Abstract

Dipeptidyl peptidase IV (DPP IV, CD26) is a homodimeric type II membrane protein that is found on the cell surface of many solid tissues and different subtypes of lymphocytes in mammals. DPP IV is a member of the prolyl oligopeptidase family (S9) within the clan SC of non-classical serine peptidases. DPP IV is known to exhibit a well characterized exopeptidase activity specific for proline residues. This doctoral thesis describes a novel, specific endopeptidase activity of DPP IV. The enzyme was isolated in a three step purification procedure that included concanavalin A chromatography and immunoaffinity chromatography. The purified DPP IV was submitted to both a gelatin zymography assay and a soluble proteolytic assay in order to demonstrate and investigate its endopeptidase activity. Substrate specificity was detected for denatured fibrillar collagens (types I, II, III and V). Denatured basement membrane collagen type IV was also cleaved, but at a lower rate, whereas native collagens, albumin, fibronectin and the enzyme itself were not digested by DPP IV at all. 1 mg DPP IV was found to degrad 7 x 10^{-6} µmol of denatured type I collagen per min under optimal conditions of 37°C at pH 7.4. Cleavage products were detected on immunoblots as multiple peptide bands in a stepladder pattern, suggesting that DPP IV recognises multiple cleavage sites within the collagen chains. Endo- and exopeptidase activities of DPP IV showed the same peptidase inhibitor profile, including similar inhibition by DFP, PMSF and diprotin A and B, which suggests that both activities of DPP IV reside in a single active site. Immunohistochemical studies using anti-collagen-pAb revealed an accumulation of fibrillar collagen structures in the space of Disse of DPP IV-deficient Fischer-344-rats, indicating a disturbed collagen metabolism in these animals. The biological relevance of DPP IV endopeptidase activity should be seen in context with other collagenases and gelatinases, as well as with DPP IV exopeptidase activity. Therefore, DPP IV might participate in processes such as final collagen degradation, cellular adhesion to collagens, cellular translocation through the ECM and resorption of proline containing peptides.