ELSEVIER

Journal of Chromatography A, 708 (1995) 263–271

JOURNAL OF CHROMATOGRAPHY A

Characterization of size-permeation limits of cell walls and porous separation materials by high-performance size-exclusion chromatography

Holger Woehlecke, R. Ehwald*

Institute of Biology, Math.-Nat. Faculty I, Humboldt-University, Invalidenstr. 42, D-10115 Berlin, Germany

First received 12 January 1995; revised manuscript received 28 March 1995; accepted 29 March 1995

Abstract

The limiting size of macromolecules for permeation through membranes (cell walls, artificial hydrophilic membranes) as well as size-dependent partitioning within hydrophilic matrices was investigated by HPLC-aided analysis of dextran permeation. The method includes (1) modification of a specially prepared polydisperse dextran probing solution (DPS) by permeation of size fractions through or into the investigated material, (2) fast flow size-exclusion chromatography (SEC) of the modified DPS on a calibrated Superdex-200 HR-column and (3) determination of permeability resp. partition parameters by comparison of the elution profiles of original and modified DPSs. By this method, the mean size limit of permeation through cell walls (cut off), size permeation parameters of hollow fibres, dialysis tubes and ultrafiltration membranes, and the size dependence of partitioning within gel particles can be determined with high accuracy in short time.

1. Introduction

The ultrafiltration properties of microbial and higher plant cell walls are attractive for polymer separation [1–3] and the physiological significance of cell wall permeability is obvious. It refers to retention of soluble polymers in the periplasma, crosslinking of the cell wall matrix, exudation of enzymes and other polymers, protoplast resistance to polymer toxins or lytic enzymes, cell–cell recognition and many other phenomena. The limiting size of polymers for permeation through the cell wall matrix may be altered in the course of extension growth and differentiation or in response to biotic and abiotic stress. Little is known about such changes, as up to now rather laborious methods have been used to measure size limits of cell wall permeation. These methods include observation of the plasmolysis/cytorhysis transition [4], identification of dye-marked polymers in the lumen of plasmolyzed or denatured cells [5,6] and permeation chromatography of polymers on beds of native cells [7], purified cell wall fragments [8] and cell wall microcapsules (CWC) [9–11]. We aimed in a convenient and fast method for estimation of size limits of exclusion resp. membrane permeation of polymers and their size-dependent partitioning, which is generally applicable to different porous and gel-like materials, especially CWC. A straightforward methodical principle was found in the early

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(95)00407-6

papers of Scherer and collaborators [12,13]. These authors used a method based on GPC fractionation of a polydisperse probing solution of polyethylene glycol (PEG) before and after its equilibration with isolated cell walls or intact cells of bacteria and yeast. The applied GPC fractionation on Biogel was time-consuming and restricted to a small range of PEG molecular size fractions. However, important advantages of Scherer's methodical principle are the possible use of HPLC and its broad applicability to both permeation through membranes and partitioning within matrices. The potency of a method based on a special dextran probing dispersion, a calibrated Superdex 200 column and a HPLC system will be shown in this paper.

2. Experimental

2.1. Materials

Cell wall capsules (CWC) were prepared by denaturation of living plant and yeast cells in ethanol and extraction of ethanol and water-soluble cell contents. Suspension cultured cells of *Chenopodium album* L. earlier used for vesicle chromatography [9], the large sphaeric cells of axenically and autotrophically grown alga *Eremosphaera viridis* De Bary (strain B 228-1 of the collection of the Institute of Plant Physiology, University Göttingen), yeast cells (*Saccharomyces cerevisiae* L., ethanol production strain "Kolin", a kind gift of the Institute of Food Technology, Technical University, Berlin) harvested from an aerobic culture on a glucose/yeast-extract mineral medium in the stationary phase, as well as 1-mm thick slices cut from a 13-mm storage parenchyma cylinder of the potato tuber *Solanum tuberosum* L. (cultivar Quarta) were extracted with 96% ethanol and kept in ethanol at 4°C until analysis. In the case of the large algae and parenchyma cells, plasmoptysis, i.e. bursting of the cell wall by fast penetration of ethanol, was prevented by denaturation of protoplasts in 10% acetic acid before incubation in ethanol. Ethanol-saturated *C. album* cell clusters, *E. viridis* and *S. cerevisiae* cells were dispersed in an excess of a cold (4°C) 1 m*M* $CaCl_2$ solution for one hour. Easily filtrable particles (swollen Sephadex gels, extracted algae cells and cell clusters prepared from the suspension culture) were equilibrated with the buffer used for HPLC by washing the packed material on a sintered glass filter with at least three bed volumes of buffer. Yeast CWCs were equilibrated with the buffer by repeated centrifugation. Vesicular packing material (VP), i.e. enzymatically purified clusters of CWCs, from which the protoplast residue was removed, were obtained from the suspension culture by the procedure earlier described [11]. Alcohol-dried VP was obtained by saturating a fixed bed of the packing material with a mixture of 96% ethanol and *n*-propanol (9:1) and evaporation of the liquid phase in a vacuum rotary apparatus at a water bath temperature of 50°C.

The investigated hollow fibre for blood dialysis consisting of regenerated cellulose [14] was obtained from Kunstseidewerk Pirna (Germany), the dialysis tube Spectra/Por 10 kD from Spectrum (USA), the ultrafilter flat membrane PM-10 from Amicon (USA) and the Sephadex dextran gel beads (G 50 fine and G 75 superfine) from Pharmacia (Sweden).

2.2. Size-exclusion chromatography

SEC was carried out using prepacked Superdex 200 HR 10/30 columns (30 cm × 10 mm I.D., bed consisting of crosslinked agarose with covalently bonded dextran, mean bead diameter 13–15 μm) provided by Pharmacia LKB Biotechnology (Sweden), coupled to a HPLC system (Model 5000 Bio-Rad) and a polarimetric detector (Chiralyser, IBZ Meßtechnik). The sample loop volume of the injector used was 50 μl. An electrolyte solution containing 0.1 or 0.01 *M* phosphate buffer (pH 7), 0.1 *M* NaCl and 0.05% NaN_3 was used as eluent. The constant linear flow-rate was 12.7 mm/min. At this flow-rate, with our system, theoretical plate numbers of 4200 and 1600 were obtained from the peak dispersion of glucose and serum albumin, respectively (the plate count of the column for acetone given by the manufacturer is 10 000).

2.3. Calibration of the molecular size scale

A set of protein standards was used to calibrate the dependence of elution time on molecular size in terms of Stokes' radius. For this purpose, data given by Laurent and Killander [15] were used for the following proteins: γ-globulin (human), alcohol dehydrogenase (yeast), serum albumin (human), peroxidase (horseradish), chymotrypsinogen (porcine), cytochrome C (equine). Providers data (Pharmacia) were available for thyroglobulin (porcine), ferritin (equine), catalase (bovine), aldolase (rabbit) and ovalbumin (hen). The log of the Stokes' radius (r_s) of standard proteins was plotted vs. K_{AV} [16] (Fig. 1). In the course of the experiments we used three Superdex 200 HR 10/30 columns which slightly differed in the elution profile of dextran and the regression parameters between K_{AV} and r_s. Therefore, each column was calibrated separately. With one Superdex column many chromatographic runs (more than 300) could be performed without detectable change in the dextran elution profile.

2.4. Dextran probing solution

From a larger range of commercial preparations, the following dextran preparations were selected as components of the DPS: dextran 162 (No. 59F-0752, Sigma), dextran 60 (control: B7), dextran 15 (control: I) and dextran 4 (control: B) from Serva.

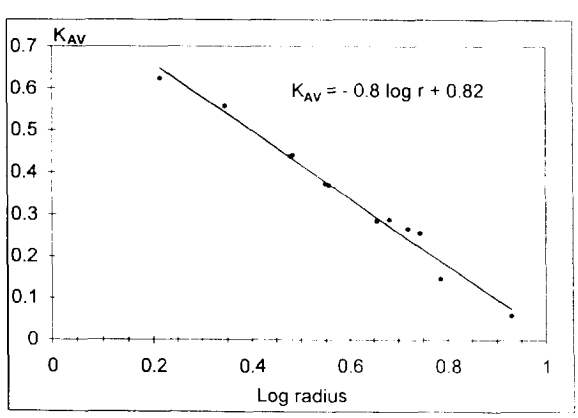

Fig. 1. Calibration curve of the Superdex-200 HR 10/30 column based on proteins with known Stokes' radius.

Computer-aided superposition of elution profiles obtained for these preparations was checked at different mixing ratios to obtain a DPS with nearly equal concentrations of the different size fractions over a wide range of molecular radii (see Results). The DPS has the following composition: 1 g/l dextran 162, 6 g/l dextran 60, 1.5 g/l dextran 15, and 2 g/l dextran 4. The dextrans were dissolved in the elution buffer.

2.5. Permeation procedures

Before dialysis of the DPS against plant CWCs or its partitioning within gel particles, mobile buffer was removed from the wet materials. In the case of the potato slices this was done by blotting on filter paper, with the other materials by suction through a glass filter (larger particles) or a 0.2-μm membrane filter (yeast). Drained materials were mixed with a small volume of DPS (about 1 ml DPS per g wet mass of drained material) and incubated for the given time. By parallel incubations in buffer it was ensured that no optically active material was lost during the incubation. After the incubation time a sample (at least 200 μl) of the external liquid phase (modified DPS) was taken and centrifuged for 10 min at ca. 3000 *g* to ensure absence of particles. In the case of easily filtrable material the modified DPS sample was taken from the outlet of an Econo column (Biorad), whereby particles were kept in suspension in order to avoid compression of soft cells by cohesion forces. In the case of yeast CWCs, the modified DPS was separated from the matrix by centrifugation (3000 *g*, 10 min). For sorption experiments, 0.1 g of alcohol-dried vesicular packing material was allowed to swell in 10 ml of the DPS (final bed volume about 6 ml). Investigation of the hollow fibres and the dialysis tube was carried out by filling their lumen with the DPS and dialysing the sample for different times in a large volume of stirred buffer. The modified DPS was taken as retentate from the lumen. In case of the flat ultrafiltration membranes, both permeate and retentate samples of the DPS were obtained by

the recommended filtration procedure (pressurizing the sample in the Amicon stirred cell 8050).

3. Results

The dextran probing solution (DPS) with a size dispersion fitted to the fractionation range of the Superdex column was obtained by mixing of commercial dextrans as described in the Experimental section. From the symmetric and narrow peaks obtained by rechromatography of the eluted fractions it may be stated, that the elution profile was not influenced by interactions other than size fractionation (Fig. 2).

The principle of the method described in this paper consists in comparison of the original DPS with a modified one, which was obtained by permeation of dextran through hydrophilic membranes or by partitioning within hydrophilic matrices. A computer was used to transform the elution time into Stokes' radius and to give the concentration quotient $Q = C_1/C_2$ of original (C_1) and modified (C_2) DPS to all pairs of pseudomonodisperse dextran fractions with equal Stokes' radius (Fig. 3).

If a volume of the resulting DPS is equilibrated with a similar volume of wet material consisting of cell wall capsules (CWCs) or gel particles, dextran fractions excluded from the cell lumina by the impermeable wall are diluted only by the buffer held in the surface film. Additional dilution by the intracellular liquid space or the intermicellar liquid is expected for permeable dextran fractions. Therefore, Q is not equal for all size fractions, the value being lower for the excluded and higher for the permeable molecules. In case of CWCs with a narrow cut-off and a large volume fraction occupied by free liquid within the cell, a step-like increase of Q at Stokes' radii between the size limits of exclusion and permeation [10] is expected. Figs. 3 and 4 demonstrate that this has indeed been found for CWCs prepared from highly vacuolated plant materials (suspension cells of *C. album*, the unicellular green algae *E. viridis*, and the parenchyma cells of *S. tuberosum*).

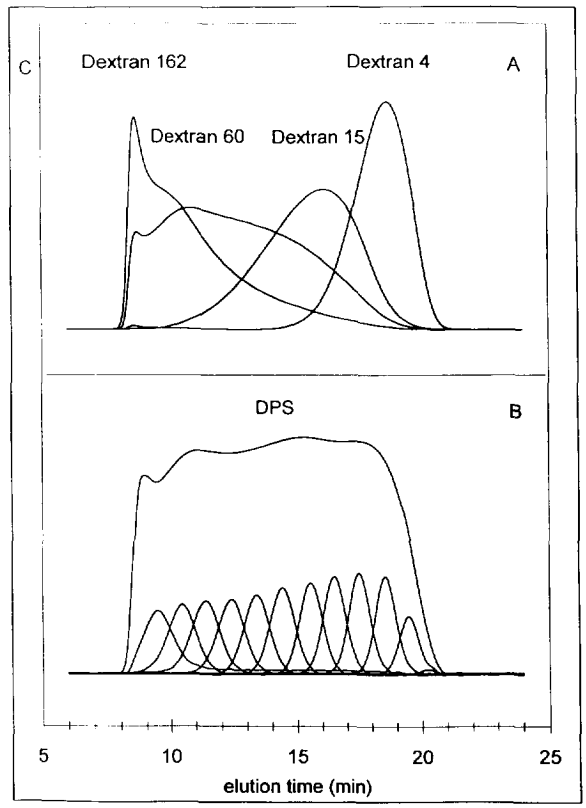

Fig. 2. (A) Elution diagrams of 1% solutions of 4 dextrans used as the components of the dextran probing solution (DPS). (B) Elution diagram of the DPS. Also shown are elution diagrams obtained by rechromatography of pseudomonodisperse fractions of the DPS eluate (0.1 ml) at different elution times (peak elution volume identical with the elution volume at sampling). The peak variance of the pseudomonodisperse fraction at elution time 17.5 min is 0.3 nm in terms of Stokes' radius. The concentration signal C (arbitrary units) is obtained by a polarimetric detector. For details of the chromatographic system see Experimental.

The difference between Q values of excluded and permeable dextran fractions is mainly caused by the large empty intracellular volume of the extracted cells which forms a size-independent dilution space for permeable molecules. The Stokes' radius of a dextran fraction which is diluted by 50% of the internal distribution space (obtained as shown in Fig. 3B) may be defined as mean size limit (MSL) of cell wall permeation. If the matrix volume of the cell wall and cell contents is small in comparison to the intracellular bulk liquid and the permeable compounds

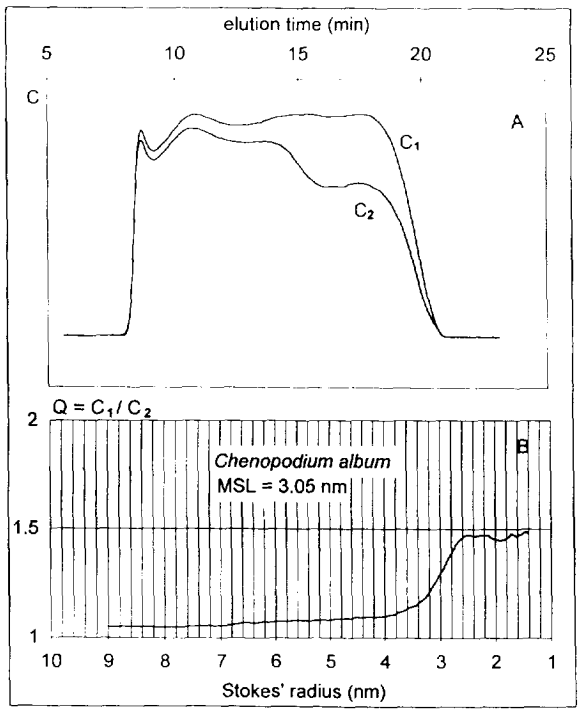

Fig. 3. (A) Diagrams of concentration of original (C_1) and modified (C_2) DPS at different elution time. Equal volumes of a wet (buffer saturated) packed mass of cell capsules prepared from *C. album* were mixed and incubated for 30 min before obtaining the modified DPS by filtration. (B) Diagram of the concentration quotient $Q = C_1/C_2$ at different Stokes' radii obtained by the calibration function shown in Fig. 1. The MSL is the Stokes' radius obtained at the middle of the step in the Q diagram.

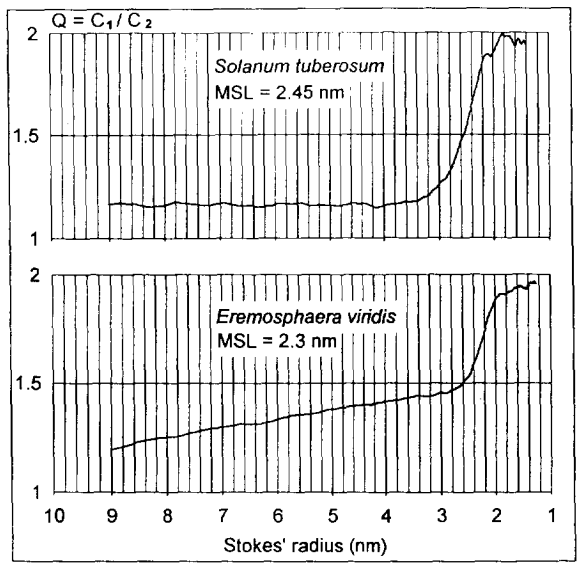

Fig. 4. Q diagrams of cell wall capsules of two different plant materials (obtained as demonstrated in Fig. 3). Incubation period: 30 min in case of *E. viridis*, 6 h in case of potato tissue slices.

have approximately reached an equilibrium partition, the MSL is the Stokes' radius allowing for permeation into half of the cells. The curves obtained with *E. viridis* CWCs did not show a clear size-independence of Q at high molecular size. This peculiarity may have been caused by a rather high content of cell wall ghosts (collapsed cell wall envelops of the mother cells, from which autospores have been released) in the preparation.

If partitioning of dextran fractions with hydrated gel particles is analyzed by our method, the dependence of Q on the Stokes' radius describes the broad size fractionation range which is typical for uniform hydrophilic gels (Fig. 5).

At a molecular size beyond the exclusion limit of the gel, Q is constant ($Q = C$). The difference $Q - C$ found for permeable size fractions is proportional to the partition coefficient of the respective dextran fraction in the matrix (K_{av} value).

$$\Delta Q = Q - C = k \cdot K_{av},$$

where k may be obtained as $(V_g - V_{ex})/(V_d + V_{ex})$, and V_g is the volume of the whole gel sample, V_{ex} the volume of the surface-bound liquid and V_d the volume of the DPS solution. V_{ex} may be derived from dilution of the excluded fractions:

$$V_{ex} = V_d \cdot (C - 1).$$

It is known that size-exclusion characteristics of a concentrated solution of soluble high-molecular-mass dextran (kept in a dialysis tube) is similar to that of a swollen gel of crosslinked dextran at equal dextran concentration [15]. Extracted and rehydrated yeast cells, which are filled with a concentrated dispersion of non-permeable protein [17], show a size fractionation curve which is similar to that of gel materials.

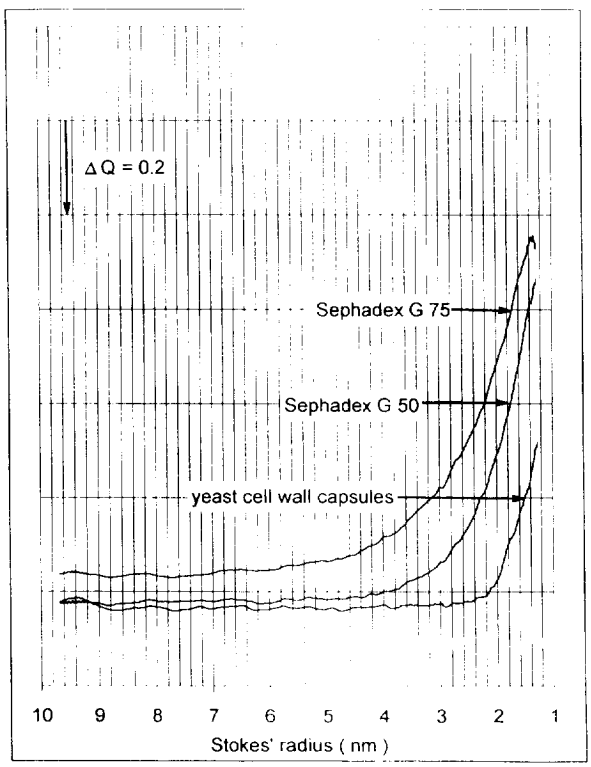

Fig. 5. Q diagrams of gel particles and ethanol-extracted yeast. For clearness, the curves are shifted on the Q scale.

Here, because of the size-exclusion effect of the concentrated polymers, the partition coefficient of permeable dextran molecules is size dependent. The size-exclusion limit obtained from such a system may be interpreted as a cell wall parameter only with caution. It should be checked whether the size-exclusion limit of the included concentrated polymer solution is larger than that of the cell wall.

In the case of yeast cells and small cell clusters of *C. album* it was proved that the values obtained for the size-exclusion limit and the MSL did not increase significantly after an incubation time of 10 or 30 min, respectively (Table 1). However, as the MSL of potato slices increased slightly even after an incubation period of 8 h, especially in case of multilayered tissue slices or cells with thick walls, it seems to be necessary to define the kinetic conditions before a comparative investigation.

In good accordance with the theoretical prediction, the MSL of *C. album* cell walls harvested in the stationary phase (Table 2) gives a value between the size-exclusion limit (ca. 3.4 nm) and the size-permeation limit (ca. 2.8 nm), which were obtained by direct permeation chromatography of polydisperse dextran with CWCs obtained from the same culture [11]. Advantages of the technique described here are the independence of the chromatographic properties of the investigated material, convenience, better reproducibility and higher accuracy. The resolution of the method is sufficient to reproduce small differences in the MSL (0.1 nm) of different batch preparations of CWCs.

In the experiments shown above, the mechanism of separation by the CWC was dialysis (diffusion through a porous membrane). If dried

Table 1
Size-exclusion limit (SEL) of denaturated and ethanol-extracted yeast cells and mean size limit (MSL) of permeation into plant cell wall capsules

Material	Incubation time	SEL, MSL (nm)					
Saccharomyces	5 min			2.2			
cerevisiae	10 min			2.2			
Chenopodium album	30 min		3.5		3.5		
150 μm cell clusters	60 min		3.5		3.4		
	2 h	2.2		2.2		2.2	
Solanum	4 h	2.4		2.4		2.4	
tuberosum	6 h	2.5		2.5		2.4	
1 mm thick slices	8 h	2.6		2.6		2.6	
	25 h	2.8		2.8		2.8	

Table 2
Reproducibility of MSL investigated for batch preparations of deproteinated vesicular packing particles (VP) and cell wall capsules (CWC) obtained from the *C. album* cell culture (incubation time 30 min)

Batch sample	VP (28)					CWC (9.6)					CWC (16.6)				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
MSL (nm)	3.1	3.2	3.2	3.1	3.2	3.0	2.9	3.0	3.0	3.0	3.2	3.2	3.2	3.2	3.1
Average MSL (nm)	3.16					2.98					3.18				
Standard deviation	0.055					0.045					0.045				

CWCs are allowed to absorb liquid from a polymer solution, the initial mechanism of size separation is ultrafiltration, a mechanism which is important for the dry column technique of vesicle chromatography [3]. Here, Q values of excluded polymers below 1 are obtained. It was questionable whether a higher porosity of the alcohol-dried cell wall might cause entrapping of larger molecules at the beginning of the sorption process (see Ref. [18]), as the size permeation limit of the dehydrated cell wall in pure ethanol or acetone (measured by permeation of polyethylene glycol) is much larger than that of the hydrated wall [19]. However, we did not find a strong difference between the size-exclusion effects of dry and wet CWCs. The MSL of vesicular particles (VP) obtained by the sorption experiments is only slightly larger than that obtained with the same material in a dialysis experiment (Table 3).

The described method can be used to characterize size separation properties for transport through membranes of hollow fibres and dialysis tubes as well as flat ultrafiltration membranes. In our dialysis experiments with hollow fibres and dialysis tubes (Fig. 6), the internal compartment was filled with the DPS.

The volume of this compartment was small in comparison to that of the stirred external compartment. The sample of the retentate (modified

Table 3
Mean size limits (MSL) of vesicular packing particles prepared from *C. album* determined by incubation of ethanol-dried material and moist material of the same batch preparation in the DPS (30 min)

	Dried material (sorption)		Wet material (dialysis)	
MSL (nm)	3.7	3.7	3.5	3.5

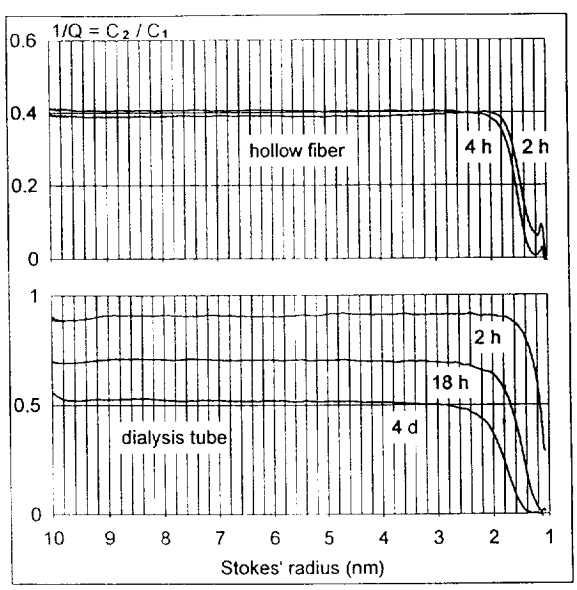

Fig. 6. Diagram of $C_2/C_1 = 1/Q$ of modified DPSs obtained by dialysis of the original DPS from a blood dialysis hollow fibre (regenerated cellulose, length 3 m, internal volume ca. 75 μl, inner diameter 190 μm) and a Spectra-Por CE dialysis tube (cellulose ester, internal volume 2 ml, diameter 7.5 mm, nominal molecular mass cut-off 10 000) into 200 ml of stirred buffer. Samples of the internal modified DPS (retentate) were obtained at the given dialysis times.

DPS) was investigated. If a permeable compound had reached equilibrium partition, its concentration in the modified DPS (C_2) decreased to approximately zero. As expected, the size range of dextran molecules with incomplete retention is clearly dependent on the incubation time. For such experiments, $C_2/C_1 = 1/Q$ is a useful parameter to describe retention. As long as $1/Q$ is constant, impermeability may be assumed (dilution may have been caused only by osmotic water uptake into the lumen). The exclusion limit of the membranes (initially strongly time dependent) may be defined by a threshold of 95% retention, i.e. the critical molecular size for reducing $1/Q$ to 95% of the constant value.

The result of ultrafiltration through a flat membrane is not only dependent on the porosity properties of the membrane but also on flow-rate, concentrations and convective flow along the membrane surface. Separation characteristics of ultrafiltration processes can be conveniently studied by the described chromatographic technique, as demonstrated in Fig. 7.

Volume flow through the membrane produces two modified DPSs: permeate and retentate. Comparison of the modified DPSs with the original one allows for the definition of ultrafiltration parameters, whereby curves of C_1/C_2 may be used in the case of the retentate and of C_2/C_1 in the case of the permeate.

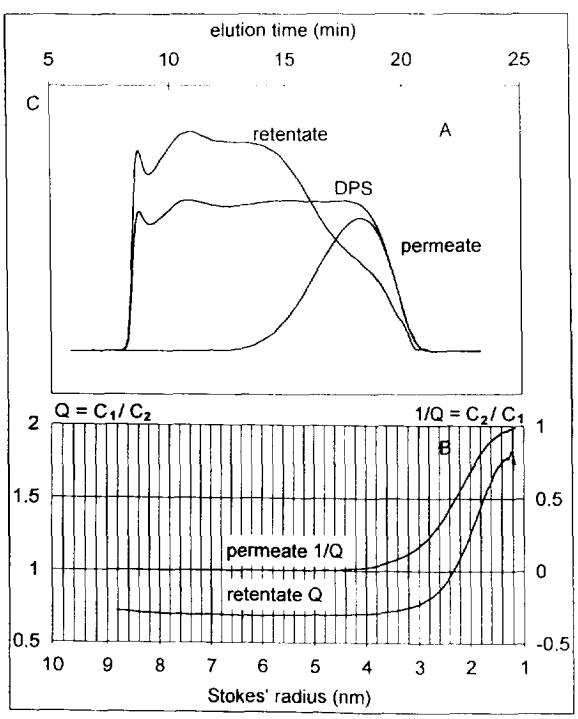

Fig. 7. (A) Diagram of the concentration of eluted dextran from modified DPSs obtained as retentate and permeate in comparison to the original DPS at different elution time. Ultrafiltration membrane: Amicon PM 10. Original volume of DPS: 10 ml, retentate volume: 3 ml. For chromatography, the retentate was diluted with buffer (1/1). (B) Diagrams of concentration quotients suitable for evaluation of the membrane cut off by modified DPDs.

4. Discussion

It should be kept in mind, that values given here as critical molecular size parameters do not refer to the size of the dextran molecules within the investigated matrices, which is difficult to estimate because of the flexibility of the highly hydrated molecules and the influence of the matrix on the large number of their possible conformations [20]. Fortunately, homogeneity of the molecular structure (branching) of the dextran polymers is not essential for our method. It was sufficient to show, that the DPS can be separated into narrow pseudomonodisperse size fractions with defined K_{AV}, as our size estimates are equivalent Stokes' radii, obtained by comparison with the Stokes' radii of the proteins used for GPC calibration. Reference to proteins is an important aspect and seems to be justified because (1) it has been shown that K_{AV} values of proteins and dextrans with equal Stokes' radius (in water) are similar or closely related [15,21,22] and (2) protein permeation into or through porous materials is most significant under both technical and physiological aspects. Cell walls are usually strongly negatively charged matrices. Therefore, the partition parameters and the size permeation limit of ampholytic polyelectrolytes as proteins in the cell wall are not only dependent on their hydrodynamic size but also on electrical field barriers or attraction forces, which are strongly dependent on ion

strength and pH [10,23]. However, as the used DPS and GPC matrix both can be regarded as uncharged, the described method is selective for the steric aspect of cell wall permeability.

In the case of CWSs the step-like change of Q is continuous over a certain range beyond and below the MSL, although for an equilibrium partition with uniform thin-walled capsules a sharp size limit (discontinuous step in Q) may be expected. The width of the experimentally observed step is partly explained by the variability of individual capsules with respect to the size limit of permeation and partly by the chromatographically caused peak variance of monodisperse fractions. Insufficient chromatographic resolution would prevent a discontinuous steep change in Q, even if the separation limit would be a point on the size scale identical for all cells and the permeable fractions were all in equilibrium. In reality, equilibrium can be approximately assumed in case of small thin-walled cells (Table 1). The peak variance of a narrow (pseudomonodisperse) dextran fraction or of a protein (0.3 nm in terms of the Stokes' radius) is similar to the variance of dQ/dr_s near the MSL (Figs. 3 and 4). Obviously, the resolution of the applied GPC system is not sufficient for an optimal description of the small variability of the cells' individual cut-off. Resolution could be improved by the choice of GPC media with a more narrow size fractionation range (and related DPSs) as well as by further improvement of the chromatographic efficiency. However, for many purposes, the variance of the individual cells of a sample with respect to the cell wall cut-off is less interesting than the mean value expressed by the MSL. Good reproducibility and resolution of even small changes in the MSL is possible with the described method (Table 2), and the applied system has a broad measuring range useful for cell wall research (MSL between 2 and 7 nm). For materials with smaller MSL of the cell wall like *Geosiphon* [24] a comparable system involving a Superdex 75 column can be recommended.

Acknowledgements

The authors thank the Deutsche Forschungsgemeinschaft for support and Mrs. Petra Heese and Mrs. Heidemarie Schneider for their excellent technical assistance.

References

[1] M. Sára and U.B. Sleytr, J. Membrane Sci., 33 (1987) 27-49.
[2] R. Kleine, H. Woehlecke and R. Ehwald, Acta Biotechnol., 12 (1992) 243-253.
[3] H. Woehlecke and R. Ehwald, Bio-Forum, 5 (1992) 174-175.
[4] N. Carpita, D. Sabularse, D. Montezinos and D.P. Delmer, Science, 205 (1979) 1144-1147.
[5] O. Baron-Epel, P.K. Gharyal and M. Schindler, Planta, 175 (1988) 389-395.
[6] E. Shedletzky, M. Shmuel, T. Trainin, S. Kalman and D. Delmer, Plant Physiol., 100 (1992) 120-130.
[7] J.P. Gogarten, Planta, 174 (1988) 333-339.
[8] M. Tepfer and I.E.P. Taylor, Science, 213 (1981) 761-763.
[9] R. Ehwald, G. Fuhr, M. Olbrich, H. Göring, R. Knösche and R. Kleine, Chromatographia, 28 (1989) 561-564.
[10] R. Ehwald, P. Heese and U. Klein, J. Chromatogr., 542 (1991) 239-245.
[11] R. Ehwald, H. Woehlecke and C. Titel, Phytochemistry, 31 (1992) 3033-3038.
[12] R. Scherrer and P. Gerhardt, J. Bacteriol., 107 (1971) 718-735.
[13] R. Scherrer, L. Louden and P. Gerhardt, J. Bacteriol., 118 (1974) 534-540.
[14] J. Gensrich and D. Paul, in J.F. Kennedy, G.O. Phillips and P.A. Williams (Editors), Cellulosics: Materials for selective separations and other technologies, Ellis Horwood, London, 1993, Ch. 14, p. 119.
[15] T.C. Laurent and J. Killander, J. Chromatogr., 14 (1964) 317-339.
[16] A.G. Ogston, Trans. Faraday Soc., 54 (1958) 1754.
[17] F.M. Klis, Yeast, 10 (1994) 851-869.
[18] B. Selisko and R. Ehwald, J. Biochem. Biophys. Methods, 27 (1993) 311-325.
[19] R. Ehwald, U. Klein, A. Jäschke, D. Cech and C. Titel, Patent EP 0412507 A1 (1991).
[20] A.M. Basedow and K.H. Ebert, J. Polym. Sci. Polym. Symp., 66 (1979) 101-115.
[21] T.C. Laurent and K.A. Granath, Bioch. Biophys. Acta, 136 (1967) 191-198.
[22] P.L. Dubin and J.M. Principi, Macromolecules, 22 (1989) 10891-10896.
[23] A. Jäschke and D. Cech, J. Chromatogr., 585 (1991) 57-65.
[24] A. Schüßler, E. Schnepf, D. Mollenhauer and M. Kluge, Protoplasma, in press.