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Rapid purification and properties of human glycodelin (endometrial α_2-globulin)

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Abstract

The method presented can easily produce milligram amounts of glycodelin from pregnancy endometrium, with a 19% yield. It involves anion-exchange chromatography, gel permeation and chromatofocusing; it results in one stainable band at M_r 28 000 after sodium dodecyl sulphate–polyacrylamide electrophoresis, as well as after immunoblot analysis, performed using an affinity-purified IgG fraction from an antiserum against glycodelin. In spite of this, the corresponding gel isoelectric focusing pattern gives four stainable bands with p*I* values between 4.55 and 5.2. Western immunoblot analysis of tissue extracts indicates the presence of glycodelin epitopes associated with materials heavier than the native protein. Circular dichroism spectra of the highly purified protein in water solutions indicate a large amount of β-sheet conformation, whereas those obtained with different proportions of 2-propanol in water, show an increased proportion of α-helix conformation.

Keywords: Preparative chromatography; Glycodelin; Glycoproteins; Globulins

1. Introduction

Glycodelin is the new name of a group of at least two glycoproteins with identical primary structure, which differ in their *N*-glycan structures [1].

Glycodelin, today designated as glycodelin-A (GdA), is a major endometrial glycoprotein synthesized in high quantities after implantation of an embryo [2], is secreted in high concentrations into the amniotic fluid of the first trimester pregnancy [2–4] and also, at much lower levels, into the blood circulation [2,5]. Further studies have shown that glycodelin is constitutively expressed by precursors of the erythroid lineage in normal human bone marrow [6] and by normal human megakaryocytes and platelets [7] and – although it was originally purified from human term placenta and designated as 'placental protein 14' [8] – is absent from the fetal side of the placenta [9,10]. A seminal plasma analogue, designated as glycodelin-S (GdS) has been recently isolated; it completely cross-reacts with antibodies to GdA from human amniotic fluid [11]. It is interesting that glycosylation seems to be gender-specific: GdA inhibits binding of human sperm to zona pellucida, whereas GdS allows it [11]. In addition, it is possible that gametes and the developing human embryo are protected by the immunosuppressive effects of glycodelin [12].

Three different methods have been described for

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the purification of glycodelin from pregnancy decidual cytosols: the procedure described by Bell [13], however, is long and complex; that followed by Bolton et al. [14] can only produce microgram amounts of the protein, whereas Westwood et al. [15] have utilized reversed-phase chromatography which yields the protein's subunits.

Glycodelin has also been purified from mid-trimester amniotic fluid with high yields by anion-exchange chromatography and gel permeation followed by reversed-phase high-performance liquid chromatography (HPLC) or by a combination of negative anti-hCG immunochromatography and hydrophobic interaction chromatography [16]. The same group also used positive immunochromatography for a single-step glycodelin extraction [17]. A potential problem with this procedure is that changes of the secondary structure of the protein might be induced by the chromatographic use of water–organic solvent mixtures or by the use of acidic and/or high-ionic strength solutions.

Data on the immunosuppressive properties of glycodelin [14,18,19], potential linkages to the human feto-embryonic defence system [12] and potential applications in fertility regulation [11], have increased the need for a simple and fast method capable of producing relatively large quantities of pure, native glycodelin.

With this aim, we have set-up a simple and rapid chromatographic procedure for obtaining, on a preparative scale, pure, native glycodelin and further defined its physico-chemical properties.

2. Experimental

2.1. Biolological material

Endometrial (decidual) tissue of the first trimester of pregnancy was obtained from women undergoing voluntary termination of pregnancy.

2.2. Purification procedure

All operations were performed at 4–8°C, unless otherwise specified.

2.2.1. Preparation of the immunogen

In early experiments, aimed at obtaining a suitable immunogen, purification of glycodelin was essentially carried out as already described [13]. This procedure involved DEAE-Sephacel anion-exchange (NaCl stepwise, increased to 0.14 *M*), Concanavalin A (ConA-)–Sepharose (Pharmacia-Biotech Europe, Brussels, Belgium) affinity chromatography (with stepwise increases to 0.5 *M* methyl-α-D-glucopyranoside) and gel filtration through Ultrogel AcA-44 (IBF, Villeneuve-la-Garenne, France).

After complete purification (see Section 3), the resulting homogeneous material was used for rabbit immunization to obtain suitable antisera to be utilized during the process of selecting a simplified method of purification.

2.2.2. Improved procedure

The procedure selected as final involved the fractionation and purification of endometrium extracts by a combination of chromatographic procedures according to the sequence: DEAE-Sephacel, Superdex-75 and Polybuffer exchanger (PBE) 94/Polybuffer (PB) 74 chromatofocusing (all from Pharmacia-Biotech).

In a typical experiment, 40 g of endometrial tissue were homogenized in 100 ml of ice-cold water in an Ultra-Turrax homogeneizer (Janke & Kunkle Staufen, Germany) at low speed for 1 min and completed in a PTFE–glass Potter homogeneizer (Thomas Scientific, Swedesboro, NJ, USA) at low speed with cooling for 2 min. The homogenate was centrifuged at 15 000 *g* for 90 min and the supernatant desalted by gel permeation through Sephadex G-25 (Pharmacia-Biotech) equilibrated and eluted in 0.1 *M* Tris–buffer solution, brought to pH 8.0 with HCl. The resulting 1200 mg of protein material were applied to a column (40×2.6 cm I.D.) of DEAE-Sephacel, equilibrated in the same Tris–HCl buffer solution. The chromatogram was developed with 550 ml of Tris–HCl buffer, with 500 ml of a 0 to 0.2 *M* NaCl gradient in the same buffer and then with 0.2 *M* NaCl always in the same buffer.

Fractions with the highest glycodelin immunoreactivity were pooled, concentrated to a small volume by ultrafiltration in an Amicon stirred-cell, equipped with a 4.5-cm diameter YM 10 membrane

(Amicon, Danvers, MA, USA), further desalted by Sephadex G-25 chromatography in 0.03 *M* NH_4HCO_3 solution and lyophylized.

At this stage, 60 mg of proteins, reconstituted in 1.5 ml of 0.1 *M* Tris, brought to pH 7.6 with HCl, made 0.1 *M* with KCl, were applied to 100×2.6 cm I.D. gel-permeation column filled with 477 ml of Superdex-75, calibrated with protein standards of known molecular mass (bovine serum albumin, M_r 67 000; egg albumin, M_r 45 000; carbonic anhydrase, M_r 29 000; myoglobin, M_r 17 000 and aprotin, M_r 6500); 1.5 ml fractions were collected.

Ten-milligram quantities of proteins from fractions containing immunoreactive glycodelin were desalted and lyophilized. Before use, they were dissolved in 2 ml 0.025 *M* imidazole–buffer solution (pH 6.6) and applied to a column of 7 ml of PBE-94 equilibrated and eluted with 21 ml of the same buffer solution and with 84 ml of PB-74–HCl, pH 4.0; 0.6 ml fractions were collected and pooled on the basis of SDS–PAGE and/or Western blot analysis [20]. Pools containing glycodelin only were mixed, concentrated on an Amicon cell equipped with a YM-30 membrane, washed with NH_4HCO_3 0.03 *M* and further freed from ampholytes by Sephadex G-75 gel permeation equilibrated and eluted in 0.03 *M* NH_4HCO_3 and then lyophilized.

This preparation was used as a standard in the immunochemical studies.

2.3. Antisera and immunoglobulins G

Antisera were obtained from adult male New Zealand white rabbits immunized with the antigen as described by Vaitukaitis et al. [21]. Specific immunoglobulins G (IgGs) were prepared, starting with 10 ml of the bleeding with the highest ELISA titre (A 4); they were chromatographed through Protein G-4 Fast Flow (Pharmacia-Biotech). Bound immunoglobulins were eluted with 0.1 *M* sodium citrate buffer solution, pH 3.0, and collected in tubes containing 1 *M* Tris–HCl, buffer at pH 9.0. The solution was neutralized and desalted by Sephadex G-25, equilibrated in 0.05 *M* NH_4HCO_3 and lyophilized. Ten-milligram quantities of the resulting powder were further purified by negative immunochromatography through a column of ActAcA (IBF), coupled to potentially contaminating antigens extracted from normal male serum by a procedure similar to that used for specific antigen preparation: DEAE-anion exchange (retarded fraction), followed by a ConA-Sepharose affinity-chromatography (bound fraction). The affinity-purified IgG fraction (A 4.1) was used as the specific antibody for all immunochemical studies.

2.4. Glycodelin analysis

2.4.1. Immunoassay

Immunological monitoring of chromatographic eluates was performed by a standard noncompetitive enzyme immunoassay [22]. Equal amounts of proteins from chromatographic eluates were absorbed onto polystyrene plates (flat-bottomed, maxisorp inununoplates; Nunc, Roskilde, Denmark). One microgram per well of specific IgG (A 4.1) followed by 100 μl anti-rabbit IgG (H+L)-HRP conjugate (Bio-Rad, Richmond, CA, USA), diluted 1:2000 as a second antibody, were used.

2.4.2. SDS–polyacrylamide gel electrophoresis

The PhastSystem was employed for SDS electrophoresis in polyacrylamide gel with a 10–15% gradient. Coomassie R-350 was used for staining. A low molecular mass calibration kit (Pharmacia-Biotech, code 17-0446-01) was used for relative mass determinations; it contains: phosphorylase B M_r=94 000; bovine serum albumin, M_r=67 000; ovalbumin, M_r=43 000; carbonic anhydrase, M_r= 30 000; soybean trypsin inhibitor, M_r=20 100 and α-lactalbumin, M_r=14 000.

SDS–PAGE and Coomassie R-250 staining of gels were performed on 0.75-mm thick polyacrylamide (12% w/v) gels in a Protean 11 vertical slab cell (Biorad), according to the specifications given by the manufacturer; α-lactalbumin, M_r=14 200; soybean trypsin inhibitor, M_r=20 100; trypsinogen, M_r= 24 000; carbonic anydrase, M_r=29 000; glyceraldheyde-3-phosphate-dehydrogenase, M_r= 36 000; egg albumin, M_r=45 000; bovine serum albumin, M_r=67 000 (Dalton Mark 11-L, Sigma, St. Louis, MO, USA) were used for relative mass determinations.

2.4.3. Isoelectric focusing

The PhastSystem (Pharmacia Biotech) was used to determine the isoelectric point of the purified antigen on a precast gel medium at polyacrylamide concentration of 5% in the pH range 4–6.5 (Pharmacia-Biotech). A calibration kit (Pharmacia-Biotech) containing a low range of p*I* markers stained with Coomassie R-350, was used. It contains: human carbonic anhydrase, p*I*=6.55; bovine carbonic anhydrase; p*I*=5.85; β-lactoglobulin, p*I*=5.20; soybean trypsin inhibitor, p*I*=4.44; glucose oxidase, p*I*=4.15; methyl red p*I*=3.75; amyloglucosidase, p*I*=3.5; pepsinogen, p*I*=2.80.

2.4.4. Western immunoblot

Western immunoblot was performed with the aid of the PhastSystem using the same SDS–PAGE (T=10–15%) indicated above for the first run. Nitrocellulose (Hybond C, 0.45 μm, Amersham International, Amersham, UK) served for protein transfer in the second run. Ponceau-S (0.2% in a 3% trichloroacetic acid solution) was used for protein staining. Hybond membrane saturation was then performed in a 3% (w/v) gelatin dissolved in 20 m*M* Tris solution, pH 7.5 containing 500 m*M* NaCl, before immunoprecipitation of the Ag by the anti-glycodelin IgG (1.65 μg/ml) in 1% (w/v) gelatin solution. Goat anti-rabbit IgG-HRP conjugate (Bio-Rad) was used as a second antibody as indicated by the manufacturer. In some experiments Western immunoblot was also performed using a Trans-Blot Cell (Bio-Rad) using 16×20-cm, 0.75-mm thick polyacrylamide (12% w/v) gel with the immunochemical reagents already described, according to the specifications given by the manufacturer.

2.4.5. Amino acid sequence determination

Samples were fractionated in 12% (w/v) SDS–PAGE and electroblotted onto ProBlott membranes (Applied Biosystems, Foster City, CA, USA) in 10 m*M* cyclohexylaminopropane sulfonate (CAPS) pH 11, containing 10% methanol as described by Matsudaira [23]. Membranes were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 50% methanol and then destained in 50% methanol. Stained protein bands were cut and sequenced in an applied Biosystems model 476A sequencer, using a Blott Cartridge (Applied Biosystems) with an optimized liquid-phase programme.

2.4.6. Circular dichroism spectra

Circular dichroism (cd) spectra were measured on a Jasco 500 A spectropolarimeter (Jasco International, Tokyo, Japan) connected to an IBM computer as data processor. Spectra were obtained for each sample in the far UV region between 200 and 250 nm at 24°C. Water and 2-propanol–water solutions (1:1, v/v) were used as protein solvents and as blanks, and the molar ellipticity [Θ] (degree×cm^2×dmol^{-1}) was calculated from $\Theta=\vartheta\times100\times l^{-1}\times c^{-1}$ where ϑ is the observed ellipticity, c is the glycodelin concentration in g/l, divided by the mean residue molecular mass of the amino acid moieties (116) and l is the pathlength in centimeters.

3. Results

3.1. Preparation of the immunogen

Following gel permeation through Ultrogel AcA-44, four glycodelin-immunoreactive fractions were obtained.

One of them, identified as Fraction 4 (Fig. 1a), corresponding to the native protein and containing the major part of the immunologically active material, was further purified by a second gel permeation through Ultrogel AcA-44.

The other three heavier fractions obtained following the first gel filtration also possess glycodelin immunoactivity, as shown in Fig. 1b.

Thus, all four fractions are immunologically active and show the same pattern in the Western Immunoblot, with a band migrating at M_r=26–27 000.

The homogeneity of the main product obtained following the second gel permeation of Fraction 4, was analyzed by SDS–PAGE in a homogeneous 12% gel where it displayed a pattern with a unique stainable band, when performed either on 10 μg of the sample prepared under non-reducing conditions or on 10 or 15 μg of the same 2-mercaptoethanol-reduced material. Such a band of homogeneous protein had a M_r of 26 000, this value being derived from the equation which fitted (with r=0.983, $t(5)$=

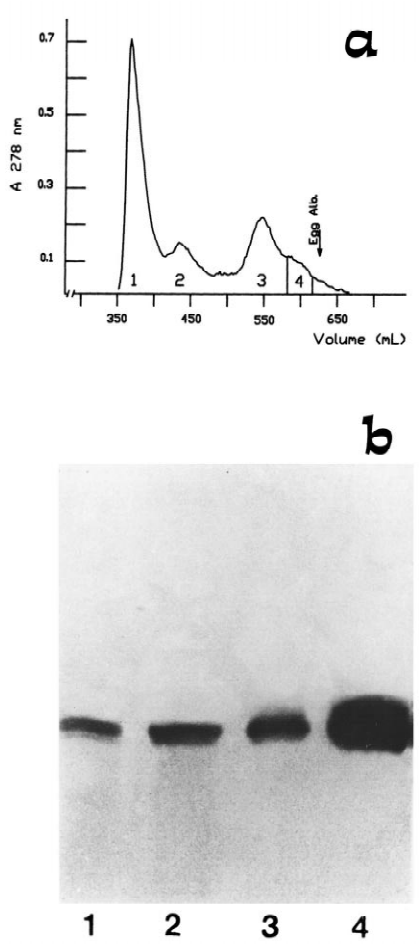

Fig. 1. (a) Gel permeation on 100×2.6 cm I.D. column filled with Ultrogel AcA-44 of 230 mg of pregnancy endometrium extracts eluted from ConA-Agarose with 0.5 methyl α-D-glucopyranoside. (b) Western immunoblot analysis of 20 μg of, from left to right, fractions 1–4.

10.8) the gel calibration curve $\mu = 6.32 - 1.1289 \log r$ (electrophoresis not shown).

3.2. Simplified purification of glycodelin

Chromatographic fractions eluted by NaCl linear gradient from DEAE-Sephacel were pooled into eight pools on the basis of their absorbance at 278 nm and of their glycodelin immuno-assay titres. Approximately 40% of the immuno-activity was present in the fraction (2.4) eluted within the NaCl concentration interval 0.100–0.130 *M*. A well-identifiable peak of albumin was present in the next fraction, coeluted with some 10% of the immunoactivity, whereas an additional 30% of the immunoactivity was coeluted with more acidic proteins between NaCl concentrations of 0.175 and 0.200 *M* (fractions 2.7 and 2.8). Fraction (2.4) was gel-chromatographed through Superdex-75 and resolved into two protein peaks. The second was eluted between bovine and egg albumins, the center of the peak corresponding to a M_r value of 54 900. This value was derived from the equation which fitted (with $r = 0.9991$, $t(4) = 40.7$) the column calibration curve: $V_e/V_o = 4.477 - 0.669 \log M_r$ and contained all the immunoactivity. When this glycodelin-rich fraction was passed through a column of PBE, a large peak of absorbance was observed between pH 5.1 and 4.0, as indicated in Fig. 2. The degree of purification and yields from the three purification steps are shown in Table 1.

The starting material, made up of 40 g of endometrial tissue, contained some 20 mg of glycodelin when evaluated by enzyme immunoassay. A total of 3.8 mg of highly purified immunoreactive glycodelin were obtained at the end of the purification process, with an overall recovery of 19%.

The protein peak was subdivided into six pools, each of them being submitted to SDS–PAGE and Western blot analysis, as shown in Fig. 3a,b. The purified material runs as a single band on reducing SDS–PAGE (Fig. 3a) and on Western blot analysis (Fig. 3b).

SDS–PAGE of the proteins present in fractions 4–6, having a lower p*I*, disclosed a second band with an M_r of 60 000. Proteins present in fractions 1–3 from chromatofocusing, containing native glycodelin were pooled. Their SDS–PAGE, was also performed by the PhastSystem and is shown in Fig. 4; glycodelin was added in two lanes. One single, stainable band was observed in the second and third lanes, loaded with 4 μg of the purified protein.

The same purified protein preparation was submitted to PhastSystem gel isoelectric focusing in the pH gradient 4–6.5 giving a pattern similar to that shown in Fig. 5. Four major protein bands were observed, exhibiting p*I* values between that of the β-lactoglobulin (5.2) and that of the soybean trypsin inhibitor (4.55).

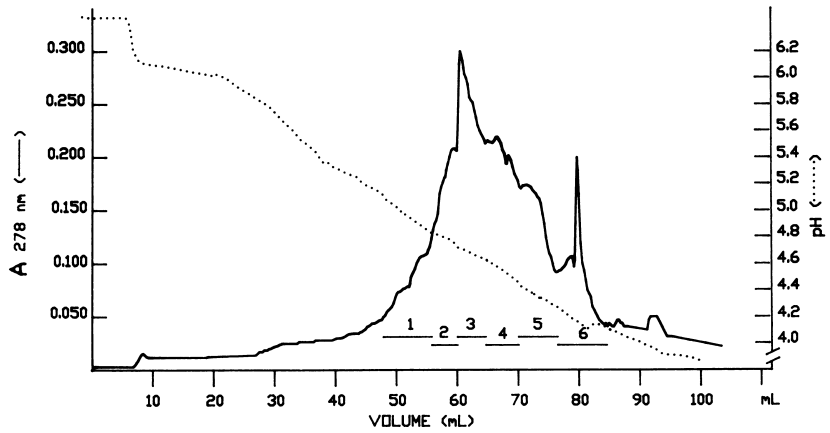

Fig. 2. Chromatofocusing of fraction 3 from Superdex-75 gel permeation in the pH range 6.0–4.0. Ten milligrams of purified glycodelin were passed through 7 ml of ion-exchanger, in the pH range 7–4. Horizontal bars 1–6 represent fractions pooled.

3.3. Amino acid sequence of glycodelin

Automated Edman degradation of the electroblotted band corresponding to a M_r of 26 000 gave an N-terminal sequence MDIPQTKQDLKELPKLAGTWSMAMATNNIA corresponding to that reported by Julkunen et al. [9] from sequenced human decidual cDNA clones encoding PP14 (glycodelin).

Furthermore, the amino acid composition of the heavier protein contained in fractions 4–6 corresponded to that of α_1-antitrypsin. Both sequences were identified by searching a protein data base (SwissProt, release 23.0) using FASTA program [24].

3.4. Circular dichroism

In order to further characterize the purified glycodelin, the final product was submitted to circular dichroism (cd) spectroscopy. The cd spectra of glycodelin in the far UV are reported in Fig. 6.

The spectrum of the aqueous solution of the protein, indicated as (a), exhibited a well-defined minimum at 217 nm. The 2-propanol-induced modifications of the secondary structure of the protein are also shown in Fig. 6: the ϑ given by a 2-propanol–water mixture (30:70) is shown as (b) and that of the 50% 2-propanol–water mixture as (c). When the glycodelin dissolved in 2-propanol–water was dialyzed against water and a new spectrum obtained (see the small diagram in Fig. 6), the large minimum at 208 nm remained.

4. Conclusions

The method proposed is a simple, rapid three-step purification procedure capable of obtaining, on a preparative scale, homogeneous glycodelin preparations. This purification system is easily reproducible, as indicated by the fact that all preparations obtained over a 3-year time interval gave identical results. The present method, represents a simplification of that originally described by Bell [13] and yields higher

Table 1
Flow sheet, with overall recoveries in terms of total proteins and glycodelin-like material, following each purification step

Purification step	Total protein (mg)	Glycodelin (mg)	Recovery (%)	Specific activity[a]
Tissue homogenate (supernatant)	1200	20	100	0.016
DEAE-Sephacell	60	10	50	0.17
Superdex G-75	20	10	50	0.50
Chromatofocussing (pH 6.5–4.0)	3.8	3.8	19	1.0

[a]Glycodelin concentration was determined by enzyme immunoassay and protein content by the Coomassie staining of Bradford [33].

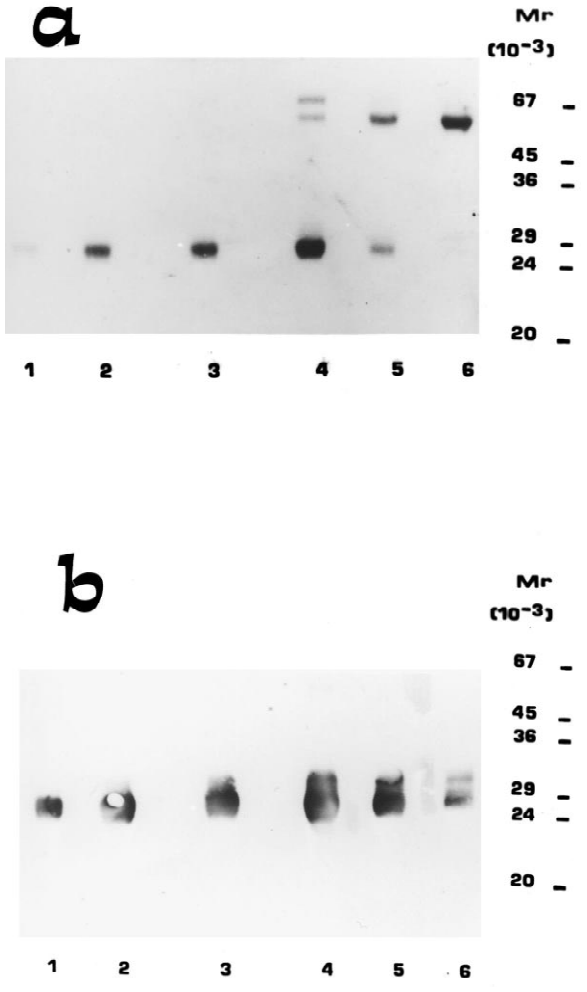

Fig. 3. (a) Monodimensional SDS–PAGE analysis of the six fractions obtained from chromatofocusing. Twenty micrograms protein/lane were analyzed and the gel was stained with Coomassie R-250. (b) Western immunoblot pattern of the same six fractions. Twenty micrograms protein/lane were loaded. Fractions 1–3 were pooled and labelled as highly purified glycodelin.

quantities of highly purified protein. The use of a different gel permeation medium (Superdex-75 instead of Sephacryl S-200) results in a higher increase of glycodelin specific activity. Furthermore, the careful performing of chromatofocusing allowed the elimination of α_1-antitrypsin which represents the heavier contamination of glycodelin, having similar mass and charge; α_1-antitrypsin is also produced by the epithelial cells of gestational endometrium [25]. In no case was the presence of contaminating traces

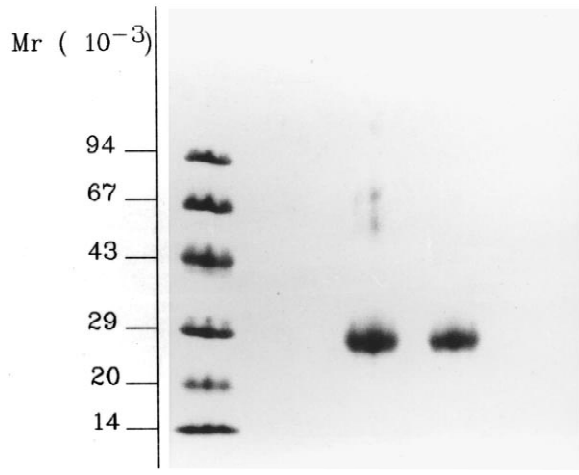

Fig. 4. PhastSystem SDS-electrophoresis in a polyacrylamide gel gradient from 10 to 15%. A total of 4 μg standard protein mixture was loaded in the first lane (left) and 4 μg of highly purified glycodelin preparation ran in lanes 2 and 3. Gels were stained with Coomassie R-350.

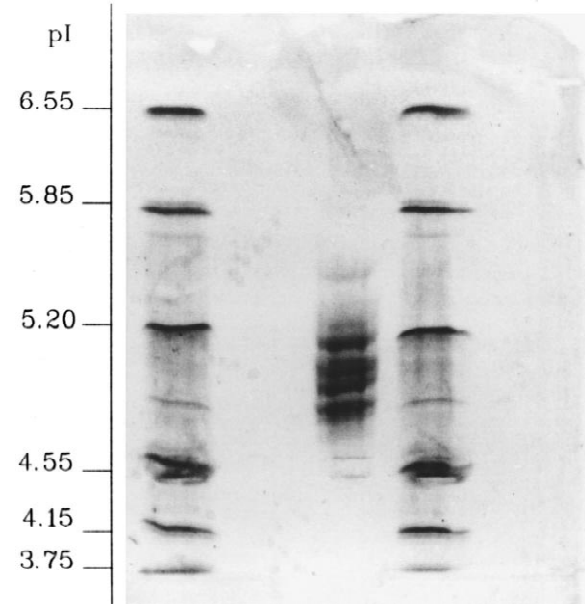

Fig. 5. PhastSystem SDS-electrophoresis in a polyacrylamide gel isolectric focusing of 4 μg of a highly purified glycodelin preparation (lane 2); lanes 1 and 3 were loaded with 4 μg of protein of known p*I*, as indicated. Gel was stained with Coomassie R-350.

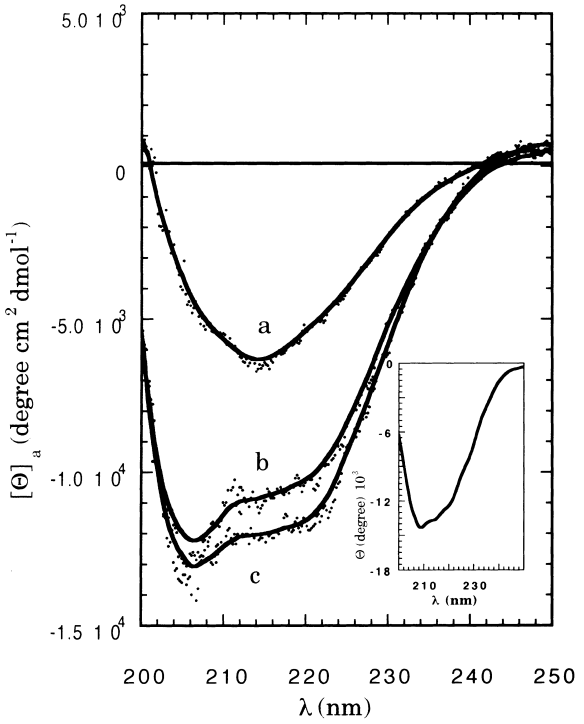

Fig. 6. Effect of solvents on the molar ellipticity [Θ] of glycodelin in the far ultraviolet. The following solvents were used: water (a); 30% 2-propanol in water (b); 50% 2-propanol in water (c). The insert shows the cd spectrum recorded after complete solvent exchange with water of the organic solution relative to spectrum c.

of hCG revealed by RIA. The first 30 NH_2-terminal amino acids of the isolated protein were identical with those deduced by sequencing the cDNA encoding PP14 [23]. This analysis identifies the glycodelin as being identical to the proteins previously described under the acronym of PP14 and isolated from the placenta, or labelled α_2-PEG and isolated from the endometrium.

When the preparation was submitted to chromatofocusing following gel permeation on Superdex-75, it resolved into six fractions, with the highest protein content being located at p*I* 4.7–4.5, and glycodelin immunoactivity spread over the p*I* range 4.1–5.1. Thus, in spite of its homogeneity in SDS–PAGE and in Western immunoblot analysis, isoelectric focusing of the purified preparation indicates a charge heterogeneity. This is consistent with the finding of Dell et al. [1], who reported that the protein is glycosylated with well-defined and substantially different heterogeneous populations of glycans. Although isoelectric focusing of glycodelin has not been reported previously, indirect evidence of heterogeneity can also be drawn from the observation of the existence of various forms of mRNA encoding α_2-PEG [26].

The secondary structure of purified glycodelin was evaluated by means of circular dichroism spectroscopy, a chiro-optical technique based on the selective absorption of right and left polychromatic visible and UV circularly polarized light. The spectrum of the aqueous solution of glycodelin exhibited a well-defined minimum at 217 nm. Similar cd spectra behaviours have been previously found in peptide or protein chains containing a large amount of β-sheet conformation [27,28]. This is consistent with the finding of Papiz et al. [29], on the prevalent β-sheet structure of the bovine β-lactoglobulin which, in turn, shows significant N-terminal amino acid sequence homology with glycodelin [30]. The increase of the α-helix conformation induced by 2-propanol might arise from the refolding of long non-structured segments of the protein. It is not known whether the α-helix transformation is associated with the monomeric form of the protein. The conformational changes induced by the organic solvent are not reversible.

A possible consequence of these findings is that the use of organic solvents (2-propanol and acetonitrile) to purify the protein by hydrophobic interaction chromatography procedures, could result in conformational changes of glycodelin and lead to a modification of its biological activity.

In the present experiments, glycodelin determinants associated with material heavier than the native protein were detected by Western immunoblot in the void volume and in the eluate of a gel permeation through AcA44. This can be interpreted as a tendency of glycodelin to multimerize or to aggregate with a higher-molecular-mass carrier. If true, the addition of one or more epitopes to a carrier molecule would not be a unique example of association between serum proteins: as is well known, the retinol-binding globulin binds to a pre-albumin tetra-

mer to yield a complex with a M_r of about 76 000 [31]. Tendency of SP1-α (pregnancy-specific α_1-glycoprotein) to aggregate with SP1-β epitopes has also recently been described [32].

The extraction method for the glycodelin described here shows significant advantages over those previously described. It allows the production, in three main chromatographic steps, of a highly purified preparation which exhibits a unique stainable band following SDS–PAGE and Western immunoblot analysis. The same procedure allows the preparation of the protein in its native form and in milligram amounts.

The purity of the protein fraction used for antibody production was also comparable to that produced by the simplified isolation procedure, when examined by SDS–PAGE. The resulting antibody did not cross-react with a protein extract from human corpus luteum and gave one stainable band with various antigen preparations.

An alternative extraction procedure, which included DEAE-Sephacell chromatography, gel permeation and again anion-exchange chromatography through Mono-Q developed with a 20 m*M* Bis-Tris propane, pH 9.5, and NaCl convex gradient from 0 to 0.35 *M*, resulted in a preparation which was not considered purified enough to be used for antibody production (Pala et al., unpublished results) or, more in general, as a purification procedure for the glycodelin protein.

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References

[1] A. Dell, H.R. Morris, R.L. Easton, M. Panico, M. Patankar, S. Oehninger, R. Koistinen, H. Koistinen, M. Seppälä, G.F. Clark, J. Biol. Chem. 270 (1995) 24116–24126.
[2] M. Julkunen, E.M. Rutanen, A. Koskimies, T. Ranta, H. Bohn, M. Seppälä, Br. J. Obstet. Gynaecol. 92 (1985) 1145–1151.
[3] S.C. Bell, Hum. Reprod. 1 (1986) 129–143.
[4] S.G. Joshi, R.A. Smith, D.K. Stokes, J. Reprod. Fertil. 60 (1980) 317–321.
[5] S.C. Bell, J. Jackson, F.D. Doré-Green, P.H. Kirwau, J.O. Drife, Hum. Reprod. 2 (1987) 389–397.
[6] M. Kamarainen, L. Riittinen, M. Seppälä, A. Patolie, L.C. Anderson, Blood 84 (1994) 467–473.
[7] D.M. Morrow, N. Xiong, R.R. Getty, Z. Rataiczak, D. Morgan, M. Seppälä, L. Riittinen, A.M. Gewirtz, M.L. Tykocinski, Am. J. Pathol. 145 (1994) 1485–1495.
[8] H. Bohn, W. Kraus, W. Winkler, Placenta 4(Suppl.) (1982) 67–81.
[9] M. Julkunen, M. Seppälä, O.A. Janne, Proc. Natl. Acad. Sci. USA 85 (1988) 8845–8849.
[10] M. Julkunen, Acta Endocrinol. 112 (1986) 271–277.
[11] H.R. Morris, A. Dell, R.L. Easton, M. Panico, H. Koistinen, R. Koistinen, S. Oehninger, M. Patankar, M. Seppälä, J. Biol. Chem. 271 (1996) 32159–32167.
[12] G.F. Clark, A. Dell, H.R. Morris, M. Patankar, S. Oehninger, M. Seppälä, Mol. Human Reprod. 3 (1997) 5–13.
[13] S.C. Bell, Human Reprod. 5 (1986) 313–318.
[14] A.E. Bolton, K.J. Clough, R.J. Stoker, A.G. Pockley, E.A. Mowles, O.M.R. Westwood, M.G. Chapman, Lancet i (1987) 593–595.
[15] O.M. Westwood, M.G. Chapman, N. Totty, R. Philp, A.E. Bolton, N.R. Lazarus, J. Reprod. Fertil. 82 (1988) 493–500.
[16] L. Riittinen, M. Julkunen, M. Seppälä, R. Koistinen, M.L. Huhtala, Clin. Chim. Acta 184 (1989) 19–30.
[17] L. Riittinen, O. Närvänen, I. Virtanen, M. Seppälä, J. Immunol. Methods 136 (1991) 85–90.
[18] A.G. Pockley, E.A. Mowles, R.J. Stoker, O.M.R. Westwood, M.G. Chapman, A.E. Bolton, J. Reprod. Immunol. 13 (1988) 31–39.
[19] N. Okamoto, A. Uchida, K. Takakura, Y. Kariya, H. Kanzaki, L. Rittinen, R. Koistinen, M. Seppälä, T. Mori, Am. J. Reprod. Immunol. 26 (1991) 137–142.
[20] R.H. Burdon, P.H. van Knippemberg (Eds.), Laboratory Techniques in Biochemistry and Molecular Biology, vol. 1, Part 3, Elsevier, Amsterdam, New York, Oxford, 1991, p. 365.
[21] J.L. Vaitukaitis, J.B. Robbins, E. Nieschlag, G.R. Ross, J. Clin. Endocrinol. Metab. 33 (1971) 988.

[22] A.M. Campbell, Monoclonal Antibody Technology, Elsevier, Amsterdam, 1987, pp. 53–55.
[23] P. Matsudaira, J. Biol. Chem. 262 (1987) 10035–10038.
[24] W.R. Pearson, D.J. Lipman, Proc. Natl. Acad. Sci. USA 85 (1988) 2444–2448.
[25] M. Seppälä, A.E. Koskimies, A. Tenhunen, Ann. NY Acad. Sci. 442 (1985) 212–226.
[26] J. Garde, S.C. Bell, I.C. Eperon, Proc. Natl. Acad. Sci. USA 88 (1991) 2456–2460.
[27] M. Barteri, B. Pispisa, Biopolymers 12 (1973) 2309–2327.
[28] W.C. Johnson Jr., Ann. Rev. Biophys. Biophys. Chem. 17 (1988) 145–166.
[29] M.Z. Papiz, L. Sawyer, E.E. Eliopoulos, A.C.T. North, J.B.C. Findlay, R. Sivaprasadarao, T.A. Jones, M.E. Newcomer, P.J. Kraulis, Nature 324 (1986) 383–385.
[30] M.L. Huhtala, M. Seppälä, A. Narvanen, P. Palomeki, M. Julkunen, H. Bohn, Endocrinology 120 (1987) 2620–2622.
[31] F.W. Putman, The Plasma Proteins, 2nd ed., vol. IV, Academic Press, New York, 1984, pp. 45–166.
[32] A. Pala, A. Di Ruzza, G. Rossetto, M. Moro, R. Strom, F. Padula, Clin. Chim. Acta 207 (1992) 87–97.
[33] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.