Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1123 (2006) 199–204
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Use of short monolithic columns for isolation of low abundance membrane proteins

Marijana Rucevic [a], James G. Clifton [b], Feilei Huang [b], Xuesong Li [b], Helen Callanan [a], Douglas C. Hixson [a], Djuro Josic [b,*]

[a] *Division of Medical Oncology, Department of Medicine, Rhode Island, Hospital, Providence, RI 02903, USA*
[b] *COBRE Center for Cancer Research Development, Rhode Island Hospital, Providence, RI 02903, USA*

Available online 20 March 2006

Abstract

Convective interaction media (CIM) monoliths provide a stationary phase with a high binding capacity for large molecules and are capable of high flow rates at a very low pressure drop. Used as anion- and cation-exchangers or with affinity ligands such as antibodies, these columns have the potential for processing large volumes of complex biological mixtures within a short time. In the present report, monoclonal antibodies against several rat liver plasma membrane proteins were bound and cross-linked to protein A or protein G CIM affinity columns with a bed volume of only 60 μL. Antigens recognized by bound antibodies and co-eluting (interacting) proteins were rapidly isolated in a single step from either total plasma membrane extracts or subfractions isolated using anion-exchange CIM disk-shaped columns. The isolated antigens and co-eluting proteins were subsequently identified by immunoblot or by LC–MS/MS.

Keywords: Plasma membrane proteins; Immunoaffinity chromatography; Monoliths

1. Introduction

Protein A and protein G affinity chromatography are especially powerful methods for the purification of antibodies. Convective interaction media (CIM) monoliths, such as CIM disks (short columns) and CIM tubes that have high binding capacities for large molecules and even for particles, enable high flow rates with low pressure drop [1,2]. With protein A and protein G ligands, these columns have the potential for rapidly processing large volumes of complex biological mixtures containing antibodies [3].

Short monolithic columns are suitable for very fast separation of proteins in different modes, e.g. in ion-exchange, hydrophobic interaction or affinity mode [1,4–6]. It is also possible to stack two or more monolithic disks with different ligands into one cartridge. We have named this separation conjoint liquid chromatography [7–9]. Using human serum as a model system, Gupalova et al. [3] described a multifunctional fractionation approach to recover IgG and serum albumin within 15 min. One IgG-binding protein G disk and one serum albumin binding disk were installed into the same cartridge. IgG and serum albumin were captured simultaneously, but the elution was performed in subsequent, separate steps. In a similar approach, hydrophobic proteins, extracted from highly enriched liver plasma membrane fraction were separated on a tandem containing an anion- and a cation-exchange monolithic disk [10].

Membrane proteins have a special position in the field of chromatographic separation due to their diversity, hydrophobicity and their behavior during the purification process [11–14]. For the analysis of membrane proteins several methods, including chromatography and different electrophoretical methods, have been used [11,14]. Methods for selective capture of plasma membrane proteins from the cell surface following biotinylation of whole cells have also been developed. Proteins from the cell surface are isolated from detergent extracts by affinity chromatography with immobilized avidin and identified with mass spectrometry (MS) [15]. Bledi et al. [16] have applied proteases to intact cells. The resulting peptide fragments were further analyzed by liquid chromatography (LC) followed by MS/MS. However, neither of these methods is applicable to the fractionation and identification of membrane proteins from whole tissues.

When the proteomes of different organelles such as plasma membranes, mitochondria or Golgi are investigated, their con-

* Corresponding author. Tel.: +1 401 444 44 27; fax: +1 401 793 89 08.
E-mail address: Djuro_Josic@brown.edu (D. Josic).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.02.053

tamination with proteins from cytoplasm and other organelles is always a problem [10,14,17]. In our laboratory, several antibodies against liver plasma membrane proteins have been raised [18,19]. After binding these antibodies to protein A or protein G supports, mainly on CIM disks, the immunoaffinity columns were used for targeted isolation of the antigen and, eventually, interacting proteins. In the present paper, we demonstrate that this method can be used for highly selective enrichment of low abundance proteins from large volumes of complex biological mixtures, such as detergent extracts of enriched plasma membranes from rat liver.

2. Materials and methods

2.1. Plasma membrane preparation

Adult Fischer rat livers (four livers, wet weight 18–20 g each) were perfused with ice-cold phosphate buffered saline (PBS), excised, minced, and dissociated using a Dounce homogenizer, in a buffer of 1 mM $NaHCO_3$, 0.5 mM $CaCl_2$, pH 7.4 (lysis buffer) and protease inhibitor cocktail (Calbiochem, San Diego, CA, USA). The membrane fraction was isolated as previously described [20]. Using this method, about 33–40 mg membrane protein/rat liver can be isolated.

Rat Morris hepatoma 7777 plasma membranes were isolated by zonal centrifugation using a Kontron centrifuge (Kontron Analytik, Munich, Germany) as described elsewhere [21]. Membrane purity was routinely checked by electron microscopy and by assaying of marker enzymes as described by Tauber and Reutter [21].

2.2. Solubilization of plasma membranes

Rat liver plasma membranes were solubilized by the method of Josic and Zeilinger [11], which utilizes different solubilization agents combined with the detergent Triton X-100 (Sigma, St. Louis, MO, USA). For the isolation of proteins of interest, the Triton X-100 extract was used.

2.3. Chromatographic separation

Solubilized membrane proteins were first separated with a system containing one anion- (DEAE) and one cation-exchange monolithic CIM disk-shaped column (BIA Separations, Ljubljana, Slovenia) as described previously [10]. The column dimensions are 12 mm I.D. × 3 mm, the bed volume was about 340 μL. The flow-rate was between 2 and 5 mL/min.

Isolation of monoclonal antibodies (mAb) was performed by use of a monolithic CIM disk column with immobilized protein G. Again, the column volume was approximately 340 μL. The antibody solution, either ascites fluid, diluted 1:10 with PBS, pH 7.3, or cell culture supernatant, was applied to the column at a flow rate 2 mL/min. The column was washed with 10 mL PBS, 10 mL PBS containing 1% Triton X-100 (Sigma, St. Louis, MO, USA) and again with 10 mL PBS. The flow rate during the washing step was 4 mL/min. The antibody was eluted with 0.1 M citric acid, pH 2.3, and the solution was immediately neutralized with 0.2 M Na_2CO_3. The flow rate during the elution was 2 mL/min. The purity of the antibody preparation was determined by SDS-PAGE.

For immunoaffinity chromatography, monolithic mini-disks (5 mm I.D. × 3 mm, about 60 μL bed volume) with immobilized protein A or protein G were used. The corresponding mAb against a targeted membrane protein was bound to one of these columns. The mAb can be cross-linked to the disk using the method of Schneider et al. [22]. Antibody solution (1–2 mg antibody/mL support) was applied to the protein G (or protein A) disk at a flow rate of 2 mL/min. The disk was subsequently washed with 20 mL PBS, 20 mL PBS containing 1% Triton X-100 and 20 mL 0.2 M triethanolamine × HCl, pH 8.0 (Sigma). The flow rate during the washing procedure was 2 mL/min. The mAb was cross-linked to the ligand with 50 mM dimethylpimelidate × 2 HCl (Pierce, Rockford, IL, USA) in 0.2 M triethanolamine × HCl, pH 8.0. For this sake, the cross-linker solution circulated through the column with the bound mAb overnight at a flow-rate of 0.5 mL/min. The reaction was stopped with 10 mL of 50 mM ethanolamine × HCl, pH 8.0 (Sigma) at flow rate 0.5 mL/min. Subsequently, the column was washed with 10 mL triethanolamine × HCl, pH 8.0, 10 mL PBS, pH 7.3, and 10 mL PBS containing 1% Triton X-100. Up to three discs with immobilized monoclonal antibodies can be placed in one cartridge. For sample application, up to 200 mL of Triton X-100 plasma membrane extract or eluate from the anion-exchange column was pumped through the column with immobilized mAb. The column was subsequently washed with: (1) 20 mL PBS, pH 7.3, containing 1% Triton X-100; (2) 20 mL PBS, pH 7.3; and (3) 20 mL PBS containing 1% octyl glucoside (Sigma). After washing the column and performing detergent exchange, the bound proteins were eluted with 0.1 M citric acid, pH 2.3, containing 1% octyl glucoside. For reuse, the column was regenerated with 20 mL PBS, pH 7.3. The flow rate during separation with these columns was 1 mL/min.

All separations were performed at 4 °C. Proteins were detected optically at 280 nm or by use of electrophoretic methods such as SDS-PAGE and/or immunoblot. For separation, a Bio-Logic Duo Flow chromatographic system (BioRad, Hercules, CA, USA) was used.

2.4. SDS-PAGE

Protein samples were solubilized in NuPAGE sample buffer and heated at 100 °C for 5 min. SDS-PAGE was performed with precast NuPAGE 4–12% Bis-Tris gels in an XCell Sure Lock Mini-Cell (Invitrogen, Carlsbad, CA, USA) acoording to the manufacturer. The gels were stained with GelCode Blue (Pierce, Rockford, IL, USA) or SYPRO Ruby and visualized by a VersaDoc Imaging System (BioRad) before excising the bands of interest for in-gel digestion.

2.5. In-gel digestion

The bands of interest were excised by extracting 6–10 plugs with a clean micropipette and extracted and digested as described previously [10].

2.6. Protein identification by LC–MS/MS

Tryptic digests (0.2–6 μL) were fractionated with a reversed-phase column (C-18 PepMap 100, 75 μm I.D. × 15 mm, 3 μm particle size, LC Packings/Dionex, Sunnyvale, CA, USA) operating at a flow of 150 μL/min. A linear gradient (5–35% solvent B in 30 min; solvent A: 0.1 parts formic acid, 5 parts acetonitrile and 95 parts water; solvent B: 0.1 parts formic acid, 5 parts water and 95 parts acetonitrile) was used to separate the tryptic peptides. The column eluate was introduced directly onto a QSTAR XL mass spectrometer (Applied Biosystems, Framingham, MA, USA and Sciex, Concord, Ontario, Canada) via electrospray ionization.

Candidate ions were selected and fragmented using a standard information dependent acquisition (IDA) method. Half-second "MS" scans (range between 300 and 1500 Thompson, Thompson (Th) = Da/z) were used to identify candidates for fragmentation during "MS/MS" scans. Up to 5 × 1.5 s MS/MS scans (range between 65 and 1500 Th) were collected after each survey scan. To be considered a candidate for fragmentation, an ion had to be assigned a charge in the range of +2 to +4. To allow more complete exploration of chromatographic peaks containing simultaneously-eluting peptides, ions whose fragmentation spectrum had just been collected were dynamically excluded for 40 s from being a candidate.

All protein identifications were performed with ProID software (Applied Biosystems). Experimental spectra were matched against in silico tryptic digests of the NCBI nr database (from September 28, 2005; ftp://ftp.ncbi.nih.gov/blast/db/FAST/nr.gz), using 0.15 Da mass tolerances for both MS and MS/MS data. Carboxamidomethyl cysteine (from iodacetoamide treatment) and oxidized methionine were the only modifications considered when performing the database matching. Results from ProID were filtered, condensed, and synthesized using the program ProGroup (Applied Biosystems).

2.7. Immunochemical methods

Western blots were performed using the monoclonal antibody (mAb) 9.2 against the integral plasma membrane protein carcinoembrionic antigen cell adhesion molecule (CEACAM1). This mAb also recognizes the denatured protein and can be optimally used for immunoblot of CEACAM1 [18,23].

3. Results and discussion

3.1. Isolation of the cell adhesion protein CEACAM1

As previously shown, DEAE CIM-disks can be used for further enrichment of relatively acidic proteins from liver plasma membranes. CEACAM1 is an integral membrane protein with relative low concentration in the liver plasma membranes [10]. To enrich the protein before the final purification step with the immunoaffinity chromatography with immobilized mAb 5.4. A previous step with anion-exchange chromatography was used. For anion-exchange chromatography, a DEAE CIM-disk with a 340 μL bed volume was used. By use of this chromatographic

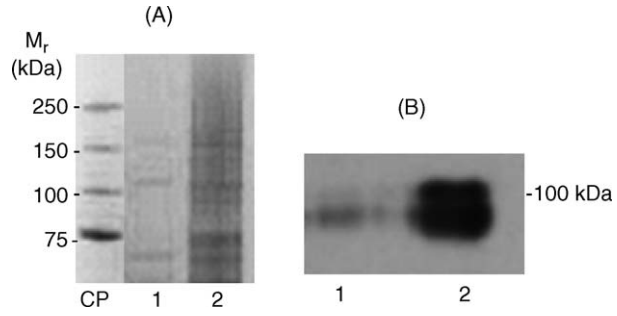

Fig. 1. Isolation of the integral membrane protein CEACAM1: first isolation step with anion-exchange chromatography. Forty milliliters of liver plasma membrane extract solubilized with 1% Triton X-100 containing 10 mg protein was applied to a DEAE CIM disk (12 mm I.D. × 3 mm, bed volume 340 μL). Bound proteins (about 8.5 mg) were eluted with 0.5 M NaCl. Twenty micrograms of eluted proteins were separated by SDS-PAGE, stained with Coomassie blue or blotted with the mAb 9.2. Left: non-bound fraction, right: fraction eluted with 0.5 M NaCl. (A) Coomassie blue stained gel and (B) immunoblot.

method, high amounts of plasma membrane extract (up to 20 mg in 80 mL solubilization buffer containing 1% Triton X-100) can be pumped through the anion-exchange disk. The model protein CEACAM1 binds to the column and can be eluted with 0.5 M NaCl (cf. Fig. 1A and B). This molecule is a highly glycosylated integral membrane protein, involved in cell–cell adhesion [18,23]. CEACAM1 can be eluted with a step gradient between 0.1 and 0.5 M sodium chloride [10]. To enable recovery of this protein in a single fraction, only one elution step with 0.5 M sodium chloride was performed. As shown in Fig. 2, the protein was subsequently captured on a column with immobilized mAb 5.4 directed against this antigen. The mAb 5.4 recognizes the native form of CEACAM1 and can be optimally used for immunoprecipitation and immunoaffinity chromatography

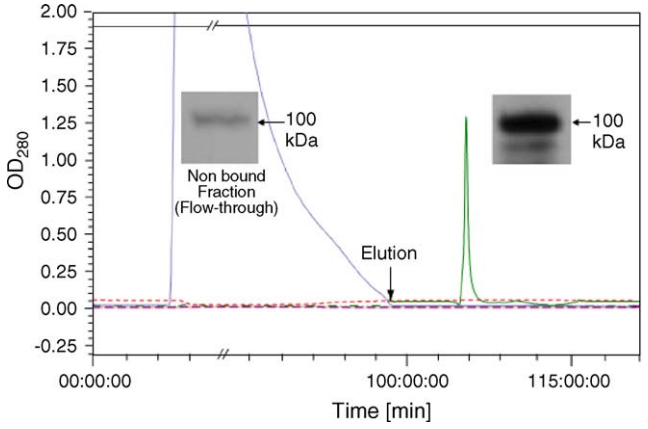

Fig. 2. Isolation of the integral membrane protein CEACAM1: second isolation step, immunoffinity chromatography with immobilized mAb. Proteins eluted from the DEAE CIM disk (about 8.5 mg in 100 mL PBS) were applied on a column containing the mAb 5.4 bound and cross-linked to a protein G CIM disk (5 mm I.D. × 3 mm, bed volume 60 μL). The antigen was eluted with 0.1 M citric acid, pH 2.3, containing 1% octyl glucoside, separated by SDS-PAGE and blotted with mAb 9.2. The majority of membrane proteins does not bind to he column. CEACAM1 was specifically bound and can be eluted, highly concentrated in very small volume. Only a small amount of this protein does not bind and is detected in the flow-through fraction. For chromatographic conditions, cf. Section 2.

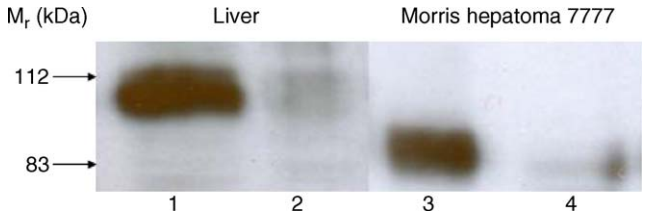

Fig. 3. Immunoaffinity chromatography with the mAb 5.4 against the integral membrane protein CEACAM1. Comparison between CEACAM1 from liver and Morris hepatoma 7777 plasma membranes. Proteins eluted (about 8.5 mg in 100 mL PBS) from the DEAE CIM disk (for chromatographic conditions, cf. Section 2 and Fig. 1) were applied to an affinity column containing mAb 5.4. bound to protein G. The antigen–antibody complex was eluted with 0.1 M citric acid, pH 2.3, and separated with SDS-PAGE. The isolated CEACAM1 band was visualized by immunoblot with monoclonal antibody 9.2. Left (lanes 1 and 2): plasma membrane extract from normal rat liver; right (lanes 3 and 4): plasma membrane extract from Morris hepatoma 7777. Lanes 1 and 3: fractions eluted from the affinity column. Lanes 2 and 4: non-bound fractions.

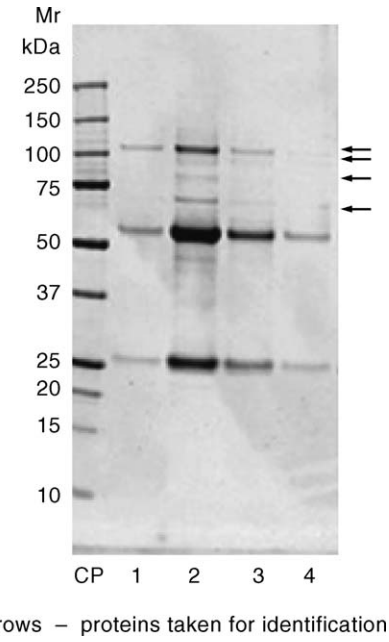

Fig. 4. Immunoaffinity chromatography with the mAb 5.12 against the integral membrane protein DPP-IV. Liver plasma extract with Triton X-100 (67.5 mg protein in 150 mL) was applied to the affinity column containing mAb 5.12 bound to protein A CIM. The antigen–antibody complex was eluted with 0.1 M citric acid, pH 2.3, and separated with SDS-PAGE. The polypeptide bands were visualized by Coomassie blue staining. The protein bands labeled with arrows were excised and taken for further identification with LC–MS/MS. CP: calibration proteins. Lanes 1–4: fractions eluted from the affinity column.

of this protein. The use of this mAb for immunoblot, where denatured protein has to be recognized is limed [18,19].

Isolation of CEACAM1 from rat liver and rat liver carcinoma Morris hepatoma 7777 is shown in Fig. 3. Because of the high degree of glycosylation, this protein is difficult to stain with Coomassie blue [23]. However, it is detected by immunoblot with the corresponding antibody No. 9.2. Because of different degree of glycosylation, the protein isolated from Morris hepatoma 7777 shows lower apparent molecular weight in SDS-PAGE than CEACAM1 isolated from normal liver plasma membranes (see Fig. 3). The investigations regarding differences in glycosylation are still ongoing.

In the experiment shown in Fig. 3, mAb 5.4 against CEACAM1 was only bound to the protein G disk, without subsequent cross-linking. If this approach is chosen, both antigen and antibody are eluted from the column with 0.1 M citric acid, pH 2.3. The disadvantages of this approach are the relatively high consumption of antibodies and the co-elution of

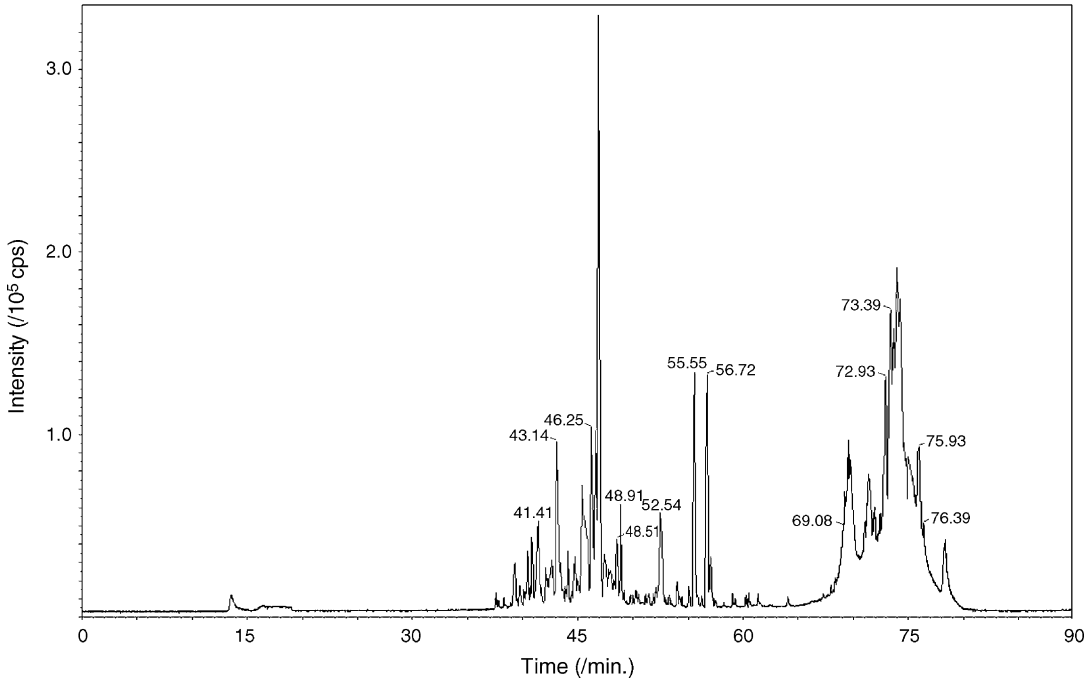

Fig. 5. Protein identification using LC–MS/MS. The total ion chromatogram from an in-gel tryptic digest of the SDS-PAGE protein band with an apparent molecular weight of 110 kDa (cf. upper arrow) shown in Fig. 4. For chromatographic conditions, see Section 2.

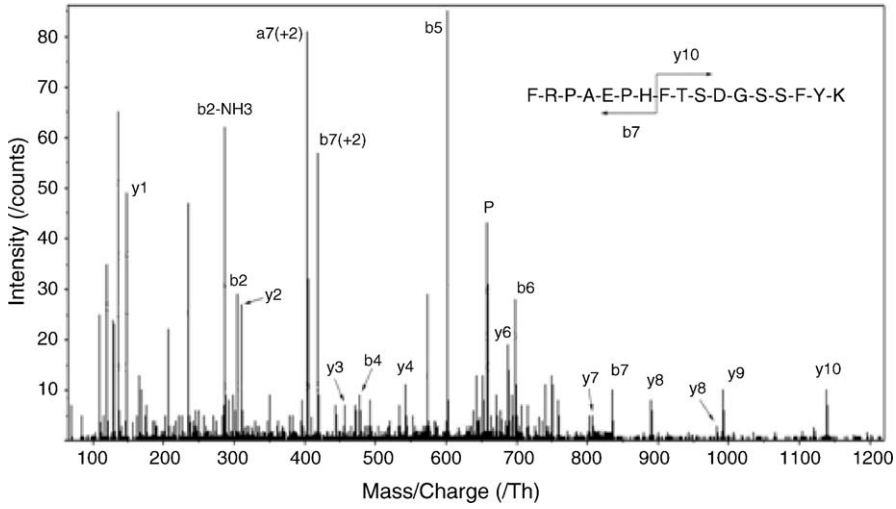

Fig. 6. One of the MS/MS spectra collected during the LC–MS run shown in Fig. 5. This fragmentation spectrum of a triply-charged parental ion with *m*/*z* 658.3 was assigned to the peptide FRPAEPHFTSDGSSFYK from rat dipeptidy lpeptidase IV. Most of the y- and b-series ions were matched and are labeled. The fragment ions have unit charge unless indicated by "+2". The precursor ion is marked by "P".

the eluted antigen with the IgG. On the other hand, the protein G column can be repeatedly used for the isolation of other antigens. Reuse is also important, especially in the case where immobilized, cross-linked antibody is not stable under given elution conditions and cannot be used for more than two to three isolations of the corresponding antigen. As mentioned above, mAb 5.4 recognizes native CEACAM1. This mAb has very high affinity for this glycoprotein and can also be used for the isolation of different form of CEACAM1 from the plasma membranes from Morris hepatoma 7777 (cf. Fig. 3). However, if immunobloting is performed, the mAb 9.2 another monoclonal antibody against this protein, has to be used, because the mAb 5.4 poorly recognizes denatured form of this protein.

In previous experiments we also used monoclonal antibodies directly immobilized to epoxy-activated CIM disk for isolation of corresponding antigens (inter-alpha inhibitor proteins, see ref. [24]). However, if such an approach was used, the capacity of the column was much lower (not shown).

3.2. Isolation of dipeptidyl peptidase IV

Dipeptidyl peptidase IV (DPP-IV, CD 26) is a glycoprotein with an apparent molecular weight of 110 kDa in SDS-PAGE that is expressed in a great variety of different cell types [25]. This enzyme is one of the glycoproteins that are expressed in the plasma membranes of hepatocytes and are at least partially lost during malignant modification [26]. Monoclonal antibody No. 5.12 against this glycoprotein was raised in our laboratory and has been used for the study of the physiological role of this protein [27,28].

As shown in Fig. 4, mAb 5.12 can be used for the isolation of DPP-IV from solubilized liver plasma membranes. In this case, 150 mL of Triton X-100 extract (protein concentration

Table 1
Peptides belonging to the integral membrane protein DPP-IV identified in the LC–MS/MS run shown in Fig. 5

Peptides assigned to DPP-IV			
Confidence[a]	Peptide sequence[b]	Mass[c]	Δ(mass)[d]
99	FRPAEPHFTSDGSSFY K	1971.87	−0.05
99	GPGLPLYTLHR	1222.66	−0.02
99	YOGLPTPEDNLDHYR	1835.77	−0.05
99	LGTLEVEDQIEAAR	1542.78	−0.01
99	WEYYDSVYTER	1509.64	0.00
86	HSYTASYSIYDLNK	1660.78	0.01
86	VWSDSEYLYK	1288.56	−0.04
18	TYTLADYLK	1086.54	−0.04
2	TVWIPYPK	1002.54	−0.01
0	ISLQWLR	914.51	−0.03

[a] Percent confidence calculated by ProID that the assignment is correct. The maximum is fixed at 99%.
[b] In addition to the normal one-letter codes, "O" stands for oxidized methionine.
[c] Experimental monoisotopic mass of the neutral peptide, in Da.
[d] Difference between experimental and theoretical masses, in Da.

0.45 mg/mL, total protein content 67.5 mg) from liver plasma membranes was used, without any previous purification step with anion-exchange chromatography. Identification of DPP-IV from the upper band with an apparent molecular weight of 110 kDa in the SDS-PAGE (cf. Fig. 4) is shown in Figs. 5 and 6 and in Table 1. Fig. 5 shown reversed-phase nano-HPLC separation of tryptic peptides from extracted protein from the excided 110 kDa band. Peptides assigned to DPP-IV were listed in Table 1. One of the representative MS/MS spectra collected during the LC–MS run is shown in Fig. 6 for the largest peptide FRPAEPHFTSDGSSFYK (MW 1971.87). In this sample, DPP-IV was identified with a very high confidence (over 99%) and a score of 11.80. Proteins coeluting with DPP-IV are identified in the lower bands (cf. Fig. 4) as liver glycogen phoshorylase, microsomal triglyceride transfer protein and mouse serum albumin. A possible interaction between DPP-IV and two first enzymes from the liver has to be investigated. Mouse serum albumin seems to be an impurity, coming likely from mouse ascites, used for production of mAb.

4. Conclusions

Specific monoclonal antibodies bound to protein A or protein G CIM disk with a bed volume of only 60 μL can be used for very rapid isolation of membrane proteins out of very large volumes of complex biological mixtures such as plasma membrane extracts.

Even very low abundance proteins can be highly enriched by use of this method.

If an antibody is bound to the protein A (G) disk without cross-linking, the use of such a separation device is not limited to the short lifetime of the immobilized antibody. However, the antigen is co-eluted with the corresponding antibody and has to be separated by SDS-PAGE.

After tryptic digestion of excised bands, isolated antigen can be identified by immunoblot or with LC–MS/MS.

Acknowledgements

This work was supported by National Institute of Health, Centers for Biochemical Research Excellence (COBRE), Grant No. P20RR017695 and NIH Grant CA 42714.

References

[1] T. Tennikova, J. Reusch, J. Chromatogr. A 1065 (2005) 13.
[2] D. Forcic, K. Branovic-Cakanic, J. Ivancic, R. Jug, M. Barut, A. Strancar, J. Chromatogr. A 1065 (2005) 115.
[3] T.V. Gupalova, O.V. Lojkina, V.G. Palagnuk, A.A. Totolian, T.B. Tennikova, J. Chromatogr. A 949 (2002) 185.
[4] H. Abou-Rebyeh, F. Koerber, K. Schubert-Rehberg, J. Reusch, D. Josic, J. Chromatogr. B 566 (1991) 341.
[5] F. Svec, M.J. Frechet, Science 273 (1996) 205.
[6] A. Strancar, P. Koselj, H. Schwinn, D. Josic, Anal. Chem. 68 (1996) 3483.
[7] A. Strancar, M. Barut, A. Podgornik, P. Koselj, D. Josic, A. Buchacher, LC–GC Int. 10 (1998) 660.
[8] D. Josic, H. Schwinn, A. Strancar, A. Podgornik, M. Barut, Y.-P. Lim, M. Vodopivec, J. Chromatogr. A 803 (1998) 61.
[9] K. Branovic, G. Lattner, M. Barut, A. Strancar, D. Josic, A. Buchacher, J. Immunol. Methods 271 (2002) 47.
[10] D. Josic, M.K. Brown, F. Huang, H. Callanan, M. Rucevic, A. Nicoletti, J. Clifton, D.C. Hixson, Electrophoresis 26 (2005) 2809.
[11] D. Josic, K. Zeilinger, Methods Enzymol. 271 (1996) 113.
[12] A. Journet, A. Chapel, S. Kieffer, M. Louwagie, S. Luche, J. Garin, Electrophoresis 21 (2000) 3411.
[13] C. Pasquali, I. Fiaka, L.A. Huber, Electrophoresis 18 (1997) 2573.
[14] L.A. Huber, K. Pfaller, I. Vietor, Circ. Res. 92 (2003) 962.
[15] Y. Zhao, S.W. Kwon, A. Anselmo, K. Kau, M.A. White, Anal. Chem. 76 (2004) 1817.
[16] Y. Bledi, A. Inberg, M. Linial, Briefing, Funct. Genom. Proteom. 2 (2003) 254.
[17] P.S. Chang, A. Absood, J.J. Lindermann, G.M. Omann, Anal. Biochem. 325 (2004) 175.
[18] M.M. Comegys, S.-H. Lin, D. Rand, D. Britt, D. Flanagan, H. Callanan, K. Brilliant, D.C. Hixson, J. Biol. Chem. 279 (2004) 35063.
[19] D.C. Hixson, R.A. Farris, N.L. Thompson, Pathobiology 58 (1990) 65.
[20] L. Lesko, M. Donion, G.V. Marinetti, J.D. Hare, Biochim. Biophys. Acta 311 (1973) 173.
[21] R. Tauber, W. Reutter, Eur. J. Biochem. 83 (1978) 37.
[22] C. Schneider, R.A. Newman, D.R. Sutherland, U. Asser, M.F. Graeves, J. Biol. Chem. 257 (1982) 10766.
[23] A. Becker, R. Neumeier, C.S. Park, R. Gossrau, W. Reutter, Eur. J. Cell Biol. 39 (1985) 417.
[24] Y.-P. Lim, D. Josic, H. Callanan, J. Brown, D.C. Hixson, J. Chromatogr. A 1065 (2005) 39.
[25] L. Gaetaniello, M. Fiore, S. de Filippo, N. Pozzi, S. Tomasi, C. Pignata, Hepatology 27 (1998) 934.
[26] C. Hanski, T. Huhle, R. Gossrau, W. Reutter, Exp. Cell Res. 178 (1988) 64.
[27] G.A. Piazza, H.M. Callahan, J. Mowery, D.C. Hixson, Biochem. J. 262 (1989) 327.
[28] D.C. Hixson, K.D. McEntire, B. Obrink, Cancer Res. 45 (1985) 3742.