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Diffusion of lipids in supported bilayers as an NMR probe for topological studies in nanoporous solids

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Abstract The diffusion of lipids in bilayers on curved supports of porous silica beads is studied by deuterium solid state NMR relaxation. We demonstrate that the combination of bilayers coated on curved silica substrates with NMR experiments and simulations which are sensitive to the diffusive motion of the fluid bilayer lipids can provide information about the substrate topology. This provides a new approach for the exploration of the complex internal surface topology of silica gels widely used in biomolecule chromatography.

Key words Bilayer · Solid support · Diffusion · Frictional drag · Relaxation

Porous solids, particularly silica or titanium oxide, with pore sizes from 50 Å up to several hundred nm are widely used as separation media in conventional gel chromatography or in high performance liquid chromatography (HPLC) (Scopes 1993). Furthermore, by coating of the external and internal surface of these chromatography gels with a single phospholipid bilayer, large and highly biocompatible surfaces are created which can be used for advanced temperature controlled bioseparation (Loidl-Stahlhofen et al. 1996 a, b). Although the pore size and shape of chromatography gels is of paramount importance for selectivity, resolution and capacity of bioseparations, their size distribution and exact geometry is largely unknown. This is mainly due to a lack of suitable experimental methods for the exploration of the inner gel surface. So far, their pore size is commonly estimated by surface area determination via gas adsorption measurements and assuming spherical pore geometry. Similarly, small angle neutron scattering (SANS) can provide correlation distances between adjacent pore centers but further analysis in terms of pore geometry and pore size distribution requires several restricting model assumptions.

Here we demonstrate a new solid state NMR approach for the assessment of internal pore size and geometry. It is based on the following four logical steps: (1) The coating of total external and internal gel surface with a single phospholipid bilayer creates a partially oriented, approximately 50 Å thick surface film (supported bilayer) separated from the solid surface by a water film of less than 30 Å thickness (Bayerl and Bloom 1990; Johnson et al. 1991; Naumann et al. 1992). (2) For a fluid bilayer on a solid support, the constituent lipids are free to diffuse laterally along the bilayer plane (Dolainsky et al. 1995). In the course of this diffusion process, each molecule samples part of the pore surface in a random walk. (3) As the pore surface is curved, the long axis of each lipid may change its angle with respect to an externally applied magnetic field at any random walk step. (4) In terms of solid state NMR, the latter corresponds to an accumulation of phase for each molecule, the amount of which depends on its path along the curved pore surface. This in turn is equivalent to a relaxation mechanism by motions (diffusion in this special case), which are evidently slow on the NMR-timescale (Dolainsky et al. 1993). Thus, the result of a suitable NMR pulse experiment which keeps track of the total accumulated phase of the diffusing lipids will inevitably contain information about the surface topology.

To test this surmise experimentally, we have coated commercial silica gels with single bilayers of selectively deuterated DPPC-d_8 (di-palmitoyl-7,7,8,8-D_2-glycero-phosphatidylcholine, special synthesis from Avanti Polar Lipids, Alabaster, AL., USA) according to procedures described in detail elsewhere (Loidl-Stahlhofen et al. 1996 a; Naumann et al. 1992) and studied the samples by a deuterium solid state NMR relaxation technique sensitive to motions such as lipid lateral diffusion (Bloom and Sternin 1987). The major advantage of using lipid bilayers to coat the pore surface over its wetting with fluid films (Stapf et al. 1994, 1995) or vapor adsorption, is that the close bilayer-silica surface contact, together with the strict confinement of the diffusing lipids within the bilayer, ensures

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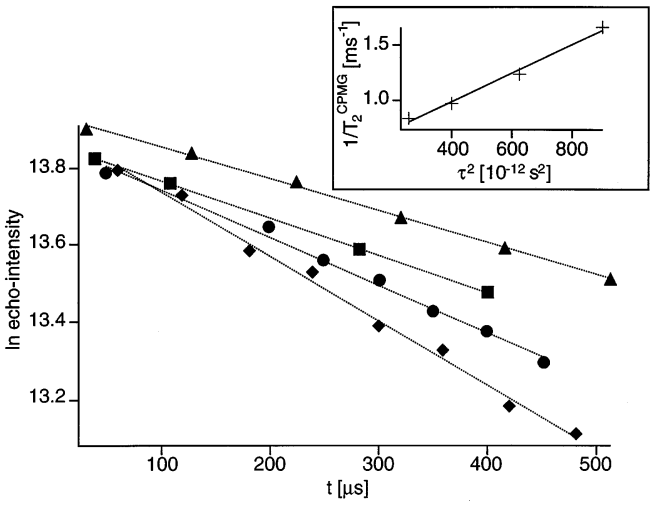

Fig. 1 Semilogarithmic plot of the CPMG echo amplitude vs. time $t = 2n\tau$ (where n is the number of the echo) at different values of the pulse spacing τ (20, 25, 30 and 35 μs starting from top) for a fluid DPPC-d_8 bilayer (T = 55 °C) coated on non-porous silica beads of radius R = (220±20) nm. The *dashed lines* represent linear fits to the data the slope of which gives $1/T_2^{CPMG}(\tau)$. The insert shows $1/T_2^{CPMG}(\tau)$ vs. τ^2 in the limit of short τ values. Here the linear fit (*full line*) provides the slope b

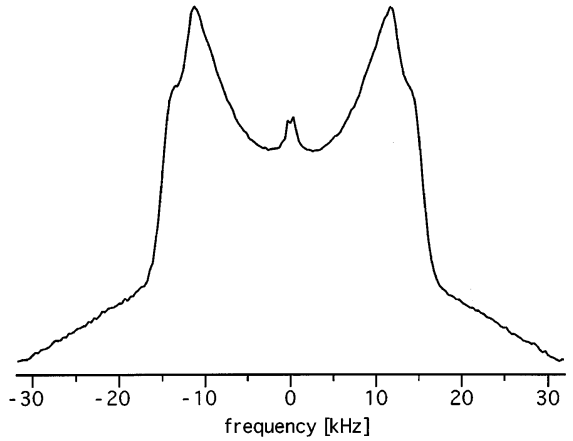

Fig. 2 A: ^{2}H-NMR spectrum of a DPPC-d_8 single bilayer coated on a porous silica gel at T = 55 °C

that only contributions arising from the surface topology will be detected. Hence, we do not need to consider Lévy-walk contributions to the evolution of the NMR relaxation signal (Stapf et al. 1995).

In a first calibration step, we have used non-porous spherical silica beads (special synthesis from Degussa AG, Anorganic Chemistry, Hanau, Germany) of R=(220±20) nm as solid support and measured the ^{2}H-NMR transverse relaxation using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence adapted for spin-1 systems. The measurements were done at 76.7 MHz using a Bruker MSL 500 solid state NMR spectrometer at a sample temperature of 55 °C according to procedures which have been described, together with our CPMG data analysis method, elsewhere (Köchy and Bayerl 1993).

Figure 1 shows the measured decay of the CPMG echo amplitude vs. time $t = 2n\tau$ (n = 1, 2, 3 . . .) for different delay times 2τ between the refocusing 90° pulses. Within the limit of short t (t <500 μs), the plot of the CPMG echo amplitude vs. t for different values of τ is exponential and the individual slopes give $1/T_2^{CPMG}(\tau)$. According to the theory of transverse relaxation with lateral diffusion being the only slow motion mechanism (Bloom and Sternin 1987), this relaxation can be described by $(T_2^{CPMG})^{-1} = [2M_{2r}D/R^2]\tau^2 + (T_2')^{-1}$ in the limit of $\tau_D \gg \tau_M$. Here M_{2r} is the residual second moment of the ^{2}H-NMR line shape, D is the lateral diffusion coefficient of the lipids, R is the radius of the bead, τ_M is the NMR timescale ($\approx 10^{-5}$ s for ^{2}H-NMR) and $\tau_D = R^2/6D$ is the diffusion correlation time on a sphere of radius R. $(T_2')^{-1}$ is the relaxation rate due to processes having correlation times τ_2' which are fast on the NMR timescale ($\tau_2' \ll \tau_M$). Hence, a plot f $1/T_2^{CPMG}$ vs. τ^2 should give a straight line with the slope $b = 2M_{2r}D/R^2$. Since D is $1.19 \cdot 10^{-11}$ m^2/s at the temperature of our measurement (T = 55 °C) (Köchy and Bayerl 1993) and $M_{2r} \approx M_2 = (4.0 \pm 0.3) \cdot 10^{-9}$ s^{-2} is determined from the ^{2}H-NMR lineshape (Bloom and Sternin 1987), we can rearrange for $R = [2M_{2r}D/b]^{1/2}$. The insert of Fig. 1 shows the plot of $1/T_2^{CPMG}$ vs. τ^2 for the case of the bilayer coated non-porous spherical bead from which we obtain $b = (1.7 \pm 0.22) \cdot 10^{12}$ s^{-3} and thus R = (235±25) nm using the above values for D and M_{2r}. This is in excellent agreement with the value of R = (220±20) nm which we determined by transmission electron microscopy (TEM) for these beads.

After this validation of the method for the determination of R in spherical systems, we proceeded by using a porous silica gel (Nucleosil 4000-10 from Machery-Nagel, Düren, Germany) of 10±4 μm bead diameter (TEM result) and R_{av} = 200 nm average pore radius according to the specification of the supplier (based on gas adsorption measurements). A ^{2}H-NMR quadrupolar echo spectrum of the DPPC-d_8 single bilayer coated on this gel and dispersed in excess (deuterium depleted) water, is shown in Fig. 2. The typical Pake powder-pattern lineshape of this spectrum is nearly identical to that obtained for the non-porous beads (not shown) but shows a slightly reduced quadrupolar splitting ($\Delta\nu_Q$ = 21.9 KHz compared to 22.7 KHz for the non-porous beads). The double peaks in Fig. 2 are not due to a non-equivalence of the labelling position (7,7,8,8) but derive from the presence of the solid support, which causes the lipids in the monolayer leaflet facing the silica surface to adopt a slightly higher molecular order (Hetzer et al. 1998). This effect of the solid support is observed for both porous and non-porous silica beads. However, the CPMG relaxation experiment reveals significant differences between the two silica gels (Fig. 3 A): The decay of the CPMG signal is not single- but multi-exponential for the porous gel, most likely due to a significant variation of curvature radii inside the porous beads. In fact, we obtained a similar decay behaviour upon mixing two or more bilayer coated non-porous bead populations of distinct radii differing by

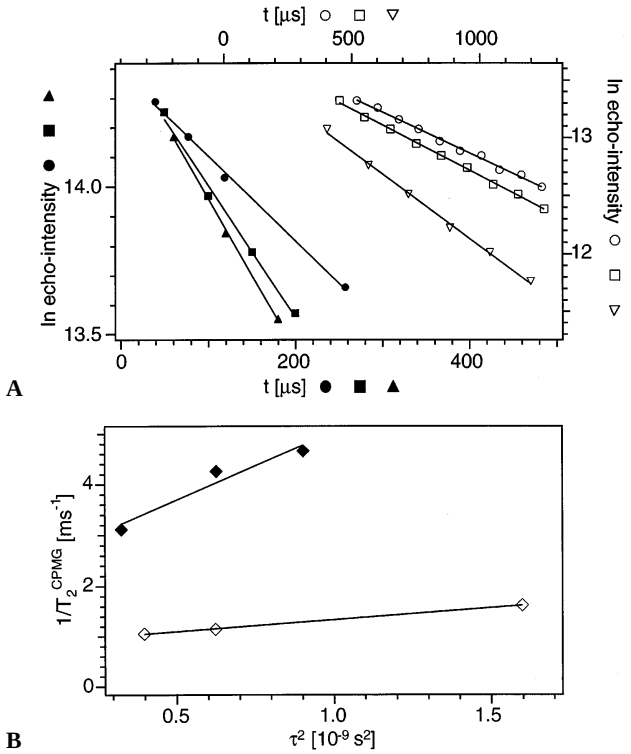

Fig. 3 **A** CPMG echo intensity vs. t for the porous silica gel sample. Dashed lines represent linear fits at short times (0–220 μs) and at long times (0.4–1.2 ms) **B** $1/T_2^{CPMG}$ vs. τ^2 in the limit of short τ values for $1/T_2^{CPMG,\ short}$ (*full squares*) and for $1/T_2^{CPMG,\ long}$ (*open squares*). Full lines represent linear fits giving b^{short} and b^{long}

a factor of two or more. Although the determination of an average pore size from the decays in Fig. 3 A seems not appropriate, its analysis is useful for estimating the limiting curvatures inside the gel beads. This was done by fitting single exponentials to the initial ($t < 250$ μs, giving $1/T_2^{CPMG,\ short}$) and the tail part (for long $t > 400$ μs, giving $1/T_2^{CPMG,\ long}$) of the decay curve. The plot of these values vs. τ^2 is shown for short τ (≤ 30 μs) in Fig. 3 B and gives slopes of $b^{short} = (2.7 \pm 0.8) \cdot 10^{12}\ s^{-3}$ and $b^{long} = (0.45 \pm 0.5) \cdot 10^{12}\ s^{-3}$. Using the above equation for R with $M_2 = 3.6 \cdot 10^9\ s^{-2}$ (calculated from the lineshape in Fig. 2) and $D = 1.19 \cdot 10^{-11}\ m^2/s$, we obtain $R^{MIN} = (180 \pm 40)$ nm and $R^{MAX} = (430 \pm 70)$ nm. Compared to the value obtained by gas adsorption (R = 200 nm), the NMR result indicates that inside the porous beads a wide distribution of pore sizes, or more generally pore curvatures, exists. The information about these limiting curvature values is not acessible by the conventional gas adsorption technique. The knowledge of the limits of such distributions is of importance for the use of these gels in chromatography since they will largely determine the capacity and the resolution of a chromatography setup. Clearly, the ideal high resolution chromatography silica gel would have a very narrow distribution of pore sizes. A noteworthy limitation of the NMR method is the case of very small pores (less than 100 nm) where motional averaging effects dominate the NMR anisotropic lineshape.

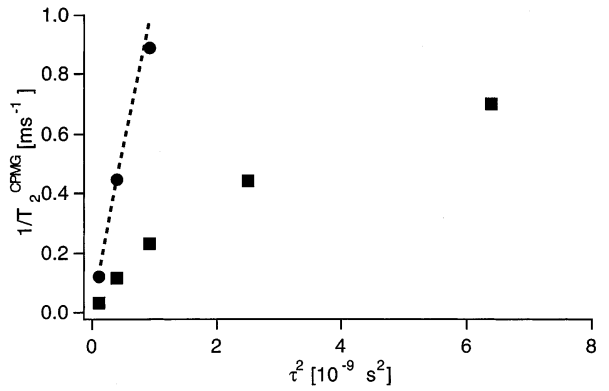

Fig. 4 Plot of $1/T_2^{CPMG}$ vs. τ^2 obtained from a random walk simulation (10^6 lipid molecules) of the CPMG experiment for two different support geometries: spherical support (*circles*) of R = 200 nm. Here the *dashed line* is a linear fit, the slope of which gives the value of R within 5% error. Cylindrical geometry (R = 200 nm) of the support (*squares*) does not allow linear fitting

Although our estimate is rather crude and the errors in R correspondingly large, this example should convey the potential of the approach. The ability of the NMR experiment to keep track of the phase evolution of all diffusing spins, together with the probing of the surface contour by the diffusing lipids may ultimately allow the determination of the full surface topology inside a solid at the mesoscopic length scale. One way to achieve this, which is presently being explored in our laboratory, relies on the comparison of the experimental CPMG relaxation spectra with "random walk" computer simulations of the CPMG experiments for different topologies. As an example, we show in Fig. 4 results of a CPMG random walk simulation experiment (Köchy and Bayerl 1993) for the case of a bilayer of 10^6 DPPC-d_8 molecules ($\Delta\nu_Q = 28.0$ KHz) diffusing ($D = 4 \cdot 10^{-12}\ m^2/s$) over surfaces of spherical (R = 200 nm) and cylindrical (cylinder radius 200 nm, cylinder axis randomly oriented) geometry in a $1/T_2^{CPMG}$ vs. τ^2 representation. The deviation of the cylinder data from a straight line even at short τ values is obvious and may provide a useful parameter for the comparison with the real NMR experiment with respect to a certain topology.

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