Anal. Chem. **2009,** *81,* 4548–4550

Isomer Resolution by Micelle-Assisted Diffusion-Ordered Spectroscopy

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Diffusion-ordered NMR spectroscopy ("DOSY") is a useful tool for the identification of mixture components. In its basic form it relies on simple differences in hydrodynamic radius to distinguish between different species. This can be very effective where species have significantly different molecular sizes, but generally fails for isomeric species. The use of surfactant co-solutes can allow isomeric species to be distinguished by virtue of their different degrees of interaction with micelles or reversed micelles. The use of micelle-assisted DOSY to resolve the NMR spectra of isomers is illustrated for the case of the three dihydroxybenzenes (catechol, resorcinol, and hydroquinone) in aqueous solution containing sodium dodecyl sulfate micelles, and in chloroform solution containing AOT reversed micelles.

NMR is a powerful tool for the elucidation of chemical structure, but has been less widely used in the study of mixtures because of the difficulty of distinguishing signals from different species. Diffusion-ordered spectroscopy[1−3] (DOSY) is one NMR method designed specifically for mixture analysis: a sequence of pulsed field gradient spin echo experiments is used to estimate the diffusion coefficients of individual signals in a spectrum, and a two-dimensional spectrum is synthesized by dispersing the signals in a second dimension according to their diffusion coefficients. This separation of the signals of different species in a mixture may be regarded as a virtual separation of the mixture that is analogous to the physical separation carried out in HPLC-NMR.[4] In a simple solution, the diffusion coefficient is determined by the effective hydrodynamic radius, so in DOSY experiments on such solutions, species of similar size and structure (e.g., isomers) are difficult or impossible to resolve. However, just as in chromatography the separation of species can be manipulated by changes in stationary and/or mobile phase, so in DOSY the average diffusion coefficients for different species may be manipulated by the addition of co-solutes or co-solvents[5−8] (or even of finely divided solids such as chromatographic stationary phases[9,10]). While the effects of surfactants on the diffusion of co-solutes are well-known, their utility for the systematic manipulation of diffusion resolution appears to have attracted surprisingly little attention.[5,8,11]

Here it is shown that isomers that have little or no difference in diffusion coefficient in simple solution may readily be resolved in DOSY experiments on solutions containing micelles or reversed micelles. The family of isomeric dihydroxybenzenes (catechol, resorcinol, and hydroquinone) was chosen as a simple test case for which hydrodynamic radii in simple solution are expected to be very similar, but interactions with micelles different. Their diffusion properties were investigated in simple D_2O and $CDCl_3$ solutions, and in solutions containing normal and reversed micelles, respectively.

EXPERIMENTAL METHODS

Aqueous samples were prepared containing approximately 12.5 mM each of catechol, resorcinol, and hydroquinone and 25 mM sodium 3-(trimethylsilyl)-1-propanesulfonate (TSP) as reference material in D_2O (sample 1), and in D_2O containing TSP as above and 150 mM sodium dodecyl sulfate (SDS, sodium lauryl sulfate) (sample 2). The sample of dihydroxybenzenes in chloroform was prepared containing 33% v/v each of saturated solutions in $CDCl_3$ of catechol, resorcinol, and hydroquinone and 1% v/v of tetramethylsilane (TMS) as reference (sample 3). Sample 4 was prepared containing 200 mM sodium bis(2-ethylhexyl) sulfosuccinate (aerosol OT, AOT), 1.1 mM D_2O and 0.2 mM of each dihydroxybenzene in chloroform, again with tetramethylsilane as reference material. All measurements were carried out on non-spinning on a 400 MHz Varian INOVA spectrometer, using a 5 mm indirect detection probe equipped with a *z* gradient coil producing a nominal maximum gradient of 30 G cm^{-1}. DOSY data were acquired using the Oneshot[12] pulse sequence with a total diffusion-

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10.1021/ac9005777 CCC: $40.75 © 2009 American Chemical Society
Published on Web 04/29/2009

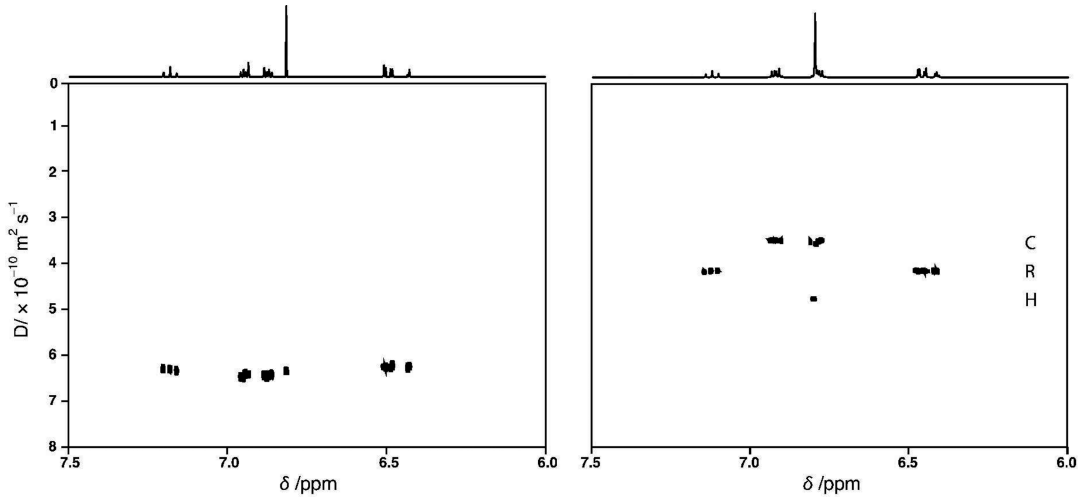

Figure 1. 400 MHz ^{1}H Oneshot DOSY spectra, with (top) 1D spectra, of samples containing 12.5 mM each of catechol, resorcinol, and hydroquinone in D_2O, with TSP as reference, with (right, sample 2) and without (left, sample 1) 150 mM SDS. 64 transients were measured for each gradient level, in a total time of 2 h. Signals of catechol, resorcinol, and hydroquinone are denoted C, R, and H, respectively.

encoding pulse duration δ of 2 ms, a diffusion delay Δ of 0.2 s for experiments in D_2O and 0.1 s for experiments in $CDCl_3$, and 10 nominal gradient amplitudes ranging from 3.0 to 27.3 G cm^{-1} chosen to give equal steps in gradient squared. Experiments were carried out without active temperature regulation, at the probe quiescent temperature of 21 ± 1 °C, to avoid convection in the $CDCl_3$ samples (convection compensation[13−15] would have entailed a 2-fold loss in sensitivity). DOSY spectra were constructed by standard methods,[1,2] using fitting to a modified Stejskal−Tanner equation parametrized to take into account the effects of pulsed field gradient non-uniformity.[2,16] Reference deconvolution[17,18] was used, with Gaussian target lineshapes chosen to optimize the resolution of neighboring signals.

RESULTS

Figure 1 shows the results of Oneshot DOSY experiments on samples of a mixture of catechol, resorcinol, and hydroquinone in D_2O, with (right) and without (left) 150 mM SDS. As expected, in simple D_2O solution the three isomers show very similar diffusion coefficients ($\sim 6.5 \times 10^{-10}$ m^2 s^{-1}).

The presence of 150 mM SDS, however, leads to a significant decrease in diffusion coefficient for the three isomers and, more importantly, to marked differences in diffusion, with the diffusion coefficients of hydroquinone and catechol differing by almost 40%. Both the TSP (not shown) and the dihydroxybenzene signals show a larger decrease in diffusion coefficient than that for HDO, indicating that the slowing of their diffusion is primarily due to a specific association with SDS micelles rather than to viscosity or obstruction effects. The strongest association between the dihydroxybenzenes and micelles is seen for catechol, and the weakest for hydroquinone; small perturbations in chemical shift are also seen for all the dihydroxybenzene signals, those for catechol again being the largest.

The low solubility of dihydroxybenzenes in $CDCl_3$ limits the signal-to-noise ratio of ^{1}H spectra, and hence leads to poorer resolution in the diffusion domain of the Oneshot DOSY spectra of Figure 2, but once again there is a dramatic change in the diffusion of the solutes on the addition of surfactant. As with D_2O, any differences in diffusion coefficient between the three isomers in plain $CDCl_3$ solution are at the threshold of detectability, although the well-resolved phenolic OH singlets encourage a tentative ordering of the diffusion coefficients catechol > resorcinol > hydroquinone, covering a range of just over 2%. With the addition of AOT and a small quantity of D_2O to create reversed micelles in the chloroform solution, the dihydroxybenzene diffusion coefficients show almost a 10-fold decrease (Figure 2 right), with the resorcinol diffusion being the most strongly affected and the hydroquinone the least. The addition of reversed micelles increases the range of diffusion coefficient spanned by the three solutes increases from 2% to over 50%.

DISCUSSION

The presence of micelles or reversed micelles can generate dramatic differential changes in the diffusion characteristics of isomeric species that are otherwise difficult or impossible to resolve by DOSY. In the simple example studied here, differential changes of 50% between isomers were seen. The effects observed were remarkably robust with respect to experimental conditions; in aqueous SDS, for example, the signals of the three isomers remained resolved in the diffusion domain of the DOSY spectrum for a very wide range of solute and surfactant concentrations.

The observation that addition of surfactant can lead to DOSY resolution of isomers is of immediate practical significance, but it is clearly tempting to speculate on the mechanism or mechanisms of selectivity involved. The retention of a single spectrum with sharp lines for each solute (and for surfactant, except at high D_2O concentrations in $CDCl_3$/AOT) indicates that the species remain in fast exchange, as is common for micellar solutions, with an equilibrium between solute in free solution and solute bound to/incorporated in/associated with micelles. A simple model for the aqueous system (samples 1 and 2) would be the incorporation of solutes into the micellar core, for which the partitioning of solute

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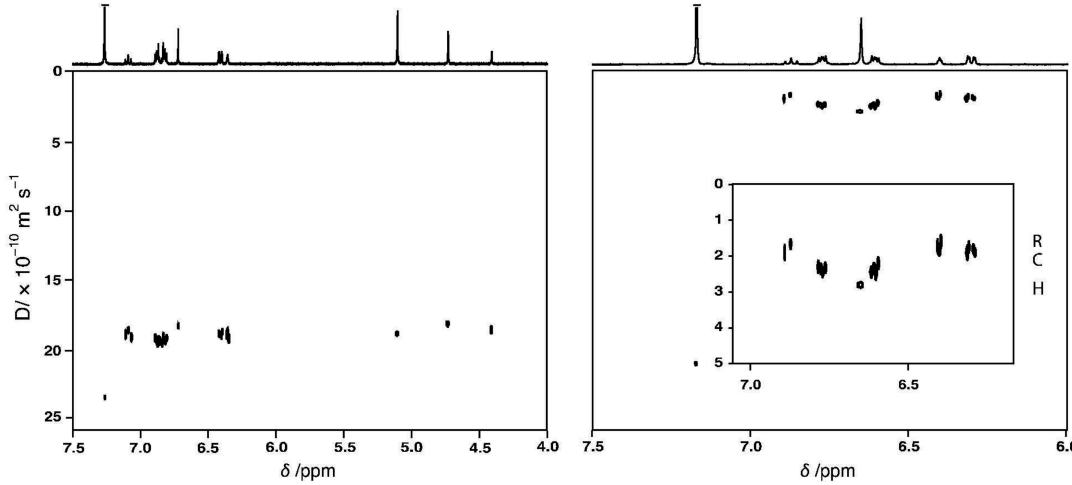

Figure 2. 400 MHz ^{1}H Oneshot DOSY spectra, with (top) 1D spectra, of dihydroxybenzene samples in $CDCl_3$, without (left) and with (right) 200 mM AOT. The inset on the right-hand side shows the dihydroxybenzene signals with the diffusion scale expanded by 250%. 64 transients were measured for each gradient level, in a total time of 2 h. Signals of catechol, resorcinol and hydroquinone are denoted C, R, and H, respectively.

between aqueous solution and micelle core will depend on hydrophobicity. The strength of binding, as evidenced by decrease in diffusion coefficient, increases in the order hydroquinone < resorcinol < catechol; this parallels the trend in estimated logP (hydroquinone 0.59; resorcinol 0.79; catechol 0.88), and is consistent with the hypothesis of incorporation into the micellar core. However, the magnitude of the changes in diffusion coefficient, and the wide range of surfactant and solute concentrations over which they are observed, suggest that this is at best a partial explanation, and other factors must be involved. While ionization of the phenolic OH's in the solutes studied should be negligible under the conditions used, there could be scope for specific hydrogen-binding interactions at the micelle core/headgroup interface, and it is possible that π-stacking effects could enhance binding to micelles at high solute concentrations. The relatively restricted repertoire of chemical shifts seen in the four proton spectra of Figures 1 and 2 suggests that medium effects on shifts may not be very informative about the local environment of the dihydroxybenzenes in this system.

Applying the same basic logic to chloroform solution (samples 3 and 4), complementary behavior would be expected in which the least hydrophobic solute should show the largest decrease in diffusion coefficient in the presence of reversed micelles. However, while the resorcinol and catechol ordering is indeed reversed compared with SDS in D_2O, the solute with the weakest binding in $CDCl_3$/AOT is once again hydroquinone. Solute behavior in $CDCl_3$/AOT might reasonably be expected to be more complex than in D_2O/SDS, since the former is actually a ternary system in which the formation of reversed micelles requires the presence of water, and indeed the diffusion ordering of the three solutes studied does depend on D_2O concentration. Again, well-resolved (in both spectral and diffusion dimensions) DOSY spectra were seen for a range of different concentrations of surfactant, solutes, and water, including samples in which the D_2O concentration was significantly greater than that of sample 4, leading to a turbid appearance.

The spectra of Figures 1 and 2 show only the aromatic region containing the signals of interest, but of course strong surfactant signals are seen in the aliphatic region. For many potential solutes the presence of these signals will be a significant problem, since even mild overlap with a strong signal will distort the apparent diffusion coefficients obtained for signals of a dilute solute. There are several possible solutions to this problem, including the use of perdeuteriated surfactants[8] (readily available because of their use in solubilizing membrane proteins for NMR structure determination) and perfluorosurfactants (for example sodium perfluorooctanoate). It may also be possible to exploit the rather different relaxation regimes of surfactants and solutes to distinguish between their signals, and/or to use selective saturation to attenuate surfactant signals through spin diffusion. In systems with relatively few components it can also be very effective to use multivariate statistical methods to separate the overlapping spectra of species with different diffusion coefficients.[19]

Part of the strength of chromatography as an analytical tool lies in the wide range of potential stationary phases and mobile phases and in the subtlety and diversity of the chemical interactions that determine chromatographic dispersion. These very preliminary results suggest that the analogies between DOSY and chromatography may extend to the use of a range of surfactant/solvent combinations to allow the systematic manipulation of diffusion resolution by NMR for analytical purposes. In addition to the exploitation of direct solute−micelle/reverse micelle interactions, ternary/quaternary systems in which interactions are mediated by complexing agents such as cyclodextrins, or by charged co-solutes, can be envisaged.

ACKNOWLEDGMENT

Support from the Engineering and Physical Sciences Research Council (grant references EP/D05592X, EP/E057888/1, and EP/E05899X) and from the Givaudan Strategic Research Fund is gratefully acknowledged.

Received for review March 19, 2009. Accepted April 13, 2009.

AC9005777

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