Pharmacuetique units/mg: Novo, Copenhagen, Denmark) and α-chymotrypsin (Choy, Paris, France) were, under appropriate conditions, added to the partially purified enzyme in final concentrations of 50 and 250 μg/ml and incubated at 37°C for different time intervals, ranging from 1 to 36 h. In all proteinase-treated preparations the DPP IV activity measured exceeded 90% of the original DPP IV activity in the samples. These results suggest that the native lymphocytic DPP IV is resistant towards proteinases.

Hydrophobic interaction chromatography was used to investigate the hydrophobic character of the enzyme. In the presence of 0.5% (v/v) (NH₄)₂SO₄ in 0.05 M-Tris/HC1/0.1% (v/v) Triton X-100, pH 7.4, the neutral gel phenyl-Sepharose CL-4B adsorbs DPP IV very strongly. Only 40% of the enzyme is eluted by the same buffer without any ammonium sulphate added. Incorporating 50% (v/v) ethylene glycol in the elution buffer resulted in an acceptable recovery (80%). Because of the poor differentiation between the adsorbed proteins in the strength of binding to phenyl-Sepharose CL-4B, this method was not incorporated in the purification scheme.

In summary, the detergent-solubilized DPP IV proved to be a hydrophobic glycoprotein, containing N-acetylglucosamine and α-α-glucosyl-related residues. Its apparent Mₐ, estimated by gel filtration, is 264 kDa and the isoelectric point, determined by chromatofocusing, is found to be situated between 5.0 and 5.1. The partially purified enzyme has a remarkably high stability towards reducing agents and proteinases. A completely purified human lymphocytic DPP IV will, by means of monoclonal antibodies, allow confirmation of its identity with CDw26.

We gratefully acknowledge the support of the Belgian NFWO and IWONL. G.V. is a recipient of an IWONL grant and I. De M. is a research assistant of the NFWO.


Received 22 November 1989

pH-induced structural transitions in β-lactoglobulin

PETER J. COUSSENS, SHARON M. KELLY and NICHOLAS C. PRICE

Department of Biological and Molecular Sciences, University of Stirling, Stirling FK9 4LA, Scotland, U.K.

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.a.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 incorporation was 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.a.d. spectra of β-lactoglobulin were recorded over the pH range 5.5–9.0, in the presence and absence of 1 mol 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., Camerann, A., Coppola, J. C., Dumnill, P., Simmons, R. M., Komorowski, E. S., Sawyer, L.,
Presence of a membrane attack complex inhibiting protein on the human epithelial cell line HeLa

B. PAUL MORGAN, MARK G. OLAVESEN and MARIA J. WATTS

Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

Several groups have recently reported the presence on the membranes of erythrocytes and other circulating cells of proteins which inhibit lysis by the membrane attack complex of complement. At least two distinct proteins have been described, one with a molecular mass about 65 kDa [1-3], the other of molecular mass 20 kDa [4-6]. Neither protein has been identified on cells other than those in the blood. The aim of the present study was to investigate whether the 65 kDa membrane attack complex inhibiting protein (MIP) was also present on cells outside the vascular space. The human epithelial cell line HeLa was chosen for the initial studies because the cell-surface complement inhibitor decay-accelerating factor, which is similarly distributed on circulating cells, has previously been identified on this cell and on a variety of other epithelial and endothelial tissues [7, 8]. In the former study HeLa cells were also shown to secrete a soluble form of decay-accelerating factor into the fluid phase. We have identified a soluble form of MIP in serum and other biological fluids [9, 10], and were therefore also interested in examining whether HeLa cells secreted soluble MIP.

HeLa cell membranes were isolated, detergent-solubilized, run on an SDS gel and Western blotted. The blots were developed with a monoclonal anti-MIP antibody (G3) and a peroxidase-labelled second antibody. As shown in Fig. 1(A), a single major MIP-reactive band was detected at about 55 kDa, the same molecular mass as erythrocyte-derived MIP when run under non-reducing conditions.

Secretion of MIP by HeLa cells was assessed using a two-site enzyme-linked immunosorbent assay [10]. Cells were washed into serum-free medium and incubated at 37°C, portions of supernatant being removed at intervals for assay. MIP-reactive material was detectable in the supernatant within 1 h and rose slowly throughout the 8 h time course. Beyond 8 h cell viability was impaired so no further measurements were made. Actinomycin D, at a final concentration of 5 μg/ml, blocked release of MIP-reactive material, providing evidence that MIP appearing in the supernatant was the product of protein synthesis de novo and secretion.

In order to further examine MIP synthesis by HeLa cells, and in particular, to determine whether the major portion of the synthesized protein was secreted or membrane-associated, biosynthetic labelling studies using [35S]methionine were performed. Cells in methionine-free medium were pulse-labelled with [35S]methionine for 2 h at 37°C, then washed and placed in medium containing unlabelled methionine. At intervals, pairs of wells were harvested, the supernatant saved and the cells were solubilized. MIP was immunoprecipitated from the supernatant and the solubilized cell membranes using solid-phase anti-MIP polyclonal antibody. The bound protein was eluted, run on an SDS gel under reducing conditions and radiolabelled protein identified by autoradiography. As shown in Fig. 1(B), a band of molecular mass 65 kDa, characteristic of MIP, was precipitated from the supernatant and the solubilized cell membranes. The time after pulse in hours is indicated in each lane. Molecular masses (in kDa) are shown on the left.

Abbreviation used: MIP, membrane attack complex inhibiting protein.

Fig. 1. Detection and biosynthesis of MIP in HeLa cells

(A) HeLa cell membranes were electrophoresed and blotted. The blots were developed with a monoclonal anti-MIP antibody (G3) and a peroxidase-labelled second antibody. As shown in Fig. 1(A), a single major MIP-reactive band was detected at about 55 kDa, the same molecular mass as erythrocyte-derived MIP when run under non-reducing conditions.

(B) HeLa cells were pulse-labelled with [35S]methionine and labelled proteins were immunoprecipitated from cells (C) and supernatants (S) at intervals during the chase. The time after pulse in hours is indicated in each lane. Molecular masses (in kDa) are shown on the left.

Received 17 November 1989