate confirmed that dimers were the predominant form.

Specificity

The purified enzyme hydrolysed Ala-Ala-2-naphthylamide at rates that were in the range 3–6 % of those obtained with Gly-Pro-2-naphthylamide as substrate, depending on the conditions of the assay. Crude preparations taken at early stages in the purification showed more activity towards the alanine substrate (10–20 %), but this was mainly attributable to the presence of aminopeptidase M (EC 3.4.11.2), an enzyme that exhibits high hydrolysis rates with substrates containing N-terminal alanine residues. Careful monitoring at each stage of one purification with both types of substrate gave no hint that the two activities could be resolved. At present one must assume that they are attributable to the same enzyme.

Two peptides with blocked α-amino groups were tested as endopeptidase substrates for dipeptidyl peptidase IV. A portion (0.5–0.9 mg) of each peptide was incubated with
0.2–0.3 mg of enzyme for 4 h at 37°C. The products were separated by high-voltage paper electrophoresis at pH 1.85. The N-terminal amino acids and the amino acid composition (after hydrolysis with 6 M-HCl) of the eluted products were identified as the dansyl derivatives. Z-Gly-Pro-Leu-Gly-Pro yielded one ninhydrin-positive product, with leucine N-terminal and containing leucine and glycine and proline. Thus the -Pro-Leu- bond had been hydrolysed. The other substrate, Z-Gly-Pro-Gly-Gly-Pro-Ala, gave rise to three ninhydrin-positive products. The first had glycine at the N-terminus and contained leucine, glycine and proline. The second was also a glycine N-terminal peptide, but contained alanine in addition to glycine and proline. The third product proved to be alanine. These results are consistent with the hydrolysis of the -Pro-Gly- and -Pro-Ala- bonds. No products were found when the incubations were repeated in the presence of 0.1 mM-diisopropyl phosphorofluoridate.

Dipeptidyl peptidase IV is a kidney microvillus-membrane serine proteinase that when solubilized exists as a dimer of subunit mol. wt. 130000 and that exhibits endopeptidase as well as dipeptidyl peptidase activity.

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