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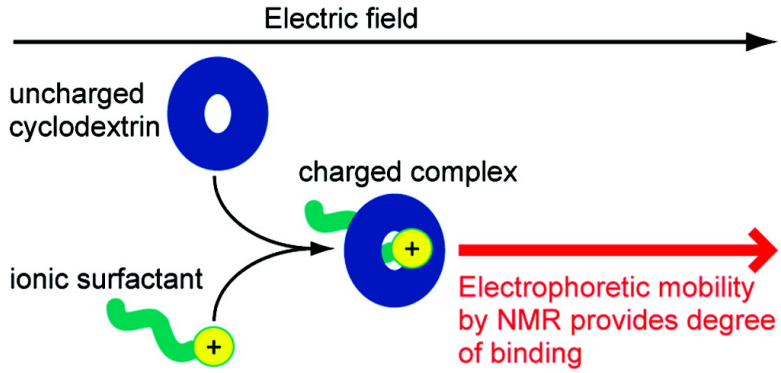

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Molecular Complexation and Binding Studied by Electrophoretic NMR Spectroscopy

Fredrik Hallberg, Christoph F. Weise, Pavel V. Yushmanov, Erik Thyboll Pettersson, Peter Stilbs, and István Furó*

Division of Physical Chemistry, Department of Chemistry, Royal Institute of Technology, SE-10044 Stockholm, Sweden

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Various forms of molecular association are of interest in wide areas of science: examples include binding of small molecules to proteins, supramolecular inclusion complexes, and solubilization in lipid and surfactant aggregates. Among methods for studying such association phenomena, approaches that employ NMR[1] and, in particular, NMR diffusion[2–5] experiments excel. The basic diffusion NMR approach,[1–5] sometimes also referred to as "affinity NMR",[6] relies on the size dependence of the translational self-diffusion coefficient D of molecules. For example, a small molecule that binds to a large protein and is, as often, in fast (on the 10−1000 ms time scale of NMR diffusion experiments) exchange between associated and nonassociated states displays an average diffusion coefficient that is lower than that measured in a neat solution without the protein. Properly performed, NMR diffusion studies may provide the binding stoichiometry and/or the binding constant. Because of the inherent chemical selectivity of NMR, all that information may also be molecularly resolved even in a complex mixture.

Association phenomena not resulting in a large change in hydrodynamic radius remain poorly determined, however. Weak association of two molecules of roughly the same size may lead to a change in D that may be below the detection limit of diffusion NMR[2,7,8] (0.5−10%, set by the precision that depends on instruments and conditions). Moreover, small molecular binding or solubilization that leads to structural changes in the host (because of either host−host association or conformational changes) is also difficult to detect or quantify. Here we present and illustrate a method, based on electrophoretic NMR (eNMR),[9–11] that may advantageously substitute and/or complement diffusion NMR methodology for binding studies.

Conceptually, eNMR is several decades old, but routine application to a wide range of problems has to date been problematic and prone to artifacts. Recently,[12,13] we found pathways toward more robust and accurate procedures, thereby enabling routine applications of the method using standard NMR probes. The basic principle of eNMR is to measure the coherent displacement of charged objects under the influence of an electric field, applied in situ to an NMR sample.[9–11] Akin to diffusion NMR, this displacement is typically (though, not exclusively[14]) detected by magnetic field gradients. Indeed, the same pulse sequences (see Figure 1) as in diffusion NMR are employed in eNMR studies with the sole significant difference of added voltage pulses. Performing the experiment in Figure 1 with gradient pulse spacing Δ and pulse length δ and at several values of the applied electric field E yields[13]

$$\varphi = \mu E \Delta \gamma g \delta \qquad (1)$$

where the phase of the complex signal φ is proportional to the electrophoretic mobility, μ of the charged entity, and the magnetogyric ratio γ of the observed nuclear spin. The electric field can be obtained as $E = U/l$ where U is the cell voltage over the electrode−electrode distance l. In ideal experiments, uncharged entities (with $\mu = 0$) should not show any phase modulation. We emphasize this latter point as an inherent advantage of eNMR: for association of charged molecules to uncharged ones, eNMR is a null experiment (i.e., no detected mobility for the uncharged component in the absence of association) and thereby very sensitive to weak association. On the other hand, electro-osmosis and thermal convection may lend mobility even to uncharged entities. As has been shown, the difference between phases of target and reference entities provides an accurate measure of the electrophoretic mobility.[13]

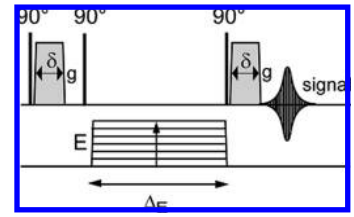

Figure 1. The schematic pulse program of an electrophoretic NMR experiment that uses the stimulated echo method for detecting the coherent displacement of charged objects under the influence of the applied electric field pulse E. Similar pulse programs but without the E pulse and with varying gradient strength g supply the diffusion coefficient D. In reality, the electrophoretic experiment was performed with the electrophoretic double stimulated echo[12] sequence with two E pulses of reverse polarity.

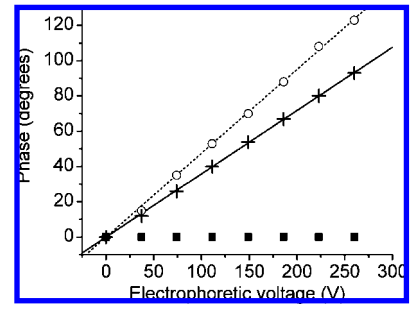

Figure 2. The electrophoretically modulated phases of the ^{1}H NMR signals of α-cyclodextrin (+), DeTAB (○), and water (HDO, ■) at compositions given in Table 1. These data, recorded with the electrophoretic double stimulated echo pulse sequence,[12] provide through eq 1 the electrophoretic mobility μ (see Table 1). The absence of measurable phase change of the HDO signal indicates the lack of sizable effects caused by electro-osmosis and thermal convection. In case these latter contributions are significant, the electrophoretic mobility is obtained from phases measured relative to the HDO phases.[13]

We here (Figure 2 and Table 1) demonstrate application of eNMR to molecular association by monitoring binding of ionic surfactants, decyltrimethylammonium bromide (DeTAB) and cesium perfluorooctanoate (CsPFO), to uncharged α- and β-cyclo-

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Table 1. Self-Diffusion Coefficients (D) and Electrophoretic Mobilities (μ) of Cyclodextrins, the Decyltrimethylammonium Ion, and the Perfluorooctanoate Ion in Their Respective Neat 10 mM Solutions and in Their Equimolar (10:10 mM *x*-CD/Surfactant) Mixtures, all in D_2O: The Nominal Charges and the Fractions of Molecules Bound in Complexes Are Derived As Described in the Text (For Experimental Details, See Supporting Information)

	D (10^{-10} m^2/s)	μ (10^{-9} m^2/Vs)	z^a	p^b	p^c
$DeTA^+$	5.51	19.9	0.93		
PFO^-	4.96				
α-CD	2.75	0			
β-CD	2.56	0			
$DeTA^+$/α-CD	3.04/2.61	10.7/8.1	0.80	0.84	0.87
$DeTA^+$/β-CD	2.87/2.42	11.0/7.8	0.82	0.85	0.84
PFO^-/α-CD	4.48/2.63	−/−1.6	−0.17	0.15	
PFO^-/β-CD	2.71/2.38	−/−8.3	−0.97	0.87	

[a] From eq 4. [b] From the tabulated diffusion coefficient data via eq 2. [c] From the tabulated electrophoretic mobility data via eq 3.

dextrins (CDs). Note that our surfactant concentrations (10 mM) are far below the corresponding critical micellar concentrations. Incidentally, complexation by cyclodextrin was the first application of diffusion NMR to (binary) molecular association.[15] In aqueous solution, the surfactant salt dissociates and the hydrophobic tail of the surfactant ion may potentially insert into the cyclodextrin cavity, as indeed detected by, for example, concentration-dependent studies of the solvent-induced ^{19}F chemical shift changes upon binding of the PFO^- ion to the β-CD host.[16,17] Hence, cyclodextrin molecules become part of charged complexes and attain a nonzero electrophoretic mobility.

The exchange of molecules between complexed and free states is fast as witnessed by the absence of multiple signals. Hence, diffusion NMR[15,18] detects the population averages $D_S = p_S D_{complex} + (1 - p_S)D_{S,free}$ and $D_{CD} = p_{CD}D_{complex} + (1 - p_{CD})D_{CD,free}$ of diffusion coefficients of surfactants and cyclodextrins in the different states, free and bound in a complex. Assuming a 1:1 stoichiometry and equimolar concentrations and exploiting $D_{CD,free}$ and $D_{S,free}$ obtained in single-component solutions, the fraction of bound molecules $p = p_{CD} = p_S$ can be expressed as

$$p = 1 - \frac{D_{CD} - D_S}{D_{CD,free} - D_{S,free}} \tag{2}$$

Since $\mu_{CD,free} = 0$ for the uncharged cyclodextrins, one obtains from the electrophoretic mobilities

$$p = 1 - \frac{\mu_S - \mu_{CD}}{\mu_{S,free}} \tag{3}$$

Hence, a comparison of eqs 2 and 3 reveals one advantage of eNMR over diffusion NMR: a potentially much higher dynamic range and a smaller inherent error when measuring p.

Another advantage of eNMR is the direct estimate of the stoichiometry of the molecular complex. Since

$$\mu = \frac{zeD}{k_B T} \tag{4}$$

where z is the (nominal) charge number and e the elementary charge, eNMR data in combination with diffusion coefficient also provide information about the nominal charge and therefore about the composition of the complexes. We illustrate these points by the data obtained in our two model systems.

Studied by diffusion NMR,[15,18] inclusion of DeTAB into the cyclodextrins is clearly observable as demonstrated by data in Table 1. However, in the case of the PFO^- ion, only a weaker association is detected to the small α-CD cavity. Note that the difference in this case between α- and β-CD, though detectable through the surfactant diffusion coefficient, leaves little mark in the cyclodextrin diffusion, which is consistent with the cyclodextrins being far larger than PFO^-. In contrast, the electrophoretic mobilities of the two cyclodextrins mixed with CsPFO differ by a factor of 5! Additional to the bound fraction calculated under the assumption of 1:1 stoichiometry, the data in Table 1 yield the nominal charge. This can be exploited as another way of obtaining p, or the finding $|z| \approx p$ can be used to provide a model-independent confirmation of the 1:1 stoichiometry.

One anticipated application of this method is to study the binding of small charged molecules to neutral and weakly charged proteins.[4,19,20] Since the current experimental setup[13] employs conventional NMR probes and tubes, experiments can be performed at concentrations far below the ones used in this demonstration. A shortcoming of eNMR is its demand on equipment that is commercially not yet available. On the other hand, there are suitable and inexpensive commercial amplifiers to include in home-built setups. The modest magnetic field gradients (20−100 G/cm) that typically suffice for eNMR are nowadays routinely available for high-resolution z-gradient probes.

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Supporting Information Available: Experimental details and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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