esters) showed the highest inhibitory activity. The special importance of these two compounds resides in the fact that they are currently used as food additives (as antioxidants) for the preservation of oils, margarines, cheeses, pastries, and sauces in concentrations ranging from 300 μM to 1 mM with the codes E311 (octyl gallate) and E312 (lauryl gallate). A further study on the effect of the compounds as growth inhibitors of different cell types will be published elsewhere.

For comparison, we have tested tyrphostins A1, A23, A25, and genistein which are widely used PTK inhibitors. These compounds, due to their low solubility in aqueous solutions, were tested in the presence of 10% dimethyl sulfoxide in a range between 0 and 200 μM. As shown in Table 1, genistein (I_{50} = 125 μM) and tyrphostin A25 (I_{50} = 150 μM) behaved as moderate inhibitors, while tyrphostine A1 or A23 did not show significant inhibitory activity at the concentration used.

An extra advantage of the compounds described above (gallic acid and derivatives) is their very low cost in comparison with other PTK inhibitors.

Finally, Fig. 1 shows that gallic acid is a potent inhibitor of cytosolic and membrane PTKs, implying that this class of compounds could be of a general use for the study of both receptor and nonreceptor PTKs. A residual activity accounting for 10–20% (in different preparations) of the total PTK activity is not inhibited even at high concentrations of the different compounds. A possible explanation for this phenomenon could be the presence of noninhibitable PTKs in the partially purified cytosolic PTK and in the crude membrane detergent extracts used in this study. Further research on the effect of this class of compounds on purified specific PTKs is planned in order to ascertain if they can be used as general inhibitors for these enzymes.

REFERENCES

1. Imoto, M., Shimura, N., Vi, H., and Umezawa, K. (1990) *Biochem. Biophys. Res. Commun.* **173,** 208–211.
2. Akiyama, T., Ishida, J., Nakgawa, H., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M., and Fukami, Y. (1987) *J. Biol. Chem.* **262,** 5592–5595.
3. Chen, Y., Yang, D. C., Brown, A. B., Jeng, Y., Tatoyan, A., and Chan, T. M. (1990) *Arch. Biochem. Biophys.* **283,** 184–192.
4. Fallon, R. J. (1990) *Biochem. Biophys. Res. Commun.* **170,** 1191–1196.
5. Fukazawa, H., Mizuno, S., and Uehara, Y. (1990) *Biochem. Biophys. Res. Commun.* **173,** 276–282.
6. Geissler, J. F., Traxler, P., Regenass, U., Murray, B. J., Roesel, J. L., Meyer, T., McGlynn, E., Storni, A., and Lydon, N. B. (1990) *J. Biol. Chem.* **265,** 22255–22261.
7. Dvir, A., Milner, Y., Chomsky, O., Gilon, C., Gazit, A., and Levitzki, A. (1991) *J. Cell Biol.* **113,** 857–865.
8. Burke, T. R. (1992) *Drugs Future* **17**(2), 119–131.
9. Lázaro, I., Gonzalez, M., Roy, G., Villar, L. M., and Gonzalez-Porqué, P. (1991) *Anal. Biochem.* **192,** 257–261.

Desalting Inositolpolyphosphates by Dialysis

Jeroen Van der Kaay and Peter J. M. Van Haastert
Department of Biochemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Received October 4, 1994

Dialysis is a separation method based on the size of the molecules. The molecular weight cutoff (MWCO)[1] of dialysis membranes specifies their pore size. Molecules with a molecular weight (M_r) exceeding the MWCO are retained by the membrane, whereas molecules with a M_r below the MWCO can diffuse through the pores of the membrane. This paper demonstrates retention of molecules with a M_r that is 50-fold less than the MWCO of the dialysis membrane. This phenomenon is solely correlated with the charge of these molecules and is not restricted to a specific class of compounds, and it is due neither to adsorption of the molecules to the membrane nor to formation of large complexes which exceed the MWCO of the dialysis membrane. A practical application of this study is the desalting of inositol(poly)phosphates, which are small multiple charged molecules, by dialysis. The amount of material to be dialyzed can be in the millimolar concentrations.

Dialysis is often used to remove small molecules, like salt ions and nucleotides, from protein preparations. During studies on the metabolism of inositol-hexakisphosphate ($InsP_6$) in *Dictyostelium,* cell lysates were dialyzed in order to remove endogenous $InsP_6$ and other inositolphosphates. The rate and extent of dialysis were monitored by addition of $[^3H]InsP_6$ to the cell lysates. To our surprise, the radiotracer was almost completely recovered in the retentate, although $InsP_6$ has a M_r of 924 Da, which is far below the MWCO of 12,000 Da. Figure 1A shows the retention of inositol, InsP, $InsP_3$, and $InsP_6$ when dialyzed against 1000 vol of dialysis buffer for 20 h. Inositol rapidly diffused through the membrane into the diffusate, and less than 1% remained in the retentate after 20 h. By contrast, $InsP_6$ was recovered for about 80% in the retentate after overnight dialysis against 1000 vol of dialysis buffer. $Ins(1,4,5)P_3$ and Ins(3)P showed intermediate dialysis rates and were recovered for 50 and 25%, respectively, after dialysis for 20 h.

The observed retention is not restricted to inositol(poly)phosphates since ATP and Glu6P showed essentially the same dialysis characteristics as $Ins(1,4,5)P_3$ and Ins(3)P, respectively (see Fig. 1C). All compounds can be fully recovered and remained in their authentic constitution as is observed by HPLC analysis and con-

[1] Abbreviations used: MWCO, molecular weight cutoff; $InsP_6$, inositol-hexakisphosphate.

ANALYTICAL BIOCHEMISTRY **225,** 183–185 (1995)
0003-2697/95 $6.00
Copyright © 1995 by Academic Press, Inc.
All rights of reproduction in any form reserved.

version with specific enzymes (1). Other dialysis membranes with higher MWCO values (10 to 50 kDa) were tested but showed no significant differences. Adsorption of the radioactive tracer to the dialysis membrane could not explain the observed retention of inositolphosphates since all tested radiotracers showed more than 97% recovery. The formation of large complexes exceeding the MWCO does not seem very likely since the extent of dialysis in the presence or absence of protein was essentially identical (data not shown). Dialysis with or without EDTA in the dialysis buffer was also performed since it is known that inositolphosphates can form complexes with bivalent cations like Mg^{2+} and Ca^{2+} (3). However, the addition of EDTA had no effect on the rate of dialysis (data not shown). Finally, dialysis showed the same kinetics in the presence or absence of phytate hydrolysate, which is a mixture of a large set of unlabeled inositol(poly)phosphates (data not shown).

The kinetics of dialysis presented in Fig. 1A seemed inversely related to the charge of the tested compounds. Therefore, NaCl was added to the samples and the dialysis buffer to overcome possible charge effects. Figure 1B reveals that in the presence of high salt concentrations the rate of $InsP_6$ dialysis was increased, resulting in nearly complete dialysis within 20 h. The effects of 0.1 and 0.5 M NaCl on the dialysis rate were almost the same, whereas 0.01 M NaCl showed about half maximal effect. The addition of NaCl also increased the diffusion rate of other compounds such as ATP and Glu6P (data not shown).

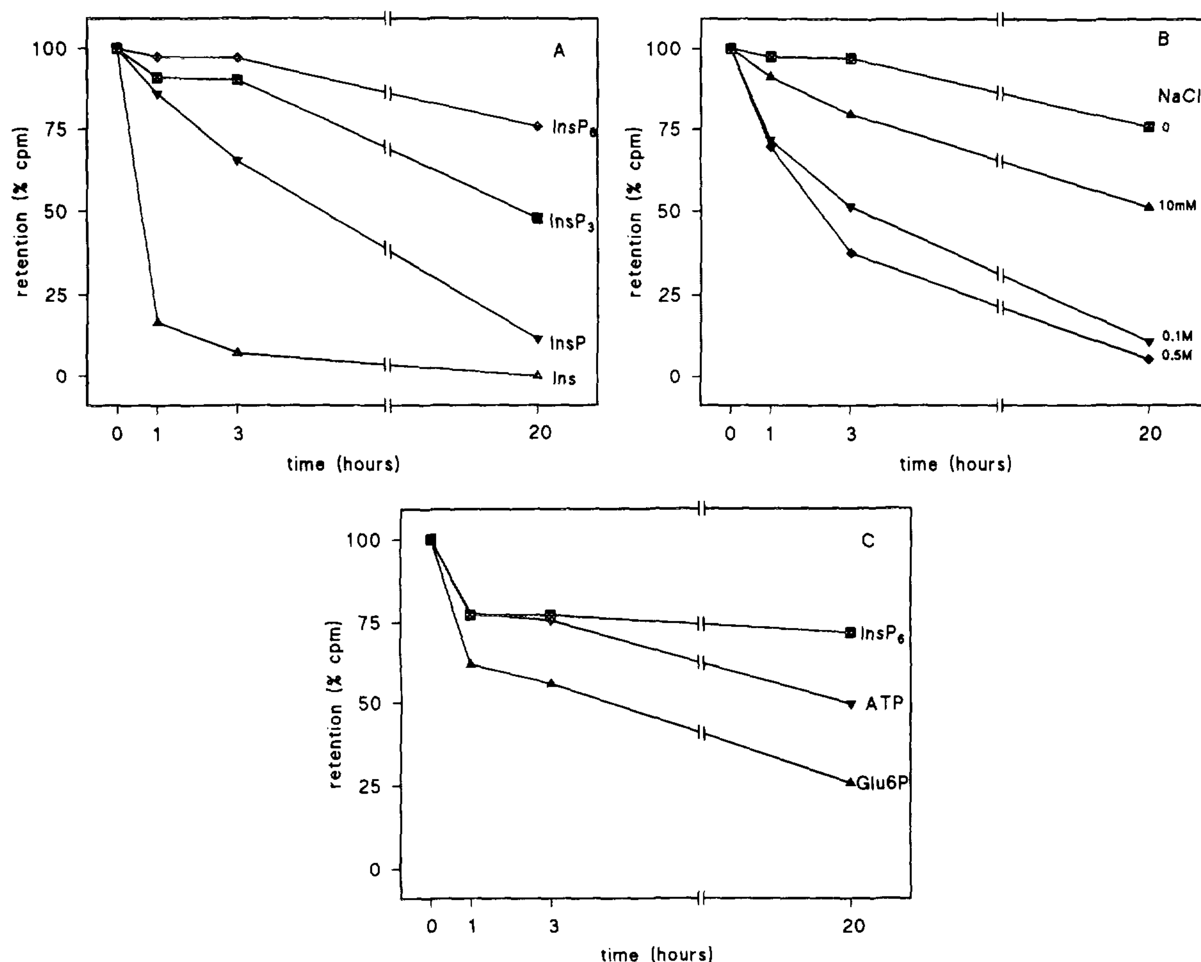

FIG. 1. [^{3}H]Inositol, [^{3}H]Ins(3)P, [^{14}C]Glu6P, [^{32}P]ATP, [^{3}H]Ins(1,4,5)P_3, and [^{3}H]$InsP_6$ were dialyzed against 1000 vol of dialysis buffer (10 mM Hepes, pH 7.1) across a dialysis membrane with a MWCO of 10-12 kDa. (A) Retention of charged compounds by dialysis. Samples of 1 ml contained 0.01 μCi of a radiotracer, 1 mg phytate hydrolysate (4), 10 mM Glu6P, and 10 mM ATP from which duplicate aliquots of 50 μl were taken at the times indicated. (B) Effect of NaCl on the retention of charged compounds by dialysis. The concentration of NaCl in the dialysis buffer was varied between 0 and 0.5 M. Samples as described above contained the same NaCl concentration as the dialysis buffer. (C) Desalting by dialysis. A mixture of [^{3}H]$InsP_6$, [^{32}P]ATP, and [^{14}C]Glu6P containing 1 M NaCl was dialyzed against 1000 vol of dialysis buffer to which no NaCl was added (the amount of radioactivity is 0.01 μCi and the concentration is 10 mM for each compound).

Finally, Fig. 1C shows the dialysis of samples containing 1 M NaCl against dialysis buffer without addition of NaCl. During the first hour, the rate of dialysis of Glu6P, ATP, and $InsP_6$ was fast, but was then reduced to a rate of dialysis approximately equal to that of these compounds in the absence of NaCl in the sample. Apparently, the initial rate of dialysis was mediated by the high NaCl concentration in the sample, which rapidly diminished and becomes too low to effectively shield the charges on the solutes, which were then retained by the dialysis membrane. This effective retention of small highly charged molecules in dialysis bags that have a MWCO which is at least 10-fold higher than their M_r was used to desalt HPLC column fractions containing inositol(poly)phosphates (1). The recoveries for $InsP_6$, $InsP_5$, $InsP_4$, and $InsP_3$ were 85, 85, 80, and 5%, respectively. Desalting by dialysis is now routinely performed in our lab and showed to be successful hundreds of times.

Two main applications arise from these observations. First, small solutes with multiple charges can be removed from enzyme preparations if at least 0.1 M NaCl is added to the dialysis buffer. The salt can subsequently be removed by further dialysis against buffer without salt. Second, dialysis can be used to remove salts from a sample of small molecules with multiple charges. The presence of a high salt concentration in the sample only gives a short-term shielding effect, because the salt ions rapidly dialyze into the diffusate. This results in a loss of a small amount of the charged compound during the first hours, whereas the remainder is retained during the subsequent period. This makes dialysis a convenient method for the desalting of inositol(poly)phosphates, avoiding the elaborate steps of desalting by columns (3). Samples can be dialyzed against any low-ionic-strength buffer, as other obvious advantages are that this method is rapid and that many samples can be processed simultaneously. This method was successfully used for the removal of excess $[^{32}PO_4]$ after *in vivo* cell labeling with $[^{32}PO_4]$ and also for the removal of salt after HPLC purification of $[^{32}P]Ins(1,4,5)P_3$ and $[^{32}P]InsP_6$. Quantitative analysis of the isolation of ^{32}P-labeled compounds was performed by using internal standards of ^3H-labeled authentic compounds. Briefly, samples of 0.1 ml were quenched with 1 vol of 3.5% perchloric acid, neutralized with 0.5 vol of potassium bicarbonate (50% saturated solution), and mixed with 0.01 μCi $[^3H]Ins(1,4,5)P_3$ and $[^3H]InsP_6$. The solid potassium chlorate was pelleted and the supernatants were dialyzed two times against 500 vol of dialysis buffer for 2 h and one time against 500 vol overnight. The samples were then applied to a HPLC SAX column eluted with ammonium phosphate, pH 3.7, as described (1). The fractions containing $Ins(1,4,5)P_3$ or $InsP_6$ were pooled and dialyzed two times against 500 vol of dialysis buffer. Optimal retention is coupled to effective desalting, which for $Ins(1,4,5)P_3$ is two times 1 h dialysis while for $InsP_6$ this may be two times 3 h.

Acknowledgments. All radioactive tracers were from Amersham, except $[^3H]InsP_6$ which was obtained from NEN Dupont. Dialysis membrane with MWCO of 12–14 kDa was from Visking. Dialysis membranes with MWCO values of 10, 15, 25, and 50 kDa were kindly provided by Spectrum Medical Industries Inc.

REFERENCES

1. Van Dijken, P., Lammers, A. A., Ozaki, S., Potter, B. V. L., Erneux, C., and Van Haastert, P. J. M. *Eur. J. Biochem.* (1994), **226**, 561–566.
2. Luttrel, B. M. (1993) *J. Biol. Chem.* **268**, 1521–1524.
3. Stephens, L. R., Hawkins, P. T., Morris, A. J., and Downes, C. P. (1988) *Biochem. J.* **249**, 282–292.
4. Wregget, K. A., Howe, L. R., Moore, J. P., and Irvine, R. F. (1987) *Biochem. J.* **245**, 933–934.

Chemiluminescent Detection of Dextran Bound to Streptococcal Glucan-Binding Lectin

Michael Y. Galperin, Maureen Lassiter, Yousheng Ma, K. G. Taylor, and R. J. Doyle[1]
Department of Microbiology and Immunology, University of Louisville Health Sciences Center, Louisville, Kentucky 40292

Received October 5, 1994

Carbohydrate-binding proteins (lectins) of animal, plant, and microbial origin are increasingly used in purification and characterization of glycoproteins and glycoconjugates (1,2). Derivatized (e.g., biotinylated or digoxigenin-labeled) lectins often retain their carbohydrate-binding capabilities, which simplifies their detection and makes lectin-binding assays especially convenient (2,3). However, as more new lectins are being described, their detection and purification still constitute a significant problem. This is especially true for bacterial lectins, where very low amounts are usually studied. Several protocols for lectin screening and detection have been recently described (3–5). We now report a simple method for detection of streptococcal glucan-binding lectins, based on staining of fluorescein-labeled dextran bound to the lectin by anti-fluorescein antibodies coupled to horseradish peroxidase (HPR).[2] The HPR-containing antibody, when mixed with substrate, enhances the fluorescence of the dextran, a process called enhanced chemiluminescence (ECL).

Glucan-binding lectin (GBL), found on the surfaces of *Streptococcus sobrinus* and several other oral strepto-

[1] To whom correspondence should be addressed. Fax: (502) 852-7163.

[2] Abbreviations used: ECL, enhanced chemiluminescence; GBL, glucan-binding lectin; GTF, glucosyltransferase; HPR, horseradish peroxidase; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

ANALYTICAL BIOCHEMISTRY **225**, 185–187 (1995)
0003-2697/95 $6.00
Copyright © 1995 by Academic Press, Inc.
All rights of reproduction in any form reserved.