Pregnancy-associated CA125 antigen as mucin: evaluation of ferning morphology

M.M. Jankovic¹ and B.S. Milutinovic

Institute for the Application of Nuclear Energy – INEP, University of Belgrade, 11080 Zemun-Belgrade, Serbia

¹Correspondence address. E-mail: miraj@inep.co.yu

CA125 antigen is a high molecular mass, mucin-type molecule expressed during embryonic development as well as in adult human tissues. This study was aimed at investigating its mucin-related property of ferning, as a general complementary way of characterization. Pregnancy-associated CA125 antigen (pCA125) was examined using light, transmission and scanning electron microscopy and compared with cancer-derived CA125 antigen (cCA125). The results obtained for spread-out, air-dried pCA125 and cCA125 samples revealed clear differences in the patterns of crystalline as well as amorphous material. Thus, the fern-like crystals were mainly sparsely distributed and their morphology was atypical. The extent of crystallization of pCA125 was moderately lower than that of cCA125 antigen, whereas variation in the size and spatial organization of fern crystals was evident. Besides the material with a crystalline appearance, differences in the organic substrate were also noticeable. In contrast to the sponge-like appearance of pCA125, cCA125 had a more compact structure. These initial data may be relevant for relating biochemical properties of CA125 antigen with its morphology as a basis for elucidating its still obscure function under different physiological conditions.

Keywords: CA125/ferning/microscopy/mucin

Introduction

CA125 antigen is a high molecular mass, mucin-type molecule, recently categorized as Muc16 (Yin and Lloyd, 2001). It has very complex molecular organization with an extremely large extracellular domain, including the N-terminus and tandem repeat region, transmembrane domain and short cytoplasmic C-terminus (O’Brien et al., 2001; Yin and Lloyd, 2001). The oligosaccharide chains, consisting of both N- and predominant O-glycans, comprise 28% of its molecular mass (Kui Wong et al., 2003). CA125 antigen occurs in a variety of molecular species ranging from approximately 100 kDa to 3.5 MDa (de Bruijn et al., 1986; Nustad et al., 1998). This heterogeneity is thought to be due to variation in its peptide backbone, i.e. the existence of splicing variants differing in the number of tandem-repeats, and also to differences in glycosylation (O’Brien et al., 2001; Yin and Lloyd, 2001; McLemore and Aouizerat, 2005).

CA125 antigen is expressed during embryonic development as well as in adult tissues, but is best known as the hallmark of serous epithelial ovarian carcinoma (Zurawski et al., 1988; Hardardottir et al., 1990). Thus, data in the available literature mostly concern investigations into its clinical and diagnostic use for screening and monitoring the progress of ovarian cancer and related gynaecological disorders (Montz, 1992). In contrast, some fundamental aspects have not been subjects of detailed study, including the structural characterization of CA125 antigen of both fetal and cancer origin. We have recently characterized the molecular forms and microheterogeneity of oligosaccharide chains of pregnancy-associated CA125 antigen (pCA125) (Jankovic and Tapuskovic, 2005). The results obtained were in agreement with its mucin nature, indicating the existence of distinct glycoisoforms and suggesting differences in comparison with cancer-derived antigen.

In this study, pCA125 antigen was further characterized by an examination of its mucin-related properties. Mucins exhibit the distinctive ability of ferning, which represents a physical characteristic of crystallization in the presence of sodium chloride. So far, the ferning phenomenon has been examined in detail in cervical mucus, where four different ferning patterns and ultrastructures, corresponding to different morphological types, were described (Odeblad, 1973, 1994, 1995; Menarguez et al., 2003). Morphological changes in cervical mucus, observed as various crystallization patterns have been used for many years in gynaecological practice as the Billing ovulation method or the amniotic fluid fern test (Eckerling et al., 1964; Billings and Westmore, 1992; Odeblad, 1994). CA125 antigen is one of the constituents of cervical mucus (de Bruijn et al., 1986; Nustad et al., 2002). However, nothing is known about the way CA125 antigen contributes to mucus arrangement or its molecular association in solution. Owing to the heavy glycosylation, the physical as well as signal and recognitive properties of mucins, including CA125 antigen, can vary widely and subsequently influence their conformation and functionality (Gum, 1992; Sheehan et al., 1995).

In this study, the issue of structural heterogeneity of CA125 antigen was addressed by comparing ferning morphology of pregnancy- and cancer-derived molecules. We have analysed spread-out, air-dried CA125 preparations by light microscopy (LM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The qualitative assessment of the patterns obtained indicated distinct differences in both inorganic material, i.e. fern crystals, as well as in the organic, i.e. proteinaceous substrates of the examined CA125
Materials and Methods

CA125 antigen

pCA125 antigen was isolated from the extract of pooled first trimester placental tissue free of blood and contaminating tissue, decidua and placental bed, as previously described (Jankovic and Tapuskovic, 2005). Cancer-derived CA125 (cCA125) antigen from ovarian carcinoma cell-line OVCAR-3 was from Biodesign (Saso, ME, USA).

The test solution was standardized to CA125 antigen in 0.1 M phosphate-buffered saline, pH 7.2, at a concentration of 6000 IU/L. The concentration was determined using ELSA CA125 II assay (Cis bio International, Gif-sur-Yvette, France).

Light microscopy

CA125 antigen solution (10 μl) was allowed to dry on a glass surface at room temperature for 30 min. The sample was deposited either as a droplet (spread out in all directions with a pipette tip) or as a uniform thin smear obtained by dragging the edge of a second slide across the solution. The preparations were examined microscopically using a Reichart Jung microscope (Vienna, Austria) and photographed using a Power Shot S50 (Cannon, Japan) camera. The ferning patterns of each of five different preparations were qualitatively assessed by two independent investigators.

Transmission electron microscopy

The CA125 antigen samples (3 μl) were placed as a droplet on carbon-coated Formvar films supported on copper grids. The excess of fluid was removed by blotting with filter paper after the sample had air-dried for 2 h. The samples were examined under a Philips CM12 electron microscope (Philips, Eindhoven, The Netherlands).

Scanning electron microscopy

The glass-deposited air-dried CA125 antigen samples were mounted on the specimen holder to be covered with gold by ion sputter coating at 30 mA for 3 min in a BAL-TEC SCD 005 instrument (BALTEC AG, Lichtenstein). The samples were analysed using a JOEL, JSM 6460 LV SEM (Tokyo, Japan) at 25 kV.

Results

The representative ferning patterns related to pCA125 and cCA125 or pregnancy- and cancer-derived CA125 antigens are shown in Figs 1 and 2, respectively. Although intrasample variation, related to the ratio of single or grouped crystals, was visible, some generalizations could be deduced and typical morphology was assigned to each of the examined antigens. In general, the fern-like crystals were sparsely distributed with no homogenous ferns and their morphology was atypical. The extent of crystallization of pCA125 was moderately lower than that of cCA125 antigen. Besides the material with a crystalline appearance, the surrounding organic substrate was also visible in both samples at higher magnification.

The fern-like crystals of pCA125 were mainly randomly distributed, but a characteristic spatial organization extending from a central axis was also present (Fig. 1a). Short branches projecting from the main curved axis were observed using LM and TEM (Fig. 1b). In addition, the organic substrate between the crystals was clearly visible with SEM. It had a sponge-like appearance (Fig. 1c) with small cavities within its structure (Fig. 1d).

The ferning morphology of cCA125 antigen, identified by LM (Fig. 2a) and TEM (Fig. 2b), differed from that of pCA125 antigen, with respect to length, branching and distribution of crystals. Thus, the branches were longer and more abundant. Grouping of crystals radiating from the centre, i.e. star-like morphology was typical and different from the organization observed for pCA125 antigen (Fig. 2a). In addition, long arrangements of crystallites were also visible. The crystals seemed not to be joined (Fig. 2c). At higher magnification, scarce organic substrate between the crystals was
noticeable. It appeared as a very compact structure with a rough irregular surface (Fig. 2d).

Discussion

The data presented here for the first time describe the ferning morphology of a mucinous molecule, CA125 antigen. It is known that mucins are highly variable due to extensive glycosylation and that their structure can be greatly influenced and altered by different collection, deposition and preparation techniques (Legget et al., 1993; McMaster et al., 1999; Ruttlant et al., 1999; Deacon et al., 2000; Round et al., 2002). In this study, we examined purified unmodified antigens, in a physiological saline buffered environment and assessed their properties by a combination of LM and electron microscopy. Under these experimental conditions, the results obtained for pCA125 antigen, isolated from first trimester placental extract, and cCA125 antigen, isolated from the OVCAR-3 carcinoma cell line, revealed distinct differences in the patterns of inorganic and organic material. Although intensive ferning, present as uniform closely branched arborisation, was not found in either sample, variation in the size and spatial organization of fern crystals was evident. We used the antigen from an ovarian carcinoma cell line and not a tissue-derived one, because it is the only CA125 antigen studied in detail and the available literature data refer to this particular antigen (O’Brien et al., 2001; Yin and Lloyd 2001; Kui Wong et al., 2003). Thus, the ferning pattern of the OVCAR-3 cell-derived CA125 antigen does not exclude the possible presence of other cancer-specific distinct ferning patterns, as the result of the heterogeneity of the mucin molecule but not a non-specific variation. Mucus consists of mucins, enzymes, other proteins and electrolytes, and its ferning depends on several factors, such as hormonal status and the ratio between salts and organic material (Wolf et al., 1980; Odeblad et al., 1994). It is intensive under estrogen dominance, whereas under the dominance of progesterone the crystals are reduced to an amorphous form. However, if the protein is denatured by heating or at low pH, no ferning occurs (Anderson, 1984). This means that the folding pattern, i.e. quaternary structure and partly aminoacid composition are also very important for this process (Anderson, 1984; Shiba et al., 2003). Generally, the pore diameter of the glycoprotein network is increased in estrogenic and decreased in progestenic mucus (Odeblad, 1994; Ceric et al., 2005). Thus, at the beginning of the menstrual cycle and during the first trimester of pregnancy a compact, dense mucus structure is present (Daunter et al., 1976; Ceric et al., 2005).

In regard to this, differences between pCA125 and cCA125 were also observed in the organic material, located in close proximity to the crystal ferns. Thus, at the ultrastructural level, in contrast to the sponge-like appearance of pCA125, cCA125 had a more compact structure, both being reminiscent of non-fertile mucus (Ruttlant et al., 2002).

The available literature data indicated that molecular topology, i.e. the physical properties of mucins, directly depend on their molecular mass and the degree of glycosylation (Silberberg, 1989; Verdugo, 1990; Viney, 1999). They generally exhibit polymorphism (Sheehan et al., 1986; Deacon et al., 2000), and in solution, they readily aggregate through covalent and non-covalent associations, although entanglement is the typical mode of association, even in dilute solution (McMaster et al., 1999). CA125 antigen is a highly glycosylated molecule and the glycans probably determine its molecular topology. Our previous investigation indicated differences between pCA125 and cCA125 antigen related to the carbohydrate composition of both N- and O-glycans (Jankovic and Tapuskovic, 2005), which might influence the observed ferning pattern. Specific determinants of O-glycans, such as ABO and Lewis antigens, seem not to be important, because villous cyto- and syncytiotrophoblast do not express these antigens and they are present at too low a level in cCA125 antigen (Ravn and Dabelsteen, 2000; Kui Wong et al., 2003). This is also supported by the fact that ferning results, used in gynaecological practice for many years, are not dependent on the subject’s blood type.

Thus, the characteristic ferning appearance commonly used for clinical purposes might reflect a more fundamental molecular

Figure 2: Ferning morphology of cCA125 antigen. Crystallization pattern observed by LM (a), by TEM (b) and by SEM (c). Organic substrate observed by SEM (d). Magnification indicated by the bar.
aspect, such as structural integrity or the presence of aberrant mucin molecules (Golding et al., 1994; Odeblad 1994; Shiba et al., 2003). If a more precise relation to this phenomenon could be established, it might possibly allow different molecular populations originating from various physiological and pathophysiological conditions to be differentiated, including those of CA125 antigen. The possibility that mucin microstructure might serve as a diagnostic tool for defects at the molecular level has already been suggested (Viney, 1999).

It is known that molecular structure is closely related to the function of any molecular species. Bearing in mind all the experimental constraints under which this study was conducted and possible influences on the examined antigens, these initial data may still be relevant for approaching the mode of CA125 interactions with different ligands as the basis of discovering its still obscure biological function.

Acknowledgments

The authors thank Prof Dr Aleksandra Korac, for help with TEM. This work was supported by the Ministry of Science and Environmental Protection of the Republic of Serbia, project code 143048: Glycans as molecular markers of cell function: expression, microheterogeneity and biosignalling properties.

References


Submitted on January 29, 2007; resubmitted on March 5, 2007; accepted on March 8, 2007.