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Nuclear Magnetic Resonance at the Picomole Level of a DNA Adduct

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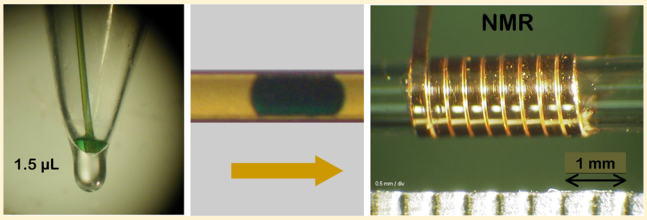
ABSTRACT: We investigate the limit of detection for obtaining NMR data of a DNA adduct using modern microscale NMR instrumentation, once the adduct has been isolated at the picomole level. Eighty nanograms (130 pmol) of a DNA adduct standard, *N*-(2′-deoxyguanosin-8-yl)-2-acetylaminofluorene 5′-monophosphate (AAF-dGMP), in 1.5 μL of D_2O with 10% methanol-d_4, in a vial, was completely picked up as a droplet suspended in a fluorocarbon liquid and loaded efficiently into a microcoil probe. This work demonstrates a practical manual method of droplet microfluidic sample loading, previously demonstrated using automated equipment, which provides a severalfold advantage over conventional flow injection. Eliminating dilution during injection and confining the sample to the observed volume produce the full theoretical mass sensitivity of a microcoil, comparable to that of a microcryo probe. With 80 ng, an NMR spectrum acquired over 40 h showed all of the resonances seen in a standard spectrum of AAF-dGMP, with a signal-to-noise ratio of at least 10, despite broadening due to previously noted effects of conformational exchange. Even with this broadening to 5 Hz, a two-dimensional total correlation spectroscopy spectrum was acquired on 1.6 μg in 18 h. This work helps to define the utility of NMR in combination with other analytical methods for the structural characterization of a small amount of a DNA adduct.



INTRODUCTION

Nuclear magnetic resonance (NMR) is important in determining the structure of DNA adducts; however, nanomole (microgram) quantities of an adduct have been required for one-dimensional (1D) ^{1}H NMR analysis in a conventional 5 mm NMR tube. While such amounts can be prepared synthetically, only much smaller amounts generally are available from biological sources without enormous scale-up. This limitation has contributed to the problem that the structures of most DNA adducts in biological samples are completely unknown. In principle, less scale-up is required if a micro NMR detector is employed. However, sample handling then becomes more challenging. To make an advance in this area, and to better define the lower limit on the amount of a DNA adduct that, once isolated, can yield NMR data, we studied the combination of a microcoil probe, as a representative micro NMR detector, and a microdroplet technique, as a way to handle a very small sample volume.

There are two basic categories of commercially available micro NMR detectors: microcryoprobes and microcoil probes. In the first category, a 1 mm high-temperature superconducting (HTS) microcryoprobe was demonstrated in 2006 to be 24 times as sensitive, per microgram of analyte, relative to a traditional 5 mm tube for small samples (5−10 μL).[1] A commercial 1.7 mm "MicroCryoProbe",[2] thought to use similar or hybrid technology, has been cited as being 15−20 times as sensitive for 30−20 μL samples, respectively.[3,4] Microcryoprobes combine their exquisitely high sensitivity with the convenience of using familiar NMR tubes. Cryoprobes of any size, however, are a major investment in themselves and further are generally installed permanently in a high-field spectrometer. These facilities require sufficient demand to cover these costs, and microcryoprobes are less versatile than 3 or 5 mm probes. Nonetheless, two to three dozen microcryoprobes have been installed in the United States and could be accessible through collaboration, including several in facilities explicitly supporting public research.

Microcoils (small solenoidal coils) are also commercially available for NMR, at a cost comparable to that of conventional probes.[5−8] Changing probes between a microcoil and conventional probe is routine, making microscale NMR readily accessible in local facilities (~200 are in the field, and they are portable). The commercial microcoils (observed volume of 1−2 μL and flow cell volume of 5 μL) theoretically have 18-fold mass sensitivity within the 1 μL observed volume (6-fold smaller diameter, multiplied by the 3-fold advantage of a solenoid over a saddle coil).[5,6] However, microcoil probes are built as flow cells, and even careful flow-injection loading of a 1 μL sample through a 8 μL dead volume results in significant dilution; therefore, the effective mass sensitivity is several-fold inferior when the sample is loaded using conventional sample handling.

The problem of sample dilution during loading of a small volume into an NMR microcoil has been overcome by a segmented flow loading method (now called "microdroplet NMR", since the establishment of droplet microfluidics as an independent field).[9−14] In this technique, the sample is carried

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through the loading tube as a droplet in an immiscible liquid, namely Fluorinert FC43, a perfluorinated fluid. FC43 is immiscible with most organic as well as polar solvents and wets Teflon surfaces aggressively, so the moving droplet is separated from the tubing inner surface by a layer of FC43 ("zero-dispersion" conditions).[15,16] This avoids the losses or carryover that can occur if the sample contacts a solid surface. The technique can deliver a small (1 μL) volume sample without dilution or dispersion and then confine it into the NMR observed volume.[9,17] FC43 has a magnetic susceptibility similar to those of aqueous solvents and functions as a Shigemi tube for the flow system, allowing sample volumes to be nearly as small as the observed volume without causing shimming problems.[9,18] Here we extend the microdroplet technique by conducting it in a simple, manual way on a small sample of a DNA adduct: a 1.5 μL total sample volume (initially in a vial) is picked up in its entirety and handled efficiently, leading to an NMR spectrum at the picomole (nanogram) level.

MATERIALS AND METHODS

Chemicals. *N*-(2′-Deoxyguanosin-8-yl)-2-acetylaminofluorene 5′-monophosphate (AAF-dGMP) was synthesized as previously described.[19] Fluorinert (FC43) was from 3M Corp (St. Paul, MN). Deuterium oxide and deuterated methanol were from Cambridge Isotope Laboratories (Andover, MA). Other chemicals were from Fisher Scientific (Pittsburgh, PA).

Instrumentation. NMR spectra were recorded using a Varian INOVA 500 MHz NMR instrument (currently Agilent, Santa Clara, CA). The microcoil NMR probes were made by Protasis/MRM (Marlboro, MA). The 1D spectra were recorded using a model H/500/1.5 probe, with an observed volume of 1.0 μL and a dead volume of 6 μL (including the flowpath from the probe inlet). Two-dimensional (2D) spectra were acquired using an inverse carbon gradient (ICG) probe with an observed volume of 2 μL and a dead volume of 8 μL, with temperature control. The silica flowpath was internally coated with tridecafluoro-1,1,2,2-tetrahydrooctyl-triethoxysilane (fluorooctylsilane (FOS), United Chemical Technologies, Lewistown, PA) as previously described.[9] The Baby-Bee syringe pump was from BASi (West Lafayette, IN).

Injection Device. To pick up the sample and inject it into the NMR probe, a homemade injection device was made as shown in Figure 1A. The needle of a 10 μL syringe (Hamilton, Reno, NV) was fit into one end of a 30 cm length of PTFE tubing [outside diameter of 1/16 in. and inside diameter of 200 μm (Cole-Palmer, Vernon Hills, IL)]. The other end of this tubing was connected, via a zero-dead volume union (P720, Upchurch, Oak Harbor, WA), to a 5 cm segment of polyimide-clad silica capillary [inside diameter of 200 μm and outside diameter of 360 μm (Polymicro Technologies, Phoenix, AZ)] that had been coated on the inside with FOS.[9]

Probe Connections. The NMR probe inlet was fit on the inlet side with 5 cm of a FOS-silica capillary (as above), then a filter (Upchurch M520), and finally 3 cm of the FOS-silica capillary. The probe outlet was fit to a waste line of 30 gauge Teflon tubing, into which plugs of dye could be injected to measure flow through the microcoil probe with a ruler. The NMR probe was flushed clean with 0.5 mL of methanol-d_4, then blown dry, and prefilled with FC43 prior to use.

Sample Injection and NMR Spectrum. The injection device was prefilled with FC43, and then 3.5 μL was ejected to waste. The AAF-dGMP sample [1.5 μL of 0.1 mM AAF-dGMP in a 90:10 (v/v) D_2O/methanol-d_4 mixture] was prepared in a polypropylene microcentrifuge tube (freshly rinsed twice with 200 μL of methanol-d_4, followed by shaking out and air-drying). The 1.5 μL came from a larger stock solution prepared by weighing AAF-dGMP. The entire sample was drawn into the injection device (with FC43 ahead of it), as illustrated in Figure 1B, using the syringe manually and viewing with a stereo microscope, according to the following sequence of steps: (1) place the tip of the pickup tube, filled with FC43, to the bottom of the sample droplet, (2) draw the entire sample droplet into the pickup tube (ejecting any air drawn after the sample), and (3) move the pickup tube to a second vial of FC43 and draw 2 μL. The injection device was then connected to the probe inlet through a union so that the two ends of fused silica capillary butted against each other (2 cm length of 30 gauge Teflon tubing, designated as "Single-Use Union" in Figure 1). The probe and union were prefilled with FC43 prior to making the connection, to avoid an air bubble, as well as fill any gap in the resulting butt joint. The syringe was then mounted onto the syringe pump, which provided smooth, constant movement of the aqueous sample droplet into the NMR coil, as illustrated in Figure 1A.

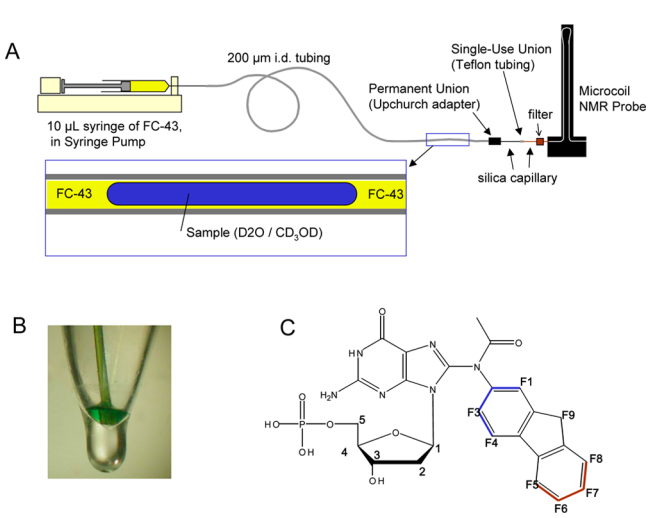

Figure 1. Microdroplet loading method. (A) Schematic drawing of the injection device, with connection to microcoil NMR probe. The inset illustrates the elongated droplet in the PTFE tubing, surrounded by FC43. (B) Photograph of a conical glass vial (for visualization purposes; a plastic vial was used for AAF-dGMP) with a model sample (aqueous dye solution) being fully drawn into the 380 μm outside diameter silica capillary of the injection device. (C) Structure of AAF-dGMP (numbered according to ref 20). Highlighted bonds are color-coded to TOCSY spectrum annotations of Figure 3.

To position the sample in the NMR observed volume, the progress of the sample was roughly monitored by movement of a dye droplet in the outlet tubing (taped to a ruler) as well as by time. Single-scan NMR spectra at 2 s intervals showed solvent NMR signals rise as the head of the plug arrived at the coil, then sharpen as the observed volume was filled. The most precise indicator of position was the lock level rising to a similar level seen in practice injections of solvent without analyte. The pump could be stopped a few seconds early, and the sample nudged forward by turning on the pump for a few seconds. If the droplet was injected too far, one could return it to an optimal position by withdrawing the injection syringe and then hastening the backflow of the solvent by applying gentle pressure to the waste end of the outlet line with a second syringe filled with FC43. One millimeter movements of the dye mark in the 30 gauge outlet line reported the position of the sample within 0.1 μL; touching up the X and Y shims could compensate 0.2 μL variations in positioning a 1.5 μL sample. After positioning and shimming, acquisition of NMR spectra then was begun. A "blank" spectrum of solvent handled like the AAF-dGMP sample was also acquired.

NMR Spectra. 1D spectra were acquired with a 45 degree pulse, a 1.02 s acquisition time, and a 0.5 s relaxation delay. Solvent suppression, where noted, was effected by setting the transmitter on the solvent resonance and presaturating at low power during the relaxation delay. 2D sequences were as provided in the vnmr6.1C library. Because full phase cycles were acquired, the indicated sequences that do not use gradients within the preparation or evolution time were chosen, only homospoil prior to the relaxation

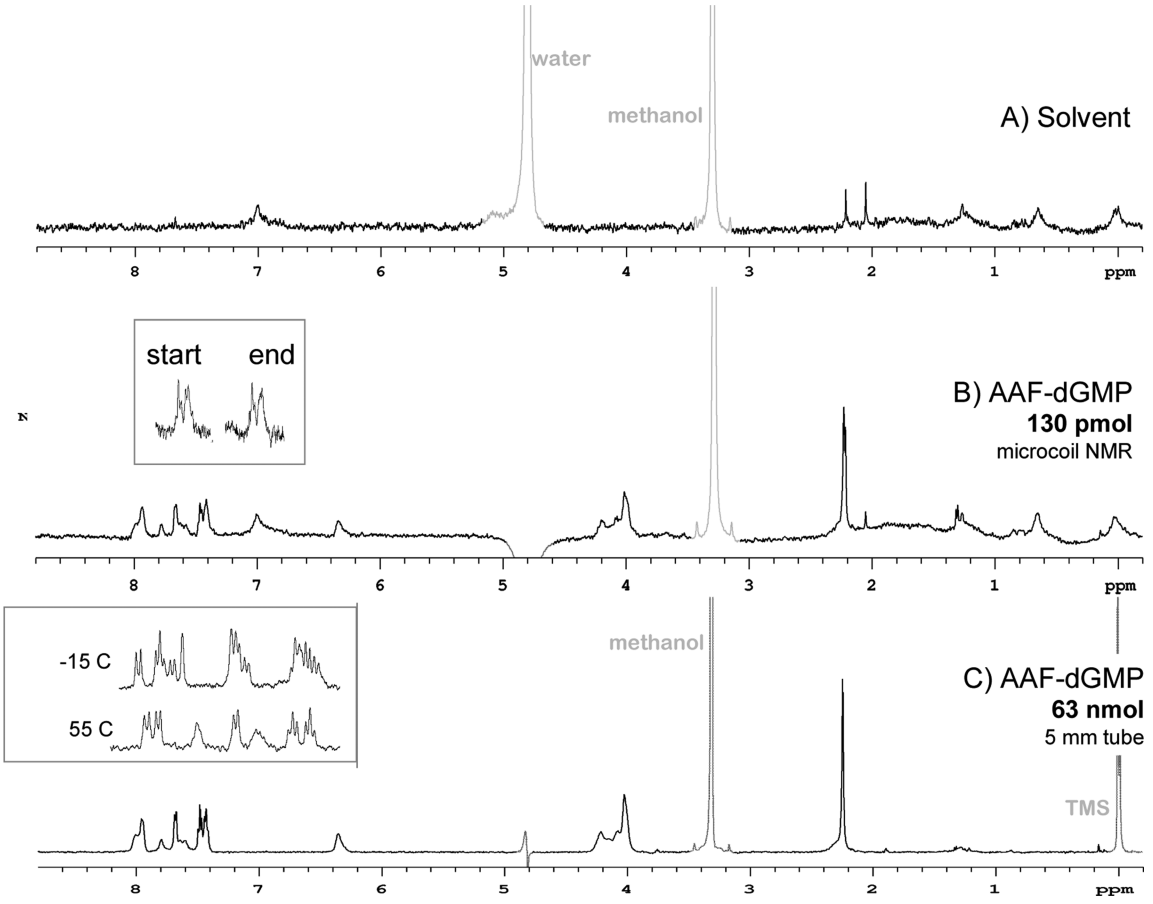

Figure 2. NMR spectra. (A) Solvent blank, 40K scans, acquired in the microcoil. Solvent peaks are colored gray. (B) AAF-dGMP, 80 ng, 100K scans acquired in the microcoil over 40 h. The inset shows the 7.4 ppm peak from the first 4 h (start) and from the last 4 h (end). (C) Reference spectrum of 39 μg of AAF-dGMP acquired using a conventional 5 mm NMR tube. The inset shows the aromatic region from spectra acquired at higher and lower temperatures. All of the signals have been assigned previously; for example, the signals above 7 ppm are 6.3 ppm (CH at ribose C-1), 4.2 ppm (CHs at ribose C-3 and C-4), 3.9 ppm (bridge CH_2 of fluorene and CH_2 at ribose C-5), and 2.2 ppm (acetyl and CH_2 at ribose C-2).[20]

delay. TOCSY at 1 mM acquired 512 points in T_2, with a relaxation delay of 1 s, 64 scans per increment, and 200 complex increments in T_1. The 2D spectra presented were processed with MestreNova version 8.0.2, applying a Gaussian of 8.5 based on a detected T_2* of 0.05 s.

RESULTS AND DISCUSSION

Any microscale NMR technique requires preparing a concentrated solution of the analyte in a small volume. Toward this goal, and also seeking to have the DNA adduct in a highly polar form in a highly polar solvent, we selected to work with a model DNA adduct in a nucleotide form and to employ D_2O containing 10% methanol-d_4 as the sample solvent. FC43 was selected as the immiscible carrier fluid because of its prior success in this area. A potential concern is that a nonpolar, bulky DNA adduct still might have limited solubility even in D_2O/methanol. Further, a bulky adduct might tend to localize at the aqueous−fluorous interface or bind to the tubing wall, leading to its loss from the droplet during transfer to the microcoil NMR observed region. Thus, we selected the bulky nonpolar DNA adduct, *N*-(2′-deoxyguanosin-8-yl)-2-acetylaminofluorene 5′-monophosphate (AAF-dGMP), as a good test case. This choice was also motivated by the fact that this adduct has been characterized previously by NMR, for comparison.[19−21]

On the basis of our prior experience with our microcoil NMR system, including acquisition of a preliminary spectrum of 1 mM AAF-dGMP, we expected that a 1D ^{1}H NMR spectrum could be obtained for this compound by overnight acquisition on a sample concentration as low as 0.1 mM. We then set up the injection device illustrated in Figure 1A. Starting with 1.5 μL of 0.1 mM AAF-dGMP in D_2O and 10% methanol-d_4, containing 80 ng (130 pmol), in a microcentrifuge tube, we used our segmented flow sample loading method to obtain the microcoil NMR spectrum shown in Figure 2B. The lack of any difference in the signal at 7.4 ppm between the first four and the last four of the 40 h of acquisition (inset) demonstrates that there is no diffusion of analyte out of the observed volume during the measurement of the spectrum. By comparison of spectra of a droplet of 1 mM AAFdG to overfilling the microcoil probe with a 1 mM solution of the DNA adduct standard (injecting 8 μL into a dry probe so the observed volume is unambiguously filled with full stock concentration), we calculate that the recovery of the adduct from the vial into the probe is essentially quantitative (additional evaluation of recovery from the probe is described later).

Shown in Figure 2C is a reference spectrum of a much larger amount of AAF-dGMP acquired in a conventional 5 mm NMR tube (39 μg in 0.55 mL, 512 scans, 10 min acquisition). As one can see, the signal-to-noise ratio (S/N) of the microcoil spectrum in Figure 2B is satisfactory for unambiguous

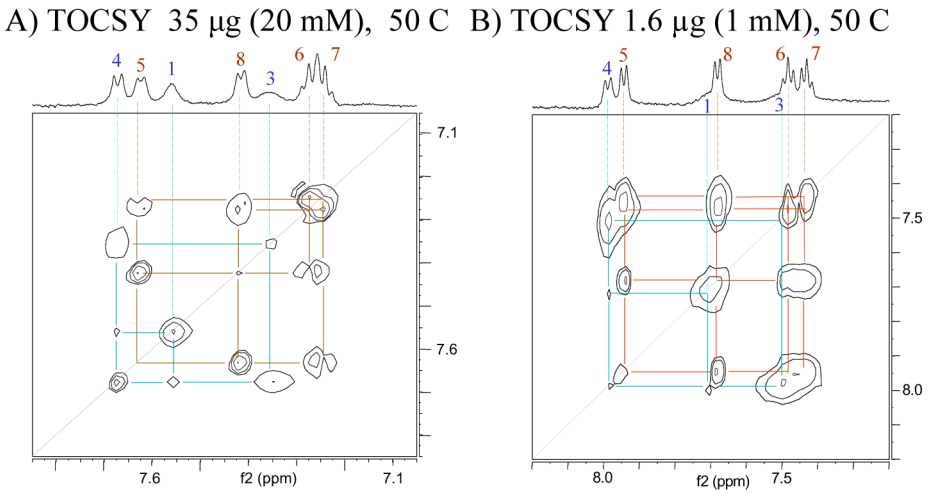

Figure 3. (A) Aromatic region of a TOCSY spectrum of 35 μg of AAF-dGMP in 3 μL (20 mM) at 50 °C. The 1D spectrum aligned above it consisted of 16 scans. (B) Aromatic region of the TOCSY spectrum of 1.6 μg of AAF-dGMP in 3 μL (1 mM) at 50 °C. The 1D spectrum aligned above consisted of 8K scans. Fluorene resonances are numbered as in Figure 1C.

comparison with the reference spectrum (Figure 2C) or for searching in a spectral database, and the two spectra are equivalent aside from the solvent and background peaks detected in the solvent blank (Figure 2A). The weakest peak of AAF-dGMP, at 6.4 ppm, has a S/N of 9.6 in the microcoil spectrum and 25 in the reference spectrum; the microcoil spectrum is of 1/488 the amount of material but was acquired for 195 longer times, so the effective mass sensitivity of the microcoil probe with a 1.5 μL sample is 13 times higher than that of the 5 mm tube, consistent with an expectation of 18 times higher within its 1 μL observed volume. The solvent peaks near 4.9 ppm (the OH moieties of water and methanol) vary among the three spectra because of different amounts of H_2O in the samples and because solvent suppression (presaturation) was applied to spectra in panels B and C. The peaks for the DNA adduct are broad (relative to sharp contaminant peaks, which indicate the shims were acceptable) because this adduct exists as a mixture of conformers that interconvert on the intermediate NMR time scale at room temperature, as described previously and discussed further below.[20,21] This explanation is confirmed in the reference spectra by resolving the conformers in slow exchange at −15 °C and merging them in fast exchange at 55 °C (inset of Figure 2C). Although the microcoil used in this 1D work does not have temperature control, newer microcoil probes in the field do, and such control can be important in optimizing resolution, as seen here with a conventional 5 mm probe.

It would be appropriate to demonstrate that the ability to acquire 2D NMR is commensurate with the 1D NMR sensitivity. For example, a common rule of thumb is that obtaining a S/N of 4 in a 1D spectrum indicates the minimal sample concentration and number of scans per increment for obtaining a COSY or TOCSY spectrum, under normal conditions. While AAF-dGMP was chosen to test the worst-case scenario of a bulky hydrophobic adduct in dissolution and sample transfer, this compound presents even greater challenges for 2D NMR because of the chemical exchange noted above. Chemical exchange can dissipate 2D NMR signals if the lifetime of a species is shorter than the evolution time of the 2D pulse sequence. As discussed in detail by Evans, AAF-dGMP has slow rotation about two bonds, the guanyl−nitrogen bond and the amide bond, resulting in four resolved conformations when exchange is slowed by the sample being cooled to −50 °C.[20] A single set of resonances is observed on heating to 50 °C, although the broadness of the lines indicates it is still in "intermediate exchange", where the exchange rate perturbs NMR spectra. Chemical exchange in NMR is a deep topic, but the results here can be clarified with just one of its conclusions.[25] The "fast exchange" regime is that in which multiple conformations present as a single sharp resonance, like most pH equilibria or bond rotations, and the average structure has a long NMR relaxation time T_2*. The transition between intermediate exchange and fast exchange is different for each proton and depends on how many hertz apart the resonances of the resolved conformations are at −50 °C, called $\Delta\delta$. In the 1D spectra at 50 °C of Figure 3A, the sharper lines (6 Hz line widths) have a $\Delta\delta$ on the order of 20 Hz; the broader F1 and F3 lines (60 and 80 Hz line widths) have $\Delta\delta$ values of >80 Hz.

To determine which 2D sequences would work with AAF-dGMP under its exchange conditions, a preliminary survey of common 2D NMR spectra was made at a relatively high concentration (35 μg in 3 μL, 22 mM) so sample data sets could be acquired quickly. An overnight acquisition afforded COSY (1 h), TOCSY (2.5 h), DQCOSY (1 h), NOESY (4 h), and ROESY (5 h) spectra. At best, partial spectra were observed: what cross-peaks were seen were only from resonances with $\Delta\delta$ values of <40 Hz, at the ends of the ribose and AAF moieties, farthest from the slowly rotating bonds. The TOCSY spectrum was able to elucidate the full spin systems of the AAF moiety (Figure 3A). Although a cross-peak was not observed directly between the broadened F1 and F3 protons, the meta coupling between them could be observed through the sharper F4 proton.

To acquire a TOCSY spectrum near the limit of detection, we calculated that 1.6 μg in 3 μL (1 mM) would provide a S/N of 4 for the 6 Hz wide aromatic protons in 64 scans. This spectrum was acquired (18 h) and is shown in Figure 3B. Despite the F1 and F3 protons becoming more broadened, relative to that at 20 mM, and moving under the F8 and F6 resonances, this spectrum was also able to resolve all cross-peaks that could be observed at higher concentration (Figure 3A). The changes in F1 and F3 between the 1D spectra at different concentrations indicate intermolecular associations are an additional challenge in studying this adduct, likely because of

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aromatic stacking of the AAF and/or dG moieties in aqueous solvent altering equilibria between the multiple conformations. (Note also the midpoint of the aromatic cluster has shifted by 0.25 ppm.) The amount of material used to acquire the TOCSY spectrum was confirmed by UV absorption of the recovered sample. The sample was recovered by back flushing it into a clean tube, followed by a clean solvent plug (that had been injected prior to the sample for this purpose of rinsing probe surfaces during recovery). Comparing UV measurements of the recovered sample to those of the stock solution revealed a recovery of 98%.

The sensitivity upon acquisition of the TOCSY spectrum was consistent with that observed in the 1D spectrum at the limit of detection. With 1.6 μg, the S/N of the aromatic region was 5 in 64 scans. The 80 ng spectrum above gave a S/N of 10 in 100K scans, which should give a 35-fold better S/N than 64 scans; 1.6 μg is 20 times more material than 80 ng. A limit of detection of 1.6 μg for a TOCSY spectrum is actually ~5-fold higher than what would be expected for a microcoil using droplet loading, or a microcryo probe. However, here the lines were >6 Hz wide, with relaxation times of ≤50 ms. The limit of detection would likely be at 5-fold lower concentration for an analyte with a favorable 1 Hz line width.

The work described above shows how a small amount of a DNA adduct can be transferred into a microcoil probe with negligible losses and how 1D or 2D NMR spectra can be acquired. Other concerns about identifying an *in vivo* DNA adduct (the most difficult case as a reference point) are discussed below. These include the amount of tissue that would have to be extracted, the integration of NMR with other analytical techniques during purification, further discussion of the scale-up required to obtain NMR beyond 1D, and the aspect of balancing the effort and cost of scale-up with that of obtaining the highest NMR sensitivity. For an adduct formed *in vitro* (where the level of exposure typically can be increased to form more adduct), the task in general would be much easier than what is presented below.

On the basis of ^{32}P postlabeling, many adducts are at the attomole to femtomole level in a few micrograms of DNA.[22] Obviously, it would be essential to isolate an adduct at this level from much more DNA. Issues with chemical noise and recovery would be critical. To obtain 100 pmol of a DNA adduct present in DNA at a level of one adduct per 10^7 nucleotides, for example, one would need ~1.5 g of DNA, assuming a 20% yield of the adduct. In turn, this would require starting with ~1 kg of tissue. Clearly, this would be a daunting project, perhaps comparable in magnitude and effort to the experiments conducted many years ago to identify hormones. No doubt, successive rounds of HPLC on different columns would be central to the purification, using the detection technique that discovered the adduct in the first place to follow the adduct of interest. Ideally, the latter technique would be mass spectrometry (MS); for example, an MS technique has been reported that can discover unknown adducts and partially characterize them at the femtomole level, furnishing accurate mass values for the molecular ion and also mass values for the fragment ions, based on an isotopologue mass-tagging method.[23,24] Because NMR cannot selectively analyze a low-level analyte in a mixture as well as MS can, the ability to obtain microscale NMR locally would be important in guiding the later stages of the purification strategy in response to the NMR interference observed, until an interpretably clean spectrum could be acquired. However, once an adduct is purified, travel to a facility with a microcryo or HTS probe would be justified if it offered even incremental sensitivity gains for multi-day acquisitions. Additionally, samples in tubes are easily recovered and reinserted, and it is likely that a precious and labile sample may need successive acquisition of additional NMR experiments.

In determining the structure of a novel adduct, MS, aside from a potential discovery role, would be the first technique to employ, contributing structural information especially via fragmentation data. 1D NMR would then provide complementary information, for example, the sites of addition to an aromatic ring. This would likely lead to hypothetical structures or partial structures for the adduct and define specific questions for targeted structural analysis. Determining chiral centers by a nuclear Overhauser effect could require 10−30-fold more material than a 1D experiment; long-range proton−carbon correlations, by means of heteronuclear single-quantum coherence/heteronuclear multiple-bond correlation, could require an additional 10−30-fold more. If additional scale-up were needed, having 1D NMR and MS data can show that an adduct, discovered at a trace level *in vivo*, is identical to an adduct present at a higher level in an alternate source, such as an *in vitro* sample subjected to an exposure that gives the adduct.

The effort and cost of scale-up of an adduct would in practice be balanced against that of obtaining higher NMR sensitivity. In principle, NMR sensitivity might be increased as much as 7-fold beyond what is reported here, reducing the amount of tissue required from 1 kg (see above) to ~150 g. Increasing the field strength from 500 MHz (used here) to a 900 MHz magnet would provide a 2.8-fold boost in sensitivity, if the instrument were outfitted with a similar microcoil detector. A microcryo or HTS probe would give comparable or slightly higher mass sensitivity. The highest mass sensitivity would come from a small microcoil: a 360 μm microcoil should give 2.5 times better mass sensitivity than a 1 mm microcoil, although it would require concentrating the sample to 50 nL at ~1 mM (in contrast with 1.5 μL at 0.1 mM tested here).[5,6,8] Whatever balance is chosen between the efforts of scale-up and NMR sensitivity, a prerequisite to accessing or developing more sensitive NMR would be demonstrating that the purity and quantity would be adequate for the proposed NMR studies.

■ CONCLUSION

Modern microscale NMR can play an important role in determining the structures of DNA adducts isolated from biological sources. The efficient sample handling technique described here for microcoil NMR in which a small aqueous volume (1.5 μL), containing a DNA adduct in nucleotide form at the picomole level (for 1D ^{1}H NMR) or nanomole level (2D NMR), is completely picked up and transferred from a vial as a microdroplet in a fluorocarbon carrier liquid can be performed with readily available equipment. The 3-fold improvement in sample efficiency is comparable to the advantage of a cryoprobe over a conventional probe and makes a microcoil probe as sensitive as a microcryo probe, allowing a 1D ^{1}H NMR spectrum to be obtained on <100 ng in a local facility without such specialized instrumentation.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

FC43, Fluorinert; AAF-dGMP, *N*-(2′-deoxyguanosin-8-yl)-2-acetylaminofluorene 5′-monophosphate; perfluorooctylsilane or PFOS, tridecafluoro-1,1,2,3-tetrahydrooctyl-triethoxysilane; COSY, correlation spectroscopy; TOCSY, total correlation spectroscopy; DQCOSY, double-quantum-filtered COSY; NOESY, nuclear Overhauser enhancement spectroscopy; ROESY, rotating-frame nuclear Overhauser effect

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