Dipeptidyl peptidase IV in human lymphocytes: molecular properties

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Dipeptidyl peptidase IV (DPP IV; EC 3.4.14.5), an ectoenzyme on the cell membranes of T-lymphocytes, has been shown to play an important role in the regulation of lymphocyte proliferation and activation. Evidence for this has been provided by the use of specific inhibitors, which revealed (a) a suppression of the proliferation of peripheral blood lymphocytes by mitogenic lectins [1], and (b) a decrease in the production of certain lymphokines, namely interleukin 2 and γ-interferon [2]. In addition, we have reported a rise in the specific activity of DPP IV in lymphocytes stimulated by phytohaemagglutinin [3]. Despite the increasing evidence suggesting that DPP IV, like other cellular proteinases, is involved in lymphocytic activation, its exact role remains unknown. Recently, four monoclonal antibodies (TII 19-4-7, 4EL1C7, B1.19.2 and TS145) were found to form a new serological cluster, named CDw26. Two of them, TII 19-4-7 (anti-DPP IV) and 4EL1C7 (anti-Tal), recognize the same or partly identical epitopes on the antigen DPP IV [4]. The use of these monoclonal antibodies will facilitate new investigations concerning DPP IV on cell surfaces. In this study we reveal some of the biochemical and molecular properties of lymphocytic DPP IV. The information obtained will be of additional aid in further defining the role of DPP IV in the immune system.

For the determination of DPP IV activity we used a sensitive fluorometric assay based on hydrolysis of Gly-Pro-4-methoxy-2-naphthylamide [5]. Lymphocytes were isolated fromuffy coats from healthy volunteers. After diluting twice with physiological saline containing 0.3% tri-sodium citrate and subsequent twofold dilution in Plasmasteril (Fresenius, Bad Hamburg, F.R.G.), the cells were sedimented, the resulting supernatant was centrifuged twice with physiological saline containing 0.3% tri-sodium citrate. The supernatant was centrifuged at 70°C.

For the solubilization of DPP IV from the cells, different methods were evaluated, namely sonication, freezing and thawing, and treatment with the non-ionic detergents 1-0-n-octylglucopyranoside, Nonidet-P40 (LKB, Bromma, Sweden) and Triton X-100 (Aldrich Chemical Co., Steinheim, F.R.G.). Detergent treatment resulted in the production of mononuclear blood cells by Lymphoprep (Nymocmed, Oslo, Norway) gradient centrifugation. The mononuclear cells were aspirated and washed twice with Ca²⁺- and Mg²⁺-free phosphate-buffered saline. After the last centrifugation step, the cell pellets were stored at −70°C.

In order to enrich DPP IV, we evaluated different chromatographic procedures (all gels used were from Pharmacia Fine Chemicals, Uppsala, Sweden). Since only small amounts of crude material are available, we were interested in highly selective separation techniques like affinity chromatography. For the purification of DPP IV from other sources, Gly-Pro-AH-Sepharose 4B is often used. After the carbodi-imide method reported earlier [6], we coupled Gly-Pro to the AH-Sepharose 4B gel. In comparative studies, at different stages of the purification, we could not detect significant differences in the interaction of DPP IV with the coupled and the original gel. Therefore the expensive and time-consuming coupling procedure was omitted. Using AH-Sepharose 4B as a first chromatographic step in the purification scheme, we could reach a four-fold purification with a recovery of 80%. By means of affinity chromatography, we proved that lymphocytic DPP IV, like the enzyme from other biological materials, is a glycoprotein with a high affinity for concanavalin A, suggesting the presence of α-D-glucosyl or sterically related residues on DPP IV. Affinity chromatography on concanavalin A-Sepharose 4B was a useful second purification step.

The binding of the enzyme to wheat germ lectin Sepharose 6 MB indicates that it contains N-acetylglucosamimyl groups. Gel filtration on Sephacryl S-300 HR removed some high and a significant amount of low-Mₙ, contaminating proteins. The DPP IV activity eluted as a single symmetrical peak. The column was calibrated with the high-Mₙ markers from Pharmacia. The Mₙ of the native, detergent-solubilized DPP IV determined by gel filtration (Sephacryl S-300 HR, eluting buffer 0.2 M-Tris/HCl, pH 7.4) was 264 kDa (mean of three experiments). In chromatofocusing on Polybuffer exchanger 94, using Polybuffer 74, the enzyme eluted at a pH between 5.0 and 5.1, which can be considered as the isoelectric point of the native enzyme under the assay conditions described.

The partially purified enzyme was treated with the reduc- ing agents dithiothreitol and mercaptoethanol for 2 h at 20°C at final concentrations of 0.1 M and 0.25 M, respectively, both in the presence of 1 mm EDTA. These reducing circumstances did not provoke enzyme inactivation. Even in the presence of 6 M-urea, treatment with the reductants only resulted in 40% inactivation of the enzyme. In contrast, the native structure was perturbed to a much greater extent by guanidinium chloride, as illustrated in Fig. 1. Incubations were carried out at 20°C, for 45 min and 12 h. Complete inactivation was obtained by 6 M-guanidinium chloride. Dialysis could not restore any enzymic activity. Other methods to induce renaturation are under current investigation. The stability of the native DPP IV towards proteases was studied. Papain (6500 units/mg, National Formulary, U.S.A.), proteinase K (27 mAnson units/mg from Merck, Darmstadt, F.R.G.), trypsin (47 Fédération Internationale...
pH-induced structural transitions in β-lactoglobulin

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β-Lactoglobulin, which occurs in the milk of many mammals, has been the subject of extensive study [1]. Of the four distinct crystal forms which have been investigated by X-ray crystallography [2], one has been characterized at high resolution [3]. The amino acid sequence has been determined [4] and there appear to be two disulphide bridges as well as a free thiol group.

β-Lactoglobulin from bovine milk has been shown to act as a substrate for guinea-pig liver transglutaminase at pH 7.5 [5]. The specificity of the enzyme for glutaminyl side-chains depends on the conformation of the polypeptide chain [6] and may be influenced by other features of the surrounding amino acid side-chains, such as charge, hydrophobicity, etc. [7]. We have used the effect of pH on the transglutaminase-catalysed reaction of primary amines with β-lactoglobulin to draw some conclusions regarding structural changes in the protein, and correlated these results with those obtained by c.d.

Typical conditions for the enzyme-catalysed reaction were: primary amine (putrescine or monodansylcadaverine), 5 mM; dithiothreitol, 1 mM; CaCl₂, 10 mM; β-lactoglobulin, 4 mg/ml; transglutaminase, 0.1 mg/ml (temperature 25°C). Incorporation of [14C]putrescine or dansylcadaverine was determined by scintillation counting using the filter disc method [8] or fluorescence spectroscopy, respectively. Reactions were performed in 50 mM-Mes buffer adjusted to the appropriate pH with NaOH.

The activity of the enzyme was also assayed using the colorimetric method of Folk & Cole [9] in which the reaction between Cbz-Gln-Gly and hydroxylamine is studied.

The rate of incorporation of [14C]putrescine into β-lactoglobulin is markedly dependent on pH. Thus after 1 h of reaction the sample incubated at pH 9.0 had incorporated 1.50 mol of amine/mol of β-lactoglobulin subunit. This value was reduced to 0.50 mol/mol at pH 7.5 and 0.07 mol/mol at pH 6.0. The highest incorporation observed (after 24 h reaction at pH 9.0) was 3.0 mol/mol. Similar incorporations were recorded using dansylcadaverine as the primary amine. The dependence of enzyme activity on pH was corrected for by dividing these rates by the activity of the enzyme in the Cbz-Gln-Gly assay at the appropriate pH. The results showed that above pH 6.0 there was a steady increase in the rate of incorporation of primary amine into β-lactoglobulin, presumably reflecting the increased accessibility of glutaminyl side-chains in the protein at the higher pH. Previous work (summarized in [1]) has shown that β-lactoglobulin undergoes a number of conformational transitions in the alkaline pH range). An analysis of the structure of β-lactoglobulin suggests that Gln-59, Gln-159 and Gln-162 may act as possible incorporation sites. There is a lack of continuous electron density beyond residue 149 [3], suggesting flexibility in the C-terminal region. Gln-59 could be exposed after rupture or rearrangement of the disulphide link between Cys-57 and Cys-70. However, these assignments require confirmation by isolation and sequencing of labelled peptides.

C.d. spectra of β-lactoglobulin were recorded over the pH range 5.5–9.0, in the presence and absence of 1 mM-dithiothreitol. In the absence of dithiothreitol considerable structure was retained over this pH range as shown by near-u.v. and far-u.v. spectra, although some differences were noted. In the presence of dithiothreitol there were marked changes in the protein between pH 7.0 and pH 9.0, which were associated with decreases in a-helical content (far-u.v.) and tertiary structure (near-u.v.). These changes might be related to the occurrence of disulphide interchange reactions which have been reported under these conditions [1].

2. Green, D. W., Aschaffenburg, R., Camerman, A., Coppola, J. C., Dunnill, P., Simmons, R. M., Komorowski, E. S., Sawyer, L.,