ELSEVIER

Journal of Chromatography B, 722 (1999) 255–262

JOURNAL OF CHROMATOGRAPHY B

Review

Isolation and separation of proteoglycans

H. Savolainen

Institute of Occupational Health Sciences, Rue du Bugnon 19, CH-1005 Lausanne, Switzerland

Abstract

Proteoglycans contain a polypeptide core and an oligosaccharide chain composed of aminohexoses and uronic acid. The glycan chain is attached to the polypeptide in a bond to serine hydroxyl. The glycan chains may contain up to 200 disaccharide units and the proteoglycan molecular mass ranges from a few thousands to millions. Their physiological functions are related to barriers limiting diffusion across the membranes, articular lubrification, blood coagulation and cellular adhesion. The tissue proteoglycans can be extracted with 4 *M* guanidine hydrochloride and purified with chromatographic techniques. The soluble proteoglycans can be precipitated with cetylpyridinium chloride, purified by chromatography or by dialysis. All proteoglycan species are amenable to electrophoresis on polyacrylamide gels, and after blotting on polyvinylidene fluoride membranes, they can be stained for glycans. Proteoglycan analyses have shown their value in clinical mucopolysaccharidosis diagnostics, in occupational toxicology and in coagulation studies. Experimental applications include cell adhesion studies in tumor biology, regeneration in neurosciences or maturation of skin and kidneys.

Keywords: Reviews; Proteoglycans

Contents

1. Introduction

Glycoproteins are polypeptides which have polysaccharide side chains [1]. Most frequently, the sugar chain is attached to the polypeptide core with an aspartyl–glucosamine link, and the polysaccharide chain may branch to form extensive antenna-like structures [2]. Polysaccharide chains do not contain

0378-4347/99/$ – see front matter
PII: S0378-4347(98)00312-0

very many sugar units. The polysaccharide moieties are added on the peptide core in posttranslational reactions, and they may serve to ensure correct tertiary structure, to provide resistance towards proteolysis or to target correctly the molecules in the cells [1].

Proteoglycans are not a subclass of glycoproteins, even if they share some biochemical characteristics. One of the most glaring principal differences is the large molecular size of the polysaccharide chain which may contain up to 400 basic saccharides [3]. Another hallmark is that the chains do not mainly branch except, perhaps, terminally. Once initially formed, chains undergo quite extensive transformation, so that the glucuronic acid moieties may be epimerized to iduronic acid [3] and the sugar moieties are also extensively sulfated [4].

Four major classes of glycan chains, as defined by the repeating disaccharide units, can be found (Table 1). All organs contain one or several types of glycan chains which are typically linked to polypeptide cores by the serine hydroxyl group. The molecular mass of the complex thus formed may range from millions to a few thousands. Because of this biochemical variability, the proteoglycans are implicated in many principal physiological functions, namely as barriers to diffusion across basement membranes, as suppliers of mechanical repulsion in the lubrication of joints, as anticoagulants and as receptors and binding sites of proteins [3].

Glycan chains can be liberated from the polypeptide cores conveniently by enzymatic proteolysis which leaves other than peptide bonds intact. The glycan chains obtained are called glycosaminoglycans because the repeating disaccharides all contain the aminosugars. The resulting glycosaminoglycans are also highly soluble in water buffers so that their manipulation and analysis are greatly facilitated as compared to parent proteoglycans. An abundant literature exists on their chemistry [3].

Because of the limited number of glycan chain variants, the basic analytical possibilities are rapidly exhausted, and they have been completed most often by kinetic tests of sulfation using radioactive precursors or by localizing studies of the glycans in the tissues and organs. The latter can be achieved by raising antibodies towards specific chain forms, e.g. heparan sulfate, or by using so-called lectins which bind to specific glycan terminal structures [5]. While versatile probes for microscopic work, the lectins do not distinguish between glycoproteins and proteoglycans.

Salivary [6] and respiratory mucins [7] are accessible sources of the very large molecular mass proteoglycans while serum [8] and urine [9] proteoglycans are of smaller size. The large sizes of mucin proteoglycans pose special requirements on analytical techniques because of the viscosity of the samples or because of difficulties of the separation of the very large species. Peptide finger printing or proteolysis to liberate glycan chains have been used in the analysis of blood and secretory proteoglycans.

With the increase in the interest for cellular adhesion mechanisms and receptor molecules, the so-called integrins [10] and syndecans [11], the analytical techniques for specific proteoglycan molecules have gained more importance. Consequently, even some of their genetic codes are known [11].

Table 1
Composition of glycan chains

Polymer	Basic units	Examples of function
Heparan	Glucosamine-(α1,4)-iduronic acid	Glomerular membrane
Heparan sulfate		Blood coagulation
Chondroitin 4/6 sulfate	Galactosamine-(β1,4)-iduronic acid	Brain adhesion molecules
Dermatan sulfate		Kidney tubular lining
Keratan sulfate	Glucosamine-(β1,3)-galactose	Skin adhesion molecules
		Corneal matrix
Hyaluronan	Glucosamine-(β1,4)-glucuronic acid	Vitreous body matrix

Further, a proposition for a simplified nomenclature of cell membrane and extracellular proteoglycans has been made [12].

2. Isolation techniques

Significant differences in the polypeptide of the proteoglycans in the membranes, of the mucins, of circulatory or of secretory proteoglycans must exist to account for their varied biochemical functions. Isolated glycosaminoglycan chains from various sources mostly display one or several of the glycan chain types. While it is interesting to know the general glycan composition of brain proteoglycans, it is not helpful in trying to assign functions to specific proteoglycan species in health or in disease [13]. This stresses the need for isolation of specific proteoglycan molecules for their biochemical and physiological study.

Because of tissue- and cell-specific differences in matrix composition and proteoglycan content, no universal method for their isolation exists. For practical purposes, the methods have been divided according to the proteoglycan source into techniques applicable to cellular and to secretory or circulatory molecules, respectively.

2.1. Isolation of tissue proteoglycans

The principal problem in the extraction of tissue proteoglycans is their localization in the cell membranes. They therefore contain lipophilic sequences which causes the circumstance that a detergent or a chaotrophic agent has to be used in the isolation. The most commonly employed chaotrope is 4 *M* guanidine hydrochloride in the isolation buffer [14–16]. The isolation buffers typically also contain inhibitors of proteolysis.

Experiments with radioactively labeled proteoglycans show that up to 95% of glycan-containing molecules can be isolated e.g. from the alveolar cells [14].

While this indicates that the fractions may be representative for metabolic or kinetic studies, further separation is often necessary to obtain well purified fractions. Further purification is mostly begun with isopycnic density gradient ultracentrifugation in CsCl/4 *M* guanidine chloride (initial density 1.4 to 1.5 g/ml) [7,15]. Centrifuged fractions can then be subjected to gel permeation chromatography [15] or affinity chromatography [17]. Preparative high-pressure liquid chromatography has also been used for the purification of corneal proteoglycans [16].

Heparan sulfate-containing proteoglycans are especially amenable to isolation and analysis by their interaction with lipoprotein lipase using the latter as fixed ligand in affinity columns [18]. Chondroitin 6 sulfate-containing proteoglycans have markedly less affinity for lipoprotein lipase so that it should not interfere with the heparan sulfate assay.

2.2. Isolation of circulating and secretory proteoglycans

Mucus proteoglycans are most often isolated by an initial extraction with 6 *M* guanidine hydrochloride followed by a CsCl density gradient centrifugation [19]. The centrifuged proteoglycan fractions are then chromatographed with 10 m*M* dithiothreitol on a Sepharose CL-2B (2.6×90-cm) column with 4 *M* guanidine hydrochloride as eluent. The isolated mucin fraction can be subjected to electron microscopic study or chemical hydrolysis and subsequent analysis [19].

Urinary proteoglycans are most conveniently isolated with the cetylpyridinium chloride. A volume of 10–20 ml of urine is needed to which 0.2–0.4 ml of 50 g/l cetylpyridinium chloride are added. After an overnight precipitation at 4°C, the ensuing precipitate is washed with absolute ethanol. The precipitates can be easily dissolved in buffers which contain 5 m*M* calcium chloride [20]. These preparations can be subjected to proteolysis or to treatment that lyse glycan chains, either by chondroitinase or by heparinase [20]. Thus, either the glycan chains or the peptide cores can be studied independently.

A similar procedure can be applied on serum samples [8] with further chemical and enzymatic hydrolysis [21]. Human serum contains considerably more proteoglycans than urine so that 1 ml of serum diluted 1:10 with physiological saline constitutes a convenient working solution [8]. It should be re-

membered that cetylpyridinium chloride powder is irritating [22].

The proteoglycans can be quantitated with dye-binding techniques directly [23,24] or by analysis of their hydrolysates for hexosamines and uronic acids by colorimetric or chromatographic techniques [21]. If dye binding methods are used, the guanidine hydrochloride should be removed by dialysis as it completely abolishes the dye binding.

3. Separation of proteoglycans

The easiest way to separate the soluble proteoglycans by their molecular mass is the use of a centrifugal concentrator (Centricon, Amicon, Beverly, MA, USA). With it, proteoglycan species with molecular mass from 3000 to 500 000 can be separated by choosing the appropriate cut-off values. An example of preparative separation of urinary proteoglycans with a 50 000 cut-off membrane is presented in Fig. 1.

An elegant high-pressure liquid chromatographic technique has been conceived for the analysis of the 4 *M* guanidine hydrochloride extracts [25]. Essentially, it is a size exclusion technique using an isocratic mobile phase of 8 *M* urea. It is a very rapid procedure even when working with molecules of more than a million relative molecular mass.

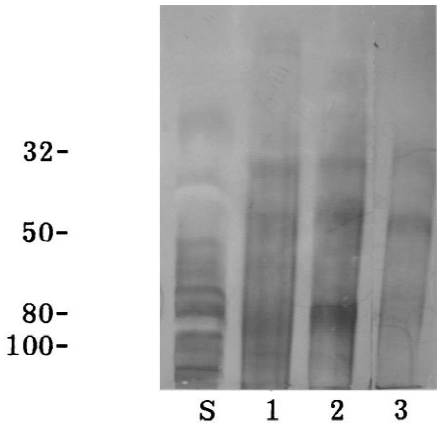

Fig. 1. Proteoglycan composition of human serum (S), proteoglycans and glycoproteins of untreated human urine (1), isolated proteoglycans of human urine (2) and partially purified small-molecular-mass urinary proteoglycans (3). All samples (5 μg protein) were first electrophoresed on 12% polyacrylamide gel then blotted on polyvinylidene fluoride membrane for glycan staining. Note that most urinary proteoglycans do not have a counterpart in the serum pattern. The small-molecular-mass proteoglycans can be conveniently prepared with the centrifuge concentrator tube method for further analysis. Scale on the left indicates the migration of molecular weight standards ($\times 10^{-3}$).

Precipitation of liberated glycan chains by simple aliphatic alcohols has been accomplished in a semi-preparative scale [26]. The isolated fractions can be analysed with agarose gel electrophoresis [26]. Based on this, a more sensitive staining method for the electrophoretically separated glycosaminoglycans has been developed using the silver staining technique [27].

Intact proteoglycan from porcine intramuscular connective tissue as isolated with the guanidine hydrochloride extraction method has been directly analysed with sodium dodecyl sulfate-containing polyacrylamide gel electrophoresis [28]. The proteoglycan bands were stained with Alcian blue which is not a very sensitive stain. To overcome the sensitivity problems, the proteoglycans can be labeled with radioactive sulfate before their extraction with a subsequent detection of radioactive bands in the electrophoretic pattern. This approach has been applied in a study on proteoglycans in a whole skin organ culture study [29]. The label seems to be associated in this case with a 350 000 molecular mass proteoglycan [29].

Use of radioactive proteoglycan precursors is not always possible so that a sensitive and glycan-specific staining method would be highly desirable. A very versatile strategy is provided by the blotting of proteoglycan bands after their electrophoresis in polyacrylamide gel on polyvinylidene fluoride membrane [30] with a subsequent detection with specific antibodies towards digitoxigenin-adducted glycans [31]. This procedure is some 1000 times more sensitive than the corresponding Alcian blue methods. It allows even a direct detection of native urine glycoprotein and proteoglycan species (Fig. 1).

Urinary proteoglycan analysis has a direct clinical utility in occupational health [30,32] and it opens interesting views on interstitial cystitis [33] and proteinuria (Fig. 2). To gain more insight in the proteoglycans associated with the renal and urinary tract pathology, the glycosaminoglycan chains can, of course, be liberated from the polypeptide cores

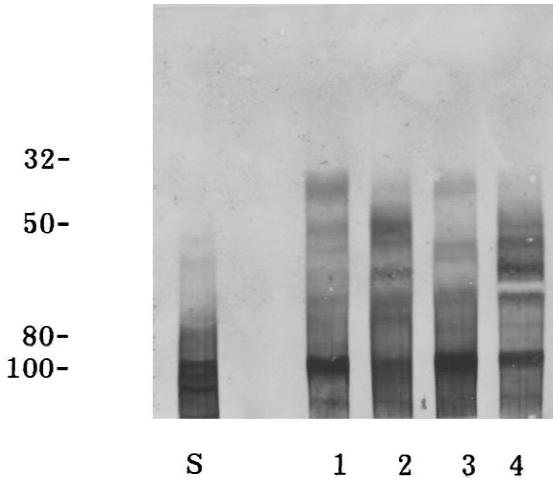

Fig. 2. Urinary proteoglycans of a control (1) and three cases of renal proteinuria (2–4). The serum proteoglycan pattern (S) is shown for reference. Note the appearance of small-molecular-mass proteoglycans in pattern 2–4 which do not have counterparts in the serum proteoglycan pattern. They are therefore of the urinary tract origin and possibly associated with the loss of the renal barrier function causing the excretion of protein in the urine. Scale on the left indicates the migration of molecular mass standards ($\times 10^{-3}$).

and analysed. This strategy has been used successfully in Wilms' tumor patients. It seems that in the case of tumor recurrence, the urinary hyaluronan excretion increases [34].

Information of the composition of the intact proteoglycan chains can be gained using their reactions with specific lectins. Commercial kits (Boehringer Mannheim) exist which allow, for example, the detection of sialic acid (Fig. 3A) or galactose (1–4) *N*-acetyl glucosamine terminals (Fig. 3B).

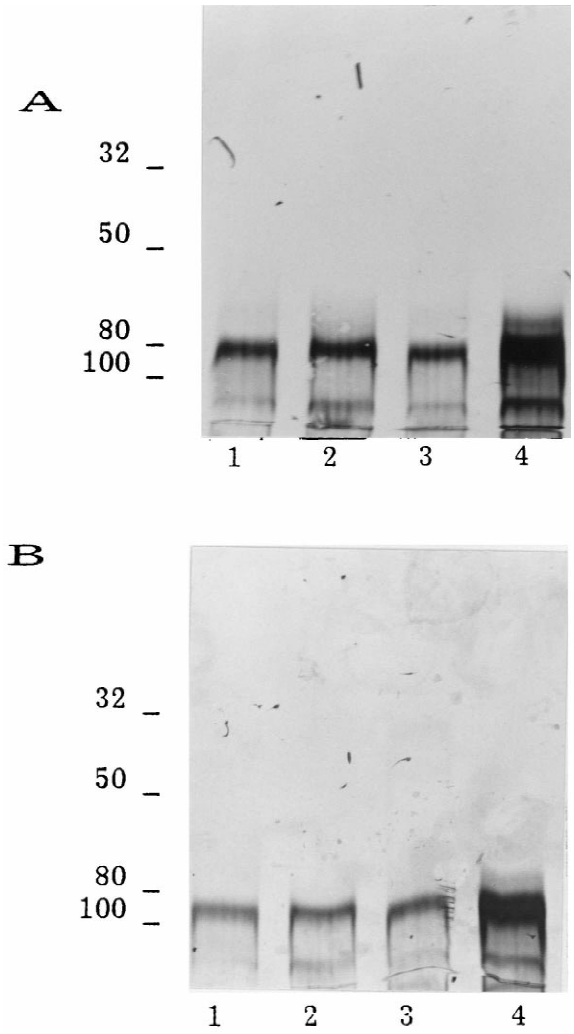

Fig. 3. Reaction of urinary proteoglycans with lectin from *Maackia amurensis* (A) in control (1) and of three patients with proteinuria (2–4, cf. Fig. 2) and of the same specimens with *Datura stramonium* lectin (B). In patterns of (A), the sialic acid terminals, and in (B) the galactose-β (1–4)-*N*-acetylglucosamine terminals are detected. Scales on the left indicate the migration of molecular mass standards. Note that the proteoglycans of a molecular mass of less than 50 000 are not positive in the lectin detection.

4. Discussion

The current analytical methods allow the isolation of human and animal proteoglycans quite well. It seems that it is no longer necessary to carry out partial hydrolysis of the peptide or glycan chains, respectively, to gain practical starting materials for chemical analysis. The data on the tissue specific proteoglycans is still, however, fragmentary. Examples of isolated proteoglycans are given in Table 2.

4.1. Physiological significance

Proteoglycans are major components of connective tissue and, for example, 4% of bone protein belong to them [35]. They are also present in connective tissue in other organs, and, notably, in the cartilage.

Table 2
Proteoglycans isolated from various model sources

Source	Glycan	Molecular mass[a]	Ref.
Brain	Chondroitin sulfate	100	[12]
Cornea	Keratan sulfate	95	[16]
Kidney	Heparan sulfate	400	[12]
Lung cell culture	Heparan sulfate	350	[14]
Muscle connective tissue	Dermatan sulfate	100	[28]
Serum	Chondroitin sulfate	112	[8]
Skin culture	Dermatan sulfate	350	[29]
Urine	Chondroitin sulfate	90	[30]

[a] Relative molecular mass $\times 10^{-3}$.

It seems that the basic functional role of the proteoglycans is to maintain and stabilise the collagen fibril morphology [36]. While the association with collagen fibres may be through the ionic forces [36], a special link protein molecule exists for the association of proteoglycans with the cartilage matrix [37].

Probably the most intriguing function of the proteoglycans is their role in the cellular adhesion [38]. The adhesion mediated by the extracellular proteoglycans is very strong [39]. Specific adhesion receptors are also known to be in some cases proteoglycans. For example, a heparan sulfate proteoglycan, called epican, in the keratinocytes is an alternative form of the CD44 molecule [40]. Thus, the proteoglycans may have an important role in adhesion and proliferation in general [41], and in tissues with a rapid cellular turn-over, like bone marrow [42] and skin [43], in particular.

The circulating proteoglycans are most probably synthesized in the liver [44]. It cannot be excluded that they are transported to other organs to serve as precursors, as e.g. in temporary repair of intimal artery lesions. The latter would be intimately related to blood coagulation where the proteoglycans have confirmed regulatory roles [45]. It is obvious that determination of heparin and dextran sulfate in patient plasma can be an important diagnostic test [46]. In this respect it might be interesting to note that exogenous chondroitin sulfate has an initial half-life in the circulation of some 25 min and a second slower phase of elimination with a half-life of 280 min [47].

A major part of urinary proteoglycans may be secreted by the kidney in the urine [48]. On the urinary tract walls, they have an important barrier function and they allow resistance against microbial invasion and infestation in bladder and urethra [49]. Urinary excretion of proteoglycans undergoes important maturational changes in early infancy [9,23], and individual variability may explain susceptibility to renal stone formation because glycosaminoglycans are known to inhibit calcium precipitation [50]. Increased calcium excretion causes increased proteoglycan excretion in healthy individuals [51].

It seems that proteoglycan isolation and analysis would have very much to offer. Obvious applications would be in clinical and experimental studies on cell and tissue maturation, wound healing, blood coagulation, platelet function, hair growth [52], among others.

4.2. Pathophysiological roles

Mucopolysaccharidoses are a heterogenous group of diseases involving inherited defects in the proteoglycan metabolism. These disorders are often associated with mental deficiency and connective tissue changes. Diagnostic tests for them include the demonstration of abnormal proteoglycans [23] or of impaired specific enzyme activity [53]. While these diseases are not very frequent, urinary screening for glycosaminoglycans is sometimes carried out in special neonatal populations.

A more important aspect of the proteoglycan metabolism is that they can be targets of toxic action of environmental chemicals or drugs thereby having a pathophysiological role in degenerative or malignant diseases. As it has been already stated, changes

in the cellular adhesion mechanisms may have a crucial role in the malignisation process of cancer cells [54]. Terminal sialyl moieties and their increased or decreased expression seem to be hallmarks of some common human tumors [54]. The clinical interpretation is still tenuous and important inter-ethnic differences may also exist [55].

Ultraviolet light is harmful to eyes [56,57]. One of the mechanisms may be the peroxidation as glycan chains can be shortened and destroyed by model oxidants [58–60].

Chronic articular lesions are associated with diminished synthesis of proteoglycans [61]. They are of different molecular size than those synthesized by normal chondrocytes [61]. D-Penicillamine in an experimental model, reverses the inhibition of proteoglycan synthesis [62]. It is an anti-inflammatory agent by suppressing the H_2O_2-mediated prostaglandin synthesis [62]. Therefore, it is not clear whether the beneficial effect is solely due to decreased peroxidative damage rather than stimulated proteoglycan synthesis.

Several di-cationic amphiphilic drugs are known to cause an accumulation of glycosaminoglycans in cell culture models [63,64]. The mechanism is likely to involve an inhibition of a lytic enzyme activity as all types of glycosaminoglycans chains are accumulated in the treated fibroblast lysosomes [64]. Theoretically, this could be beneficial in cases with decreased proteoglycan synthesis whereas this cell model might be more useful in research on mucopolysaccharidoses. Anyway, inhibitors of proteoglycan synthesis offer a very useful tool in experimental studies of pulmonary emphysema [65].

Altered synthesis of a chondroitin sulfate proteoglycan is a salient feature in cerebral injury models [66]. The cells expressing increased proteoglycan formation are glia adjacent to the trauma site, and they may be responsible for unsuccessful axonal repair [67] and for the formation of inclusion bodies in neurological degenerative diseases [68]. An in-depth review on the role of proteoglycans in Alzheimer's disease has been published [69].

In conclusion, current analytical techniques allow the isolation of proteoglycans in sufficient quantity and purity for their chemical analysis. Useful clinical and experimental applications exist already, and there is excellent scope for further applications in tumor biology, in neurosciences and in the biology and medicine of the locomotor system.

Acknowledgements

I thank Ms. Nicole Turesky for her technical assistance and Ms. F. Valceschini for typing this article.

References

[1] G. McDowell, W.A. Gahl, Proc. Soc. Exp. Biol. Med. 215 (1997) 145.
[2] M. Covarrubias, C. Kopta, J.H. Steinbach, J. Gen. Physiol. 93 (1989) 765.
[3] S. Ernst, R. Langer, C.L. Cooney, R. Sasisekharan, Crit. Rev. Biochem. Mol. Biol. 30 (1995) 387.
[4] M. Salmivirta, K. Lidholt, U. Lindahl, FASEB J. 10 (1996) 1270.
[5] S.S. Spicer, B.A. Schulte, J. Histochem. Cytochem. 40 (1992) 1.
[6] A.M. Wu, G. Csako, A. Herp, Mol. Cell. Biochem. 137 (1994) 39.
[7] J.R. Davies, H.V. Hovenberg, C.-J. Lindén, R. Howard, P.S. Richardson, J.K. Sheehan, I. Carlstedt, Biochem. J. 313 (1996) 431.
[8] H. Savolainen, Res. Commun. Mol. Pathol. Pharmacol. 88 (1995) 359.
[9] H. Savolainen, Glycosyl. Dis. 1 (1994) 191.
[10] D.J. Carey, Biochem. J. 327 (1997) 1.
[11] G.S. Hillis, A.M. MacLeod, Clin. Sci. 91 (1996) 639.
[12] R.V. Iozzo, A.D. Murdoch, FASEB J. 10 (1996) 598.
[13] S. Hockfield, R.G. Kalb, S. Zaremba, H. Fryer, Gold Spring Symp. Quant. Biol. 55 (1990) 505.
[14] W.M. Maniscalco, M.H. Campbell, Am. J. Physiol. 263 (1992) L348.
[15] J.A. Ripellino, R.U. Margolis, J. Neurochem. 52 (1989) 807.
[16] J.L. Funderburgh, G.W. Conrad, J. Biol. Chem. 265 (1990) 8297.
[17] A. Lookene, R. Savonen, G. Olivecrona, Biochem. 36 (1997) 5267.
[18] M. Okamoto, S. Mori, M. Ishimaru, H. Tohge, Y. Nakata, H. Endo, Life Sci. 60 (1997) 1811.
[19] J.K. Sheehan, P.S. Richardson, D.C.K. Fung, M. Howard, D.J. Thornton, Am. J. Resp. Cell Mol. Biol. 13 (1995) 748.
[20] H. Wlad, W. Fenrych, K. Lacka, W. Sikorska-Horst, J. Clin. Chem. Clin. Biochem. 26 (1988) 259.
[21] N. Gässler, C. Reissner, N. Janzen, H. Kähnert, K. Kleesiek, Eur. J. Clin. Chem. Clin. Biochem. 31 (1993) 503.
[22] G.H.Y. Lin, K.A. Voss, T.J. Davidson, Fd. Chem. Toxic. 29 (1991) 851.
[23] L.P. Thuy, W.L. Nyhan, Clin. Chim. Acta 212 (1992) 17.

[24] V.P. Bhavanandan, A. Martin, D.R. Erickson, Clin. Chim. Acta 251 (1996) 207.
[25] P.Y. Johnson, D.A. Blake, J. Chromatogr. B 688 (1997) 27.
[26] N. Volpi, J. Chromatogr. B 685 (1996) 27.
[27] C.H.A. van de Lest, E.M.M. Versteeg, J.H. Veerkamp, T.H. van Kuppevelt, Anal. Biochem. 221 (1994) 356.
[28] T. Nishiumi, T. Fukuda, T. Nishimura, J. Agric. Fd. Chem. 45 (1997) 2978.
[29] A.-L. Martikainen, M. Tammi, R. Tammi, J. Invest. Dermatol. 99 (1992) 623.
[30] H. Savolainen, Res. Commun. Chem. Pathol. Pharmacol. 82 (1993) 241.
[31] A. Haselbeck, W. Hösel, Glyconjug. J. 7 (1990) 63.
[32] H. Savolainen, Rev. Env. Health 11 (1996) 167.
[33] R.E. Hurst, J.B. Roy, K.W. Min, R.W. Veltri, G. Marley, K. Patton, D.L. Shackelfort, P. Stein, C.L. Parsons, Urology 48 (1996) 817.
[34] R.Y. Lin, P.A. Argenta, K.M. Sullivan, R. Stern, N.S. Adzock, J. Pediatr. Surg. 30 (1995) 304.
[35] J.T. Triffitt, Clin. Sci. 72 (1987) 399.
[36] C. Dell'Orbo, G. De Luca, L. Gioglio, D. Quacci, C. Soldi, Histol. Histopathol. 10 (1995) 583.
[37] Q. Nguyen, G. Murphy, P.J. Roughley, J.S. Mort, Biochem. J. 259 (1989) 61.
[38] D. Spillmann, M.M. Burger, J. Cell. Biochem. 61 (1996) 562.
[39] U. Dammer, O. Popescu, P. Wagner, D. Anselmetti, H.-J. Güntherodt, G.N. Misevic, Science 267 (1995) 1173.
[40] L.C. Kugelman, S. Ganguly, J.G. Haggerty, S.M. Weissman, L.M. Milstone, J. Invest. Dermatol. 99 (1992) 381.
[41] J.-P. Zanetta, A. Badache, S. Maschke, S. Kuchler, Histol. Histopathol. 9 (1994) 385.
[42] J.J. Minguell, M. Tavassoli, Blood 73 (1989) 1821.
[43] X. Guillonneau, J. Tassin, E. Berrou, M. Bryckaert, Y. Courtois, F. Mascarelli, J. Cell. Physiol. 166 (1996) 170.
[44] A.M. Gressner, S. Schäfer, J. Clin. Chem. Clin. Biochem. 27 (1989) 141.
[45] M.-C. Bourin, U. Lindahl, Biochem. J. 289 (1993) 313.
[46] L.B. Jacques, S.M. Wice, L.M. Hiebert, J. Lab. Clin. Med. 115 (1990) 422.
[47] A. Conte, M. de Bernardi, L. Palmieri, P. Lualdi, G. Mautone, G. Ronca, Drug Res. 41 (1991) 768.
[48] R.E. Hurst, World J. Urol. 12 (1994) 3.
[49] R.E. Hurst, R. Zebrowski, J. Urol. 152 (1994) 1641.
[50] D. Grant, W.F. Long, F.B. Willamson, Biochem. J. 259 (1989) 41.
[51] J. Laitinen, J. Liesivuori, H. Savolainen, Occup. Envir. Med. 53 (1996) 595.
[52] G.E. Westgate, A.G. Messenger, L.P. Watson, W.T. Gibson, J. Invest. Dermatol. 96 (1991) 191.
[53] M.R. Natowitz, P.M. Short, Y. Wang, G.R. Dickersin, M.C. Gebhardt, D.I. Rosenthal, K.B. Sims, A.E. Rosenberg, New Engl. J. Med. 335 (1996) 1029.
[54] E.F. Hounsell, M. Young, M.J. Davies, Clin. Sci. 93 (1997) 287.
[55] G. Lindberg, H. Iso, L. Rastam, A. Lundblad, A.R. Folsom, Int. J. Epidemiol. 26 (1997) 58.
[56] H.R. Taylor, S.K. West, F.S. Rosenthal, B. Munoz, H.S. Newland, H. Abbey, E.A. Emmett, New Engl. J. Med. 319 (1988) 1429.
[57] E. Lapcik, L. Omelka, K. Kubena, A. Galatik, V. Kello, Gen. Physiol. Biophys. 9 (1990) 419.
[58] K.R. Hightower, J.P. McCready, D. Borchman, Photochem. Photobiol. 59 (1994) 485.
[59] R. Moseley, R. Waddington, P. Evans, B. Halliwell, G. Embery, Biochim. Biophys. Acta 1244 (1995) 245.
[60] M. Li, L. Rosenfeld, R.E. Vilar, M.K. Cowman, Arch. Biochem. Biophys. 341 (1997) 245.
[61] R. Shuckett, C.J. Malemud, Proc. Soc. Exp. Biol. Med. 190 (1989) 275.
[62] M.S. Baker, J. Feigan, D.A. Lowther, Biochem. Int. 16 (1988) 1153.
[63] R. Lüllmann-Rauch, M. Ziegenhagen, Virch. Arch. B Cell Pathol. 60 (1991) 99.
[64] J. Fischer, Biochem. Pharmacol. 52 (1996) 1331.
[65] T.H. van Kuppevelt, C.H.A. van de Lest, E.M.M. Versteeg, R. Dekhuijzen, J.H. Veerkamp, Am. J. Respir. Cell Mol. Biol. 16 (1996) 75.
[66] J.M. Levine, J. Neurosci. 14 (1994) 4716.
[67] R.J. McKeon, A. Höke, J. Silver, Exp. Neurol. 136 (1995) 32.
[68] D.A. De Witt, P.L. Richey, D. Praprotnik, J. Silver, G. Perry, Brain Res. 656 (1994) 205.
[69] B. Leveugle, H. Fillit, Mol. Neurobiol. 9 (1994) 25.