Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 809 (2004) 323–329
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Lectin affinity capillary electrophoresis in glycoform analysis applying the partial filling technique

Maria Bergström[a,*], Mikael Nilsson[a], Roland Isaksson[a], Ingvar Rydén[b], Peter Påhlsson[c], Sten Ohlson[a]

[a] *Department of Chemistry and Biomedical Sciences, University of Kalmar, SE-39182 Kalmar, Sweden*
[b] *Department of Clinical Chemistry, Kalmar County Hospital, SE-39185 Kalmar, Sweden*
[c] *Department of Biomedicine and Surgery, Division of Cell Biology, Linköping University, SE-58185 Linköping, Sweden*

Received 5 April 2004; received in revised form 25 June 2004; accepted 28 June 2004

Available online 28 July 2004

Abstract

The study of protein glycosylation and its significance in biological interactions is a field of growing interest. This work demonstrates a lectin-based separation of protein glycoforms of α_1-acid glycoprotein (AGP or orosomucoid) with capillary electrophoresis. Glycoform analysis was performed with a "partial filling technique" with the lectin Concanavalin A (Con A) as affinity ligand. Con A separated human AGP into two peaks; the first peak included AGP glycoforms without biantennary glycans, and the second peak represented the fraction that had one or more biantennary glycans. The applicability of the method was demonstrated with the analysis of AGP from clinical samples and AGP treated with *N*-glycosidase F. The AGP separation was also used as a reporter system to estimate the dissociation constant (K_D) between Con A and a competing sugar.

Keywords: Partial filling technique; α_1-Acid glycoprotein; Orosomucoid

1. Introduction

In recent years, increasing evidence have emerged indicating the great importance of carbohydrates in cell differentiation, signalling and immune response [1]. Glycosylation is the most common post-translational modification in eukaryotes and it has a great impact on the functional, biophysical and biochemical properties of the glycosylated product. Glycoproteins can exist in a high number of different glycoforms, differing in number of carbohydrate chains, branching, length and modifications such as sialic acid and fucose. This is also referred to as glycoprotein microheterogeneity. The relative proportions of the different glycoforms of a specific protein are normally constant in a healthy individual. Changes in the glycosylation pattern can be seen in e.g. malignant and inflammatory diseases and have the potential to be used as diagnostic and prognostic tools [2,3]. The physiological significance of these findings is not yet fully understood and studies of carbohydrate interactions in biological system are of high importance [4]. The analysis of glycoform heterogeneity in the production of recombinant glycoproteins is also of special interest to assure biocompatibility. To determine glycoform distribution it is desirable to analyse the intact glycoprotein rather than the free carbohydrates. The large number of possible glycoforms of an individual protein makes this a difficult task [5]. Capillary electrophoresis (CE) is a promising tool to study both intact glycoproteins, glycopeptides as well as released carbohydrate chains [6].

The heavily glycosylated acute phase protein α_1-acid glycoprotein (AGP or orosomucoid) is a major constituent of human plasma but its function is still obscure [7]. AGP has five glycosylation sites with *N*-linked complex-type oligosaccharide chains that can be of biantennary, triantennary as well

* Corresponding author. Fax: +46 480 446262.
E-mail address: maria.bergstrom@hik.se (M. Bergström).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.06.042

as tetraantennary type [8]. The high number of sialic acid groups on AGP is the reason for its low isoelectric point (p*I*) of 2.9–3.3 [9]. Disease-associated changes in glycosylation of AGP involve branching of the carbohydrate chains as well as the amount of fucose and sialic acid [10]. An increase in branching is typical in chronic inflammatory conditions as rheumatoid arthritis (RA). The opposite, a decrease in branching, is connected with acute inflammatory conditions. Crossed affino-immunoelectrophoresis (CAIE) with lectins such as Concanavalin A (Con A) has been extensively used to study specific changes in AGP glycosylation [8]. In these studies, it was found that approximately 58% of the AGP from a healthy individual have at least one biantennary glycan.

Con A has several isoforms and a p*I* of 4.5–5.5. It exists as a tetramer at neutral pH but disintegrates into a dimer at pH below 5.6 [11]. The monomeric molecular mass is approximately 26 kDa. AGP glycoforms with only one biantennary chain is known from affinity chromatography studies to interact reversibly with Con A-Sepharose and to elute under isocratic conditions while glycoforms with two or more biantennary glycans are bound to Con A-Sepharose. A competitor such as methyl α-D-mannopyranoside (α-Me-Man) can be used to elute these glycoforms [12,13]. The interaction between Con A-Sepharose and glycopeptides bearing different biantennary variants has been studied in detail [14]. Con A interacts with the trimannosyl core structure Manα1–6(Manα1–3)Man-, present in the branching point of all *N*-linked carbohydrate chains. The affinity towards tri- and tetraantennary chains is considerably lower and can be neglected in most cases. The affinity constant (K_A) between Con A-Sepharose and the complete biantennary structure *N*-linked on a peptide is about $5 \times 10^6\,M^{-1}$ [14]. The K_A between Con A and α-Me-Man has been estimated both in solution and in systems where either the carbohydrate or Con A has been immobilised. Titration microcalorimetry and fluorescence anisotropy measurements report values of 7.6×10^3 and $5.5 \times 10^3\,M^{-1}$ [15] and affinity capillary electrophoresis estimate the K_A to $9.6 \times 10^3\,M^{-1}$ [16]. Frontal affinity chromatography on a Sepharose-immobilised system reports a higher affinity of $2.2 \times 10^4\,M^{-1}$ [17]. All measurements above were performed at pH 7.0–7.9.

Affinity capillary electrophoresis (ACE) can be designed in a number of ways depending on the properties of the affinity system and the problem under investigation. In the classical affinity capillary electrophoresis approach is the affinity ligand added to the background electrolyte (BGE) [18] and the sample is injected as a narrow zone. Components in the sample that interact with the affinity ligand will exhibit a mobility shift during the passage through the capillary filled with the affinity ligand, provided that the mobility of the affinity complex is different from the mobility of the sample. The experimental design is very similar to weak affinity chromatography [19,20] but the affinity ligand is not immobilised in ACE. A major drawback with classical ACE is the interference from the affinity ligand with the detection of the sample when the affinity ligand is present in BGE. It is for instance impossible to couple a mass detector to CE with this approach. To overcome these problems the "partial filling technique" was introduced [21]; with this technique the capillary is only partly filled with the affinity ligand in BGE prior to injecting the sample. The pH of the BGE is selected to have the affinity ligand and the sample to move in opposite directions in a neutral coated capillary with a low electroosmotic flow (EOF) [22,23]. The sample migrates through the affinity ligand zone when voltage is applied, leaving the affinity ligand behind, and reaches the detector window without any affinity ligand that can disturb the detection. The partial filling technique has been used mainly in enantiomer separations of small drug molecules with proteins as chiral affinity ligands (selectors). Affinity constants are possible to estimate from the change in migration time when the absolute amounts of the affinity ligand is varied [24]. The affinity separation can also be used as a reporter system for the estimation of K_A between the affinity ligand and other substances, competing for the same binding site [25].

This work aims to demonstrate the use of the partial filling technique in lectin ACE for the analysis of large molecules as glycoproteins. We show that Con A ACE has the capacity of separating glycoforms of the intact glycoprotein according to carbohydrate composition. We also demonstrate the possibility to use the separation of AGP as a reporter to estimate the affinity constant between Con A and a inhibiting sugar such as α-Me-Man that compete for the same binding site as AGP.

2. Experimental

2.1. Materials

Polyvinylpyrrolidone K30 (PVP), 3-(trimethoxysilyl)propyl methacrylate, human AGP, Con A (type IV), methyl α-D-mannopyranoside (α-Me-Man) and *N*,*N*-dimethylformamide were purchased from Sigma (St. Louis, MO, USA). Recombinant *N*-glycosidase F was obtained from Roche Diagnostics (Mannheim, Germany). Polyclonal rabbit anti-AGP antibodies (A0011) were provided by DAKO (Glostrup, Denmark). Chromatography materials like PD-10 desalting columns, HiTrap NHS-activated column and Con A-Sepharose were from Amersham Biosciences (Uppsala, Sweden). Ultrafiltration devices (Vivaspin 6, 10K MWCO PES) were obtained from Vivascience (Hannover, Germany). Capillaries (outer diameter: 375 μm, inner diameter: 50 μm) were from Polymicro Technologies (Phoenix, AZ, USA). Filtropur S 0.45 μm syringe filters (25 mm diameter) and Acrodisc LC 13 PVDF 0.2 μm syringe filters (13 mm diameter) were purchased from Sarstedt (Nümbrecht, Germany) and Gelman Sciences (Ann Arbor, MI, USA), respectively. Slide-A-Lyzer MINI Dialysis units 10 K MWCO were from Pierce Biotechnology (Rockford, IL, USA). All chemicals were of analytical grade and acquired from commercial sources.

2.2. Purification of AGP from clinical samples

AGP from blood donors and two clinical samples from RA patients were purified with affinity chromatography. An affinity column was prepared with 2.3 ml polyclonal anti-AGP antibodies immobilised on a 5 ml HiTrap NHS-activated column according to the manufacturer's recommendations. EDTA-plasma was diluted 10 times with 0.1 M NaCl, 0.1 M sodium phosphate pH 7.0 and applied on the column. Unbound fraction was eluted with 0.1 M NaCl, 0.1 M sodium phosphate, pH 7.0 and AGP was eluted with 0.2 M glycine–HCl, pH 2.0. Fractions were immediately adjusted to pH 6.0 with 0.2 M sodium phosphate, pooled and concentrated with ultrafiltration. The concentrated pool was desalted on PD-10 according to the manufacturer's recommendations. Purity of the eluted material was confirmed with sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

2.3. Fractionation of AGP with Con A-Sepharose chromatography

A column (1.6 cm × 5 cm) with 10 ml Con A-Sepharose was prepared according to the manufacturer's recommendations. The column was equilibrated with 50 mM sodium acetate, 1 mM $CaCl_2$, 1 mM $MnCl_2$, pH 5.0 and loaded with 0.5 ml (10 mg/ml) human AGP. By changing the elution conditions AGP was separated into three separate pools assigned AGP pool I, II and III, respectively. AGP pool I was eluted as a narrow peak with the column void by changing to high ionic strength buffer by adding 0.5 M sodium chloride to the equilibrating buffer. AGP pool II was eluted isocratically as a broad peak in approximately 20 column volumes with the same high ionic strength buffer. AGP pool III was eluted with 0.1 M α-Me-Man added to the previous buffer. Finally, AGP pool I, II and III were concentrated and desalted with ultrafiltration.

2.4. Con A capillary affinity electrophoresis; Con A ACE

The capillary electrophoretic system was a Beckman P/ACE system 5510 (Beckman instruments, Palo Alto, CA, USA) equipped with an UV diode array detector and detection was performed at 204 nm. Instrument control and data collection was done with P/ACE Station software (Beckman instruments). Capillaries were 27 cm (20 cm effective length) with an inner diameter of 50 μm. Fused silica capillaries were coated with 3-(trimethoxysilyl)propyl methacrylate in combination with polyvinylpyrrolidone K30 (PVP) as described elsewhere [26]. BGE in the capillary electrophoresis separation was 50 mM sodium acetate, pH 5.0 supplemented with 1% of $CaCl_2$ and $MnCl_2$, 100 mM each. The sodium acetate buffer was prepared from MilliQ water, concentrated acetic acid and 6.0 M sodium hydroxide. BGE were degassed and passed through a 0.45 μm syringe filter before use, a small 0.2 μm syringe filter was used for sample filtration. New capillaries were equilibrated with BGE for 30 min. The instrument temperature was maintained at 20 °C and it was operated under reversed polarity with detection at the anode. Con A was dissolved in BGE, without $CaCl_2$ and $MnCl_2$, and introduced into the capillary at low-pressure mode (0.5 psi; 0.03 bar), i.e. "partial filling" the capillary, prior to injection of the AGP sample. AGP (1–2 mg/ml) was injected with low pressure for 10 s and constant voltage was set to −15 kV giving rise to a current of 40 μA. Between each separation the capillary was rinsed at high-pressure mode (20 psi; 1.4 bar) with 1% Tween 20 in BGE (5 min) and then equilibrated with BGE (5 min). The concentration of Con A in BGE, after filtration, was calculated from the absorbance at 280 nm, using the extinction coefficient $E^{1\%}$ (280 nm) = 11. The concentration of Con A was varied between 4 and 12 mg/ml and the separation zone length was varied between approximately 4 and 14 cm. The different zone lengths were calculated from the effective capillary length and the time that Con A needed to reach the detector window when it was injected with low pressure. The filling time was not affected by the concentration of Con A. The effect on the AGP separation from varying the separation zone was evaluated by subtracting the migration time of the first peak (AGP glycoforms without biantennary glycans) from the second peak (AGP glycoforms with one or more biantennary chain) and this value (Δt) was plotted versus the Con A concentration or the separation zone length in the experiment.

2.5. Con A ACE inhibition experiment; α-Me-Man added to Con A

The capillary was partially filled (120 s ∼ 14 cm) with Con A (12 mg/ml), prior to injecting AGP, and the separation was performed as described earlier. In the next step 2 or 4 μl α-Me-Man (20 mM) was successively added directly to the Con A solution (200 μl) used in the analysis and the AGP separation was repeated. This procedure was repeated until the AGP separation was completely inhibited. The added volume of α-Me-Man in each step was in the same range as the evaporation during the separation so the volume and concentration of Con A was constant in the experimental series. The separation of the two AGP peaks (Δt) was plotted versus the concentration of the inhibitor α-Me-Man [I] in the experiment and the dissociation constant (K_D) between Con A and inhibitor α-Me-Man was estimated from the nonlinear regression curve fit according to Nilsson et al. [25] using the equation

$$\Delta t = \frac{\Delta t_0 K_D}{K_D + [I]}$$

Δt_0 is the migration time of biantennary AGP without α-Me-Man present. The AGP separation is in this case used as a reporter system for the estimation of K_D between Con A and the inhibitor α-Me-Man.

2.6. N-glycosidase F experiment

AGP was analyzed with Con A ACE (12 mg/ml, separation zone length 120 s ∼ 14 cm) as described previously. 5 μl *N*-glycosidase F (1000 U/ml) was added directly to the CE-vial with 100 μl AGP (2.5 mg/ml). The deglycosylation was done under native conditions at a relatively low concentration of *N*-glycosidase F. The successive loss of biantennary glycans was followed with repeated Con A ACE analysis during 30 h. The CE-vial with the mix of AGP and *N*-glycosidase F was incubated at 37 °C between the analyses.

3. Results

3.1. Electrophoretic mobility of Con A and AGP

EOF was measured in all newly PVP-coated capillaries by injecting 0.1% *N*,*N*-dimethylformamide. The coating procedure was very reproducible with an EOF of about 1.1×10^{-5} cm^2/(V s) in 50 mM sodium acetate, pH 5.0. The pH of the BGE was selected to have Con A and AGP moving in opposite directions when voltage was applied. The apparent electrophoretic mobility at these conditions was 1.1×10^{-4} cm^2/(V s) for Con A and -0.8×10^{-4} cm^2/(V s) for AGP. In other words, Con A had a mobility towards the cathode and AGP had a mobility towards the anode. For that reason all affinity separations were done with the instrument at reversed polarity, with detection at the anodic end of the capillary.

3.2. Con A affinity capillary electrophoresis and the partial filling technique

Con A ACE separated AGP into two peaks in less than 15 min. The resolution was increased with higher concentration of Con A and a longer separation zone (Figs. 1 and 2). The separation zone length (with constant Con A concentration) was directly correlated to the separation of the two peaks with a linear relationship (Fig. 1). The correlation with the concentration of Con A in the separation zone was less obvious. No linear relationship between the Con A concentration and the separation of the two peaks was found in the concentration range studied. A concentration of Con A above approximately 10 mg/ml did not improve the separation (Fig. 2). A concentration of 12 mg/ml Con A and a separation zone from 120 s injection (14 cm) was used as standard conditions.

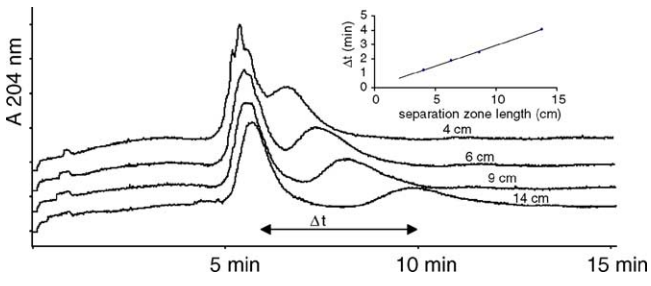

Fig. 1. Electropherogram of AGP in Con A ACE with increasing separation zone length; 35 s, injection (4 cm), 55 s, injection (6 cm), 75 s, injection (9 cm) and 120 s, injection (14 cm). Separation was performed with 12 mg/ml Con A in the separation zone. ACE conditions: PVP fused silica capillary (27 cm total length, 20 cm effective length, 50 μm i.d.); BGE, 50 mM sodium acetate (pH 5.0) with $MgCl_2$ and $MnCl_2$, 1 mM each; applied voltage −15 kV; detection, UV absorption at 204 nm; sample concentration 1–2 mg/ml; injection, 0.5 psi. for 10 s.

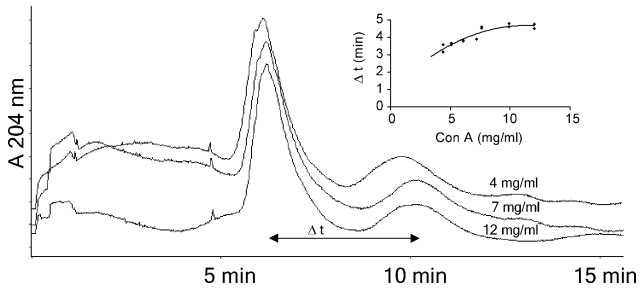

Fig. 2. Electropherogram of AGP in Con A ACE with increasing concentration of Con A in the separation zone; 4, 7 and 12 mg/ml. Separation zone length was 120 s (14 cm). ACE conditions as in Fig. 1.

3.3. Comparison of Con A chromatography and Con A affinity capillary electrophoresis

Fractionated AGP from Con A chromatography (AGP pool I, II and III) were analysed with Con A ACE (Fig. 3). AGP pool I (not bound by Con A-Sepharose at high ion strength) was eluted in the position of the first peak in Con A ACE. AGP pool II and III (retarded and bound to Con A-Sepharose, respectively) were both eluted in the position of the second peak. AGP pool II eluted earlier than pool III in Con A ACE but it was not possible to completely resolve the two peaks.

3.4. Inhibition of Con A ACE with α-Me-Man

When an increasing concentration of α-Me-Man was included in the separation zone in Con A ACE the separation

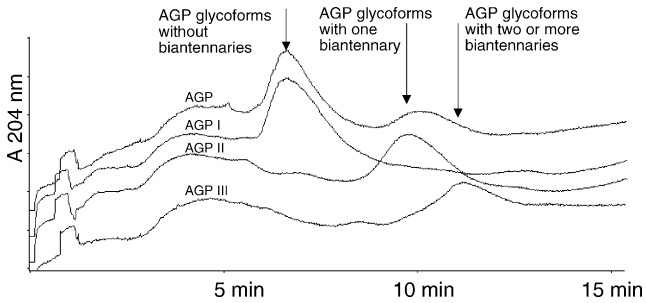

Fig. 3. Electropherogram of AGP pool I, II and III (from Con A chromatography) in Con A ACE; AGP not fractionated on Con A-Separose is included for a comparison. Con A concentration was 12 mg/ml and separation zone length was 120 s (14 cm). ACE conditions as in Fig. 1.

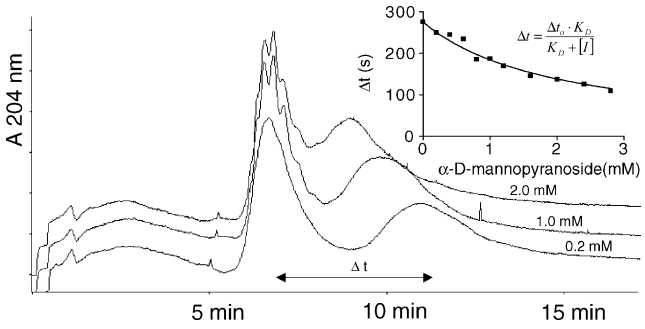

Fig. 4. Electropherogram of AGP in Con A ACE with 0.2, 1.0 and 2 mM α-Me-Man in Con A separation zone. Con A concentration was 12 mg/ml and separation zone length was 120 s (14 cm). ACE conditions as in Fig. 1.

was successively lost (Fig. 4). The two AGP peaks were not possible to resolve above a concentration of approximately 3 mM α-Me-Man in Con A separation zone (Con A concentration 12 mg/ml = 0.5 mM monomeric Con A). The dissociation constant (K_D) between Con A and α-Me-Man was estimated to 2.0 mM ($K_A = 0.5 \times 10^3\ M^{-1}$) from the plot of Δt versus α-Me-Man concentration (Fig. 4).

3.5. Applications; separation of clinical samples and deglycosylated AGP

AGP from blood donors and patients with RA was purified with affinity chromatography, as described earlier, and analyzed with Con A ACE (Fig. 5).

AGP from the two groups had a similar separation profile, as had been found earlier in analyses of commercial AGP, but the relative area of the biantennary peak was lower in AGP from RA-patients compared to the blood donors. The blood donors had a relative area of 42% of the biantennary peak and the two RA-patients had an area of 35–36%.

The addition of *N*-glycosidase F to AGP had a great impact on the appearance of AGP in Con A ACE (Fig. 6). Most of the biantennary glycans was lost in approximately 3.5 h while the rest of the glycans seems to be unaffected at this point, as the migration time of the first peak was constant. After approximately 14 h no further deglycosylation could be detected and there was a shift of the remaining AGP-peak

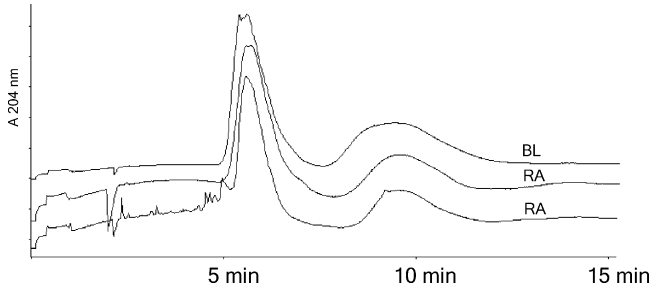

Fig. 5. Electropherogram of two clinical samples of AGP in Con A ACE. RA; RA-AGP and BL; blood donor AGP. Con A concentration was 12 mg/ml and separation zone length was 120 s (14 cm). ACE conditions as in Fig. 1.

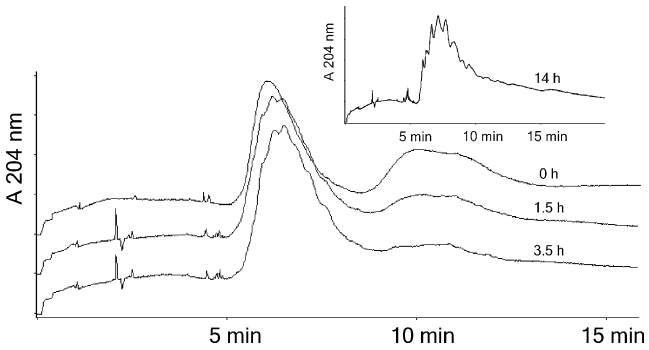

Fig. 6. Electropherogram of AGP, with the addition of *N*-glycosidase F, in Con A ACE. Analyses at 0, 1.5, 3.5 and 14 h (inserted electropherogram). Con A concentration was 12 mg/ml and separation zone length was 120 s (14 cm). ACE conditions as in Fig. 1.

towards later eluting (less acidic) glycoforms due to the loss of sialic acids from the protein (Fig. 6).

4. Discussion

Some prerequisites are necessary to achieve an affinity-based separation with the partial filling technique. The pH of the BGE should be in-between the p*I* of two compounds that are interacting and a coated capillary with a low EOF has to be employed, in order to get a migration of sample and affinity ligand in opposite directions. The capillary coating should be compatible with the affinity ligand and sample and prevent interactions of the proteins with the capillary wall. We found that the coating with PVP was reproducible and easy to perform; the lifetime of the coated capillary was about 100 injections.

In our case both AGP and Con A are heterogeneous, having p*I* values between 2.9–3.3 and 4.5–5.5, respectively. The distribution in p*I* is the explanation for the slightly heterogeneous appearance of the first AGP peak, containing the glycoforms not interacting with Con A. The pH of the BGE is at the p*I* of Con A but the electrophoretic mobility was anyway found to be in the cathodic direction (backwards in the capillary because of the reversed polarity). The separation zone length and the concentration of Con A in the separation zone were varied to optimize the separation. A plateau was reached at a Con A concentration of about 10 mg/ml. Con A has been reported to exist mainly as a dimer at pH below 5.6 [11]. A successive formation of tetrameric, or even larger multimers of Con A, would have an impact on the separation in Con A ACE and can possibly be an explanation to why the separation did not improve with increased concentration of Con A. The low ionic strength of BGE may also contribute to the inactivation of Con A at elevated concentrations.

The separation of AGP with Con A ACE was compared to Con A chromatography at conditions that were as similar as possible. An identical buffer (50 mM sodium acetate, pH

5.0) was used in both methods and the concentration of Con A was in the same range; 10–16 mg/ml in Con A-Sepharose (according to the manufacturer) and 12 mg/ml in Con A ACE. The elution order of AGP pools I, II and III was identical in Con A-chromatography and Con A ACE. The elution conditions were however not identical; AGP pool I was bound at low ionic strength to Con A-Sepharose and it was necessary to use α-Me-Man to elute AGP pool III from Con A-Sepharose in contrast to Con A ACE where these interactions were reversible at low ionic strength. The differences between Con A-chromatography and Con A ACE can be due to the formation of multimeric Con A as discussed earlier or possibly an effect of the immobilisation to Sepharose. A modified Con A, with a homogenous and monomeric appearance, would probably improve the resolution in Con A ACE. In our experience, it is generally an advantage to keep the protein concentration in ACE at a moderate level and to increase the separation zone length to improve separation, if necessary.

It is however clear from the comparison of the two methods that the first peak in Con A ACE is identical with the AGP pool I in Con A-chromatography. This peak has been shown earlier to include the glycoforms without biantennary glycans. The second peak in Con A ACE comprises both AGP pool II and III from Con A chromatography, which has been shown to includes AGP glycoforms with one or more biantennary chain [12,13].

In the inhibition experiment, increasing concentrations of α-Me-Man was included in the separation zone in Con A ACE. The inhibition, measured as the change in Δt, was found to be correlated to the α-Me-Man concentration. This fact implies that the separation is based of the affinity interaction between Con A and mannosyl groups on AGP. The dissociation constant (K_D) between Con A and α-Me-Man was estimated to 2 mM, an affinity about ten times weaker than has been reported earlier [15]. Published data is from experiments performed at neutral pH and it is reasonable to assume that the affinity is considerably lower at pH 5.0, as in our experiment. The affinity between Con A and other inhibiting molecules, competing for the same binding site as AGP, can easily be studied in the same way using AGP as a "reporter molecule". The material and time consumption for the analysis is very low when the "reporter system" is established. The affinity between Con A and the biantennary glycans on AGP was not possible to calculate because of the uncertainties about the concentration of active Con A in the separation zone.

Con A ACE was used to analyse the glycoform distribution of AGP in two different applications; the separation of AGP glycoforms of RA patients and to follow the deglycosylation of AGP with *N*-glycosidase F. Severe RA is connected with an increase in branching of the carbohydrate chains of AGP and thereby a decrease in biantennary chains and a concomitant increase of tri- or tetrantennary glycoforms [27]. These high-branched glycoforms do not interact with Con A and it was expected that AGP from RA-patients would show a decrease in relative peak area of the biantennary peak compared to normal AGP. This was also found when the relative area of the biantennary peak was compared between RA-patients and healthy individuals, even if the difference was less than has been estimated earlier with CAIE [28]. As the purpose of the analysis of the clinical samples was mainly to demonstrate the applicability of Con A ACE, no efforts were made to further investigate these discrepancies.

N-glycosidase F has the capacity of releasing all *N*-linked carbohydrate chains from a glycoprotein, converting aspargine to aspartic acid on the same time. As most *N*-linked carbohydrate chains has two or more sialic acids as a capping structure, the deglycosylatation will have an impact also on the p*I* of the protein. AGP is known to be difficult to deglycosylate and it is usually done under denaturing conditions at very high *N*-glycosidase F concentrations. The conditions in our experiment were not expected to promote a complete deglycosylation of AGP but rather to indicate if *N*-glycosidase F had a preference for biantennary glycans. This seems to be the case as the biantennary peak almost disappeared in 3.5 h. The peak without biantennary glycans was not affected at this time. After deglycosylation in 14 h a general shift towards less acidic glycoforms of AGP was obvious and the electropherogram show only one broad peak of partly deglycosylated protein.

5. Conclusions

Lectin partial filling ACE has the potential of specific and quantitative analysis of a chosen glycoform in an automated way. Con A ACE was successful in separating human AGP into two peaks according to the biantennary content of the glycoforms. It was possible to detect a decrease in biantennary content in two AGP samples from RA patients and to follow the deglycosylation of AGP with Con A ACE. The separation of AGP with Con A ACE was also possible to use as a reporter system for the estimation of the affinity constant between Con A and the competing sugar α-Me-Man. The partial filling technique has mostly been used in chiral separation of small drug molecules but this work demonstrates that it is possible to analyse also large molecules as glycoproteins with this approach.

The main obstacle with the partial filling technique is to have the desired mobility of the interacting compounds. Construction of "carrier ligands", having an optimal electrophoretic mobility, which then can be combined with a desired affinity ligand, would greatly facilitate the development of a fast and general method for studying affinity interactions in solution.

Acknowledgements

We thank the University of Kalmar and the Health Council in the Southeast of Sweden for financial support.

References

[1] N. Sharon, H. Lis, Sci. Am. 268 (1993) 82.
[2] G. Durand, N. Seta, Clin. Chem. 46 (2000) 795.
[3] W. van Dijk, G. Turner, A. Mackiewicz, Glycosylat. Dis. 1 (1994) 5.
[4] P.K. Qasba, Carbohydr. Polym. 41 (2000) 293.
[5] S. Kishino, K. Miyazaki, J. Chromatogr. B 699 (1997) 371.
[6] K. Kakehi, S. Honda, J. Chromatogr. A 720 (1996) 377.
[7] T. Fournier, N.N. Medjoubi, D. Porquet, Biochim. Biophys. Acta 1482 (2000) 157.
[8] W. van Dijk, E.C. Havenaar, E.C. Brinkman-van der Linden, Glycoconj. J. 12 (1995) 227.
[9] E.G. Berger, S.R. Wyss, R.B. Nimberg, K. Schmid, Hoppe Seylers'Z. Physiol. Chem. 361 (1980) 1567.
[10] S.K. Moule, M. Peak, S. Thompson, G.A. Turner, Clin. Chim. Acta 166 (1987) 177.
[11] D.F. Senear, D.C. Teller, Biochemistry 20 (1981) 3076.
[12] M.F. Bierhuizen, M. De Wit, C.A. Govers, W. Ferwerda, C. Koeleman, O. Pos, W. Van Dijk, Eur. J. Biochem. 175 (1988) 387.
[13] M.J. Treuheit, C.E. Costello, H.B. Halsall, Biochem. J. 283 (1992) 105.
[14] J.U. Baenziger, D. Fiete, J. Biol. Chem. 254 (1979) 2400.
[15] R.V. Weatherman, K.H. Mortell, M. Chervenak, L.L. Kiessling, E.J. Toone, Biochemistry 35 (1996) 3619.
[16] K. Shimura, K. Kasai, Anal. Biochem. 227 (1995) 186.
[17] Y. Oda, K. Kasai, S. Ishii, J. Biochem. 89 (1981) 285.
[18] N.H. Heegaard, S. Nilsson, N.A. Guzman, J. Chromatogr. B 715 (1998) 29.
[19] L. Leickt, M. Bergström, D. Zopf, S. Ohlson, Anal. Biochem. 253 (1997) 135.
[20] S. Ohlson, M. Bergström, P. Påhlsson, A. Lundblad, J. Chromatogr. A 758 (1997) 199.
[21] L. Valtcheva, J. Mohammad, G. Petterson, S. Hjerten, J. Chromatogr. 638 (1993) 263.
[22] Y. Tanaka, S. Terabe, J. Chromatogr. A 694 (1995) 277.
[23] Y. Tanaka, S. Terabe, J. Biochem. Biophys. Methods 48 (2001) 103.
[24] M. Nilsson, V. Harang, M. Bergström, S. Ohlson, R. Isaksson, G. Johansson, Electrophoresis 25 (2004) 1829.
[25] M. Nilsson, G. Johansson, R. Isaksson, Electrophoresis 25 (2004) 1022.
[26] K. Srinivasan, C. Pohl, N. Avdalovic, Anal. Chem. 69 (1997) 2798.
[27] A. Mackiewicz, T. Pawlowski, A. Mackiewicz-Pawlowska, K. Wiktorowicz, S. Mackiewicz, Clin. Chim. Acta 163 (1987) 185.
[28] J.E. Hansen, T.C. Bog-Hansen, B. Pedersen, K. Neland, Electrophoresis 10 (1989) 574.