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Protein binding to supported lecithin bilayers controlled by the lipid phase state: a new concept for highly selective protein purification

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Chromatography of proteins often requires the application of ion exchange or affinity techniques. Here we report a fundamentally new concept for protein separation that offers distinct advantages over the above mentioned techniques. It is based on the phase transition between the gel phase and the fluid phase of phospholipid bilayers on a solid support. We show that this approach allows the purification of "bindin", an amphiphilic peripheral membrane adhesion protein from sea urchin sperm. This protein binds to the supported bilayer of dielaidoyl-*sn*-glycero-3-phosphatidylcholine (DEPC) in its gel phase and dissociates from it on raising the temperature to values above the main phase transition. Other proteins of the mixture studied do not show this gel phase affinity and thus a separation of bindin is achieved simply by applying the protein mixture at gel phase conditions and eluting the bindin in the fluid phase.

The phase transition in phospholipid model membranes between the crystal-like L_β phase and the fluid-like L_α phase is a well known phenomenon and has been studied in great detail over the past two decades (Mabrey and Sturtevant 1976; Cevc 1993). In spite of the accumulated wealth of information, the lipid phase transition remained a subject of mainly academic interest. The recent introduction of phospholipid bilayers on a solid spherical support allowed not only the first solid state NMR studies of supported membranes (Bayerl and Bloom 1990) but also allowed new approaches in the field of bilayer-protein interaction (Reinl and Bayerl 1993). We demonstrated that single phospholipid bilayers on a spherical solid support (silicon or silica) exhibit a phase transition behaviour similar to that of the classical model membrane systems (Naumann et al. 1992) and showed that dynamic features of the bilayer are not significantly altered by the presence of the solid support (Köchy and Bayerl 1993; Dolainsky et al. 1993).

The fact that the bilayer is immobilized on a macroscopic support in such systems allows the use of the bilayer as a binding matrix for water soluble proteins in a well defined manner. This is because the supported bilayers can be packed into a column similar to that used in classical gel chromatography for protein separation, and dissolved proteins can pass the immobilized bilayers via the flow of the buffer medium through the column (cf. Scopes 1994 for a review). Since some amphiphilic proteins exhibit different affinities for electrically neutral bilayers, depending on whether they are in the gel or in the fluid phase, the phase transition may be employed for separating such proteins in a thermostated column setup. Moreover, the bilayer coverage of the silica surface should have the additional benefit of protecting the protein from contacts with the inorganic silica surface and thus retaining its activity to a degree which is not usually achieved with classical chromatography packing materials. In this work, we demonstrate that the lipid main phase transition in combination with the specific advantages of supported bilayers can be used for the purification of the amphiphilic protein bindin, a 24 kDa protein which is responsible for the adhesion of sperm to sea urchin eggs (Vacquier and Moy 1977).

The bilayer coated silica gel Transil-N (pore size 4000 Å, diameter 10±1.5 μm) is commercially available from Nimbus Biotechnologie GmbH, Leipzig, Germany. The silica bead is coated with a single bilayer or DEPC (dielaidoyl-*sn*-glycero-3-phosphatidylcholine). The bindin enriched protein fraction was kindly provided by Prof. C. G. Glabe, Irvine, California, USA (Vacquier and Moy 1977). Protein concentrations were determined using the method of Lowry (Lowry 1951).

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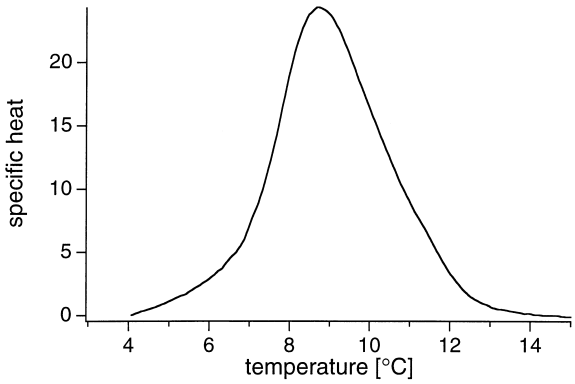

Fig. 1 Differential scanning calorimetry (DSC) endotherms of Transil-N. Transil-N is a single phospholipid bilayer of DEPC coated on a silica gel (10±1.5 μm diameter and a (400±100) nm pore size)

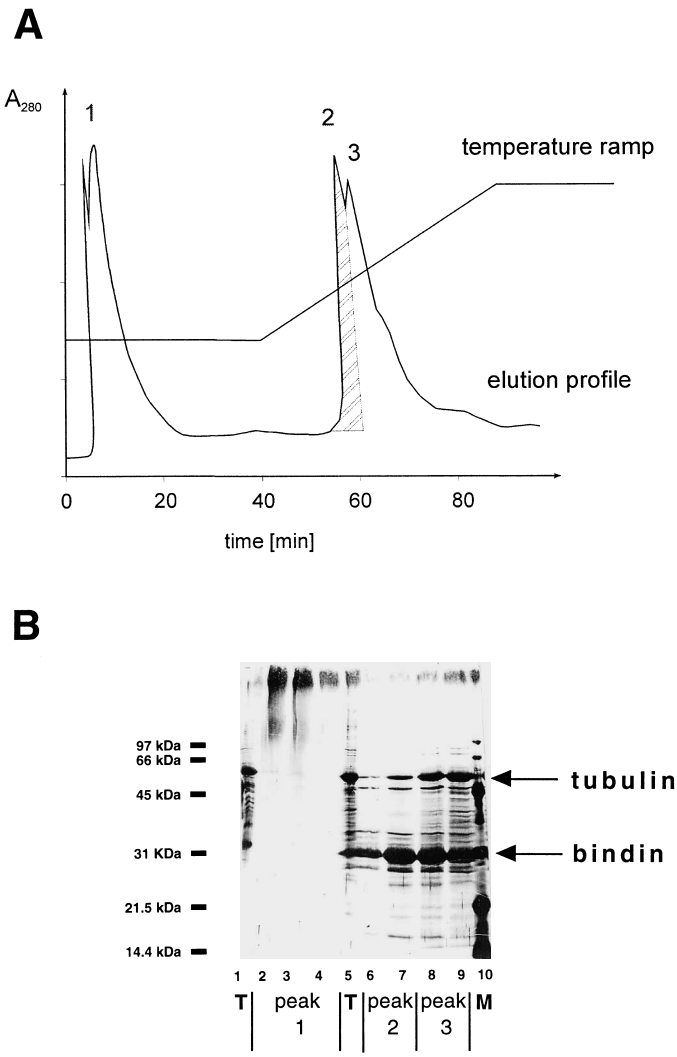

Fig. 2 **A** UV monitor absorbance of the Transil-N column during the bindin purification experiment, giving the peaks 1 (flow through, T = 4 °C, L_β phase of the supported bilayer) and 2,3 (eluate, T = 17–22 °C, L_α phase of the supported bilayer). **B** SDS PAGE results (silver stain) of the protein mixture applied (lanes 1, 5) and of fractions collected from peaks 1 (lanes 2–4), 2 (lanes 6, 7) and 3 (lanes 8, 9); apparent molecular weight of bindin: 30.5 kDa

A GradiFrac-System (Pharmacia, Uppsala, Sweden) was used for protein chromatography. This consisted of column, controller and fraction collector unit, UV-monitor and peristaltic pump. Thermostating of the chromatography column was achieved via an external thermostat (Julabo, Seelbach, Germany) interfaced to a PC. Temperature control was achieved by the NIMBUS software product CT-CON, allowing simultaneous column temperature control and UV-monitor data acquisition.

Transil-N (1.5 g) was dispersed in 50 mM 2-morpholinoethane-sulfonic acid (MES), pH 6.5, 1 M NaCl (buffer A) and packed in the 10 mm diameter standard chromatography column. All chromatography was done with this buffer. The crude bindin fraction was dissolved in buffer A at a concentration of 5 mg/ml. The flow rate was 0.5 ml/min during sample application and 0.7 ml/min during chromatography. The size of the collected fractions was 1.4 ml, proteins were detected by UV monitoring at $\lambda = 280$ nm. The temperature program (4°→ 25 °C; 0.7 °C/min) was started after the flow-through absorbance peak reached baseline level. Electrophoresis was performed according to Laemmli (Laemmli 1970) on a 14% polyacrylamide separating gel and proteins were detected by silver stain (Wray 1981). The DSC endotherm of Transil-N (Fig. 1) was acquired using a Microcal MC-2 microcalorimeter (Microcal, Amherst, MA, USA) in the ascending temperature mode at a scan rate of 20 °C/h according to procedures described previously (Naumann et al. 1992).

The DSC measurement gives a phase transition temperature T_m for Transil-N of 8.5 °C (Fig. 1), which is ≈2 °C lower than for DEPC in multilamellar dispersions, and the width of the transition is a factor of 4 higher. This effect is well known for supported bilayers and has been linked to the buildup of lateral stress in the bilayer owing to the shrinking of lipid molecular area at the transition to the gel phase (Naumann et al. 1992).

The equilibrated Transil-N column was loaded at 4 °C, well below T_m, with 3.5 mg of the bindin containing protein mixture obtained from the sperm of the sea urchin *Strongylocentrotus purpuratus* after subcellular fractionation. This mixture generally contains substantial amounts of bindin and tubulin, and minor amounts of other proteins. Bindin is an amphiphilic protein and shows a pronounced affinity for lipid bilayers in the gel state but not in the fluid state (Glabe 1985). Thus, by keeping the Transil-N column at temperatures $<T_m$ in the gel phase, bindin should couple to the bilayer coated gel surface to a much higher extent than any of the other protein constituents of the mixture. Consequently, the latter proteins should be found in the bindin depleted flow-through fraction (peak 1 in Fig. 2 A). After the return of the UV monitor signal at the column exit to baseline level, the temperature of the column was raised to 25 °C ($T > T_m$) at a rate of 1 °C/min. The phase transition of the Transil-N coating lipids (DEPC) leads to the elution of a protein fraction characterized by a double peak (peaks 2 and 3 in Fig. 2 A). SDS PAGE shows that peak 1 fractions (flow-through) contain mainly high molecular weight proteins but no bindin or tubulin (Fig. 2 B, lanes 2–4). In contrast, peak 2 consists of purified bindin

(Fig. 2 B, lanes 6–7) while peak 3 originates from a bindin enriched mixture. With increasing fractionation time the amount of bindin in the peak 3 fractions decreases while that of other proteins (mainly tubulin) increases (peak 3, Fig. 2 B, lanes 8, 9).

The data in Fig. 2 provide clear evidence that the phase state of the electrically neutral DEPC bilayer is indeed a major factor in determining its affinity for bindin. The results do not provide any clues for an understanding of the binding/unbinding effect on a molecular scale but they show that it clearly exists. Possible explanations are: (1) a change of the lecithin headgroup conformation between the two phase states. It is conceivable that the P-N dipole assumes an orientation relative to the protein in the gel phase which does not completely cancel out the electric field components arising from the P and N charges in the choline headgroup, thus giving rise to an attractive Coulomb force. After all, it is well known that the bilayer dipole potential changes at T_m (Gawrisch et al. 1992). However, the fact that buffer A contains 1 M NaCl makes surface electrostatics rather unlikely as the dominating mechanism since the Debye screening length is well below 10 Å under these conditions. (2) Bindin could exhibit an affinity for grain boundaries and defect lines in the lecithin bilayer via hydrophobic loops penetrating into the bilayer. Such defect lines are perfectly conceivable in a gel phase bilayer, and their number might be even higher in a solid supported bilayer owing to the inevitable buildup of lateral stress along the bilayer plane after the transition to the gel state. This stress is mainly caused by the shrinkage of the molecular area of the lecithin bilayer while the solid support remains unchanged, and has been demonstrated to result in a reduction of T_m (Naumann et al. 1992). The healing of such defects at the transition to the L_α phase might then be the driving force for the dissociation of bindin. However, hard evidence for the validity of one of the above surmises could only be provided by methods that allow a simultaneous monitoring of the secondary structure of bindin and of the bilayer structure and dynamics during the binding and dissociation events. A combination of IR-ATR experiments with NMR and neutron reflection techniques might be suitable to tackle this problem.

Nevertheless, the protein binding/dissociation mechanism discussed above provides a new, very sensitive tool for protein purification by column chromatography. We denote this method as Phase Transition Chromatography (PTC). The major difference compared to the classical chromatographic techniques, such as ion-exchange chromatography, is the fact that switching between protein binding and unbinding is achieved by a change of the column temperature rather than the by a change of the buffer ionic strength. The need to change the ionic strength of the buffer medium for the elution is a drawback since it can cause partial protein denaturation, a problem which PTC elegantly bypasses by replacing the buffer change by a temperature ramp. Moreover, the use of a lipid bilayer to coat the silica gel, i. e. a highly biocompatible surface, may further prevent protein denaturation during chromatography.

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References

Bayerl TM, Bloom M (1990) Physical properties of single phospholipid bilayers adsorbed to micro glass beads. Biophys J 58: 357–362

Cevc G (1993) Material transport across permeability barriers by means of lipid vesicles. In: Lipowski R, Sackmann E (eds) Biophysics handbook on membranes. Structure and conformation. Springer, Berlin Heidelberg New York

Dolainsky C, Möps A, Bayerl TM (1993) Transverse relaxation in supported and nonsupported phospholipid model membranes and the influence of ultraslow motions. A 31P-NMR study. J Chem Phys 98: 1712–1720

Gawrisch K, Ruston D, Zimmerberg J, Parsegian A, Rand P, Fuller N (1992) Membrane dipole potentials, hydration forces, and the ordering of water at membrane surfaces. Biophys J 61: 1213–1223

Glabe CG (1985) Interaction of the sperm adhesive protein, bindin, with phospholipid vesicles. I. Specific association of bindin with gel phase phospholipid vesicles. J Cell Biol 100: 794–799

Köchy T, Bayerl TM (1993) Lateral diffusion coefficients of phospholipids in spherical bilayers on a solid support measured by 2H-nuclear-magnetic-resonance relaxation. Phys Rev E 47: 2109–2116

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature 227: 680–685

Mabrey S, Sturtevant JM (1976) Investigation of phase transitions of lipids and lipid mixtures by high sensitivity DSC. Proc Natl Acad Sci USA 73: 3862–3866

Naumann C, Brumm T, Bayerl TM (1992) Phase transition behavior of single phosphatidylcholine bilayers on a solid spherical support studied by DSC, NMR and FT-IR. Biophys J 63: 1314–1319

Reinl HM, Bayerl TM (1993) Interaction of myelin basic protein with single bilayers on a solid support – An NMR, DSC and polarized infrared ATR study. Biochim Biophys Acta 1151: 127–136

Scopes RK (1994) Protein purification: principles and practice. Springer, New York Berlin Heidelberg

Vacquier VD, Moy GW (1977) Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. Proc Natl Acad Sci USA 74: 2456–2460

Wray W, Boulikas T, Wray VP, Hanckock R (1981) Silverstaining of proteins in polycarylamide gels. Analytical Biochemistry 118: 197–203