Observation of ^{18}O solvent-induced isotope shifts in ^{19}F NMR signals

John R. P. Arnold*[a]† and Julie Fisher[b]

[a] *School of Biology, University of Leeds, Leeds, UK LS2 9JT*
[b] *School of Chemistry, University of Leeds, Leeds, UK LS2 9JT*

$H_2{}^{18}O$ solvent-induced isotope shifts, which have differing dependence on the level of ^{18}O-enrichment, have been observed in the ^{19}F NMR signals of F^- and 5-fluorouridine, but are absent in 5-fluorouracil.

The chemical shift of an ^{19}F NMR signal is highly sensitive to its environment, and as such it is well-suited to the observation of the solvent-induced isotope shift (SIIS). This is the shift observed when the solvent is changed to a heavier isotope form, usually by an H to D substitution, and most commonly this is a change from H_2O to D_2O.[1] Fluorine-modified amino acids and heterocyclic bases have been incorporated into biomolecules, and subsequent H_2O/D_2O SIIS measurements have enabled evaluation of the solvent exposure of specific residues in proteins[2,3] and nucleic acids.[4–7] An alternative heavy isotope form of water is $H_2{}^{18}O$. Thirty years ago a study of NaF, made without the benefit of today's high magnetic fields and FT techniques, gave the $H_2{}^{16}O/H_2{}^{18}O$ SIIS (herein the ^{18}O-SIIS) as being '< 0.05 ppm' upfield.[8] The origin of the fluorine H_2O/D_2O SIIS has been considered in terms of hydrogen bonding with the solvent and the different vibrational behaviour of H_2O and D_2O.[9] It has been calculated that an OH bending vibration can account for about 30% of the H_2O/D_2O SIIS observed for F^-.[10] Lower frequency librational modes are also potential contributors to the SIIS, but a failure to detect any ^{18}O-SIIS for 50 mM F^- led to the conclusion that these librational modes do not play a significant role in the SIIS of fluorine.[9] Here we report a re-examination of the ^{18}O-SIIS of fluorine.

We investigated the fluoride ion, which has been used in a number of previous studies of ^{19}F SIIS,[1,8–10] together with 5-fluorouridine and 5-fluorouracil, which represent subunits commonly used to incorporate fluorine into nucleic acids to probe structure and function, as well as being important anti-cancer chemotherapy agents.[11] The experimental arrangement placed fluoride, fluorouridine and fluorouracil in the same sample, so that the solution conditions for each were identical.‡ With the ^{18}O enrichment at 86%, the fluoride and fluorouridine resonances both displayed small but distinct upfield shifts, whilst fluorouracil showed no such effect (Fig. 1 and Table 1).§ On reducing the ^{18}O enrichment to 53%, the fluoride SIIS was reduced whilst the fluorouridine SIIS was *downfield*; with fluorouracil still showing no isotope shift. A further decrease in ^{18}O enrichment to 43% reduced the isotope shifts of F^- and of fluorouridine (still downfield). Further significant reduction of the ^{18}O content was impractical due to the difficulty in measuring the diminishing isotope shifts. Thus the effect of ^{18}O enrichment of the solvent water for F^- is an upfield shift proportional to the level of ^{18}O enrichment (within experimental error). For fluorouridine the effect is more complicated, but can be explained by the observed isotope shift being the sum of one term which is upfield and proportional to the ^{18}O content, and a second term which is downfield and non-linear. The change from an upfield to a downfield isotope shift with decreasing ^{18}O enrichment of the solvent meant that at some point the ^{18}O-SIIS would be zero. This behaviour is illustrated by Fig. 2, where at 66% ^{18}O, the ^{18}O-SIIS was not detectable (*i.e.* being less than the linewidth). Both solvent-induced and intra-molecular isotope shifts are generally upfield on heavy atom substitution.[1] However, a few cases of downfield isotope shifts have been reported,[1,12] and other than where noted above, we know of no previous reports of ^{18}O-induced isotope shifts of ^{19}F, nor of ^{18}O-SIIS for any other NMR-active nucleus.

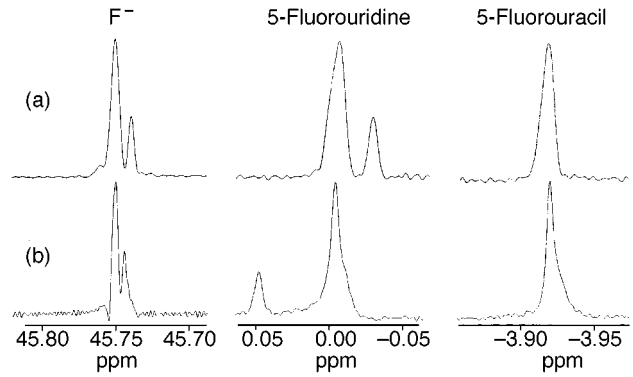

Fig. 1 ^{19}F NMR spectra (^1H-decoupled) of fluoride ion (left), 5-fluorouridine (centre) and 5-fluorouracil (right). Smaller signals are due to ^{18}O-induced shifts. (a) ^{18}O-enrichment in central chamber of concentric tube was 86%, solute concentrations 4 mM fluoride, 1 mM for both fluorouridine and fluorouracil. (b) ^{18}O-enrichment 53%, concentrations 30 mM fluoride, 1 mM for both fluorouridine and fluorouracil.

Table 1 SIIS values for fluoride, 5-fluorouridine and 5-fluorouracil‡§

Solvent system	Concentrations[a]/mM	Enrichment[b] (%)	SIIS[c] (ppm) F^-	5-Fluorouridine	5-Fluorouracil
$H_2{}^{16}O/H_2{}^{18}O$	4, 1, 1	86	−0.0120	−0.0227	0
$H_2{}^{16}O/H_2{}^{18}O$	4, 1, 1	53	−0.0068	+0.0089	0
$H_2{}^{16}O/H_2{}^{18}O$	4, 1, 1	43	−0.0060	+0.0080	0
$H_2{}^{16}O/H_2{}^{18}O$	30, 1, 1	53	−0.0056	+0.050	0
$H_2{}^{16}O/H_2{}^{18}O$	50, 1, 1	53	−0.0040	+0.044	0
H_2O/D_2O	4, 1, 1	100	−2.771	−0.243	−0.199
H_2O/D_2O	50, 1, 1	100	−2.679	−0.255	−0.190

[a] In the order of fluoride ion (as NaF), 5-fluorouridine, 5-fluorouracil, present in the same sample. [b] Enrichment of the heavier-isotope solvent in its chamber of the concentric NMR tubes. [c] Negative values indicate upfield shift for heavier-isotope solvent, positive for downfield shift. ^{18}O-SIIS values have estimated error span of ±0.0005 ppm. H_2O/D_2O SIIS estimated error margin ±0.001 ppm.

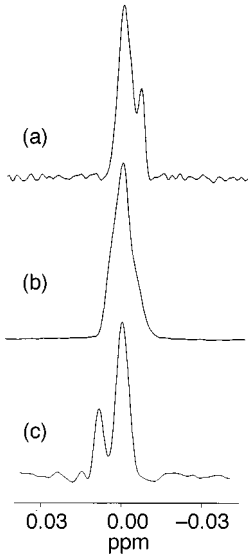

Fig. 2 ^{19}F NMR spectra (^{1}H-decoupled) of 5-fluorouridine (5 mM) with varying ^{18}O-enrichment in the central chamber of concentric tubes: (a) 87, (b) 66 and (c) 44%. ^{18}O-SIIS values are −0.0060, 0 and +0.0084 ppm respectively (all ± 0.0005 ppm).

In order to approach the conditions used in a previously reported study[9] (*i.e.* 50 mM NaF), we increased the flouride ion concentration from 4 to 30 and then 50 mM, whilst using 53% ^{18}O. With this increasing concentration, the SIIS of fluoride ion decreased in an approximately linear fashion so that it was only 0.004 ppm at 50 mM. The combination of lower magnetic field strengths and relatively high fluoride concentration would thus account for the previous negative results.[9] Increasing the fluoride ion concentration also made the SIIS of fluorouridine significantly more pronounced, but did not cause any change to the absence of a perceptible ^{18}O-effect for the resonance of fluorouracil (Table 1 and Fig. 1).

The H_2O/D_2O SIIS values are also given in Table 1; those for F^- are in fair agreement with previous reports.[1,8,10] Values for fluorouridine and fluorouracil have not previously been reported, but 0.39 ppm has been given for closely related 2'-deoxy-5-fluorouridine[6] for 10–100% D_2O (since seperate tubes were used) under different sample conditions. Our value for fluorouridine is some 25% larger than that for fluorouracil. This may be related to the absence of an observable ^{18}O-SIIS for the latter, if the internal vibrations of solvent water make a stronger contribution to the ^{19}F chemical shift of fluorouridine than to that of fluorouracil. Alternatively, for fluorouracil, upfield and downfield contributions to the ^{18}O-SIIS may more consistently balance than would seem to be the case in fluorouridine. Pyrimidine bases have been noted to weakly self-associate in aqueous solution,[13] but with the relatively low concentrations used here the effect of this should be small (*i.e.* in the order of 0.1% of the molecules being associated, based on typical association constants[13]).

We have shown that an ^{18}O-SIIS for ^{19}F is detectable, and can be of relatively significant magnitude. Thus the librational motions within the water molecule cannot be discounted from contributing to the fluorine chemical shift, but clearly other terms are also involved, at least for fluoropyrimidines. The ^{19}F ^{18}O-SIIS may prove to be a useful complement to the H_2O/D_2O SIIS in the study of fluorine-labelled proteins and nucleic acids.

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Notes and references

† E-mail: genjrpa@leeds.ac.uk

‡ Samples for NMR analysis were prepared as follows: a solution of NaF (4 mM), 5-fluorouridine (1 mM), 5-fluorouracil (1 mM), and EDTA (0.1 mM) in 25 mM sodium phosphate buffer, pH 6.8 was divided into portions which were then lyophilised to dryness. The residues were then re-dissolved in appropriate volumes of 90% $H_2{}^{16}O$/10% D_2O or 90% $H_2{}^{18}O$/10% D_2O. In this way the ^{16}O and ^{18}O portions contained identical concentrations of all solutes. The solutions were placed in an NMR tube containing a concentric insert, with typically 80 µl of ^{18}O-enriched sample in the central chamber, and 330 µl of ^{16}O sample in the outer part. An ^{18}O-shifted signal is thus identified by its size compared to the ^{16}O resonance. Reduction of the ^{18}O enrichment was achieved by diluting with the ^{16}O solution. The concentration of NaF was increased by evaporating to dryness appropriate volumes of NaF solution in H_2O, and then redissolving these in the ^{18}O and ^{16}O solutions. For measurements of H_2O/D_2O effects, lyophilised solutions were redissolved in either H_2O or D_2O, and placed in concentric NMR tubes.

§ All ^{19}F NMR spectra were acquired on a GE-Omega 500 spectrometer at 470.5 MHz using a 5 mm ^{19}F/^{1}H probe. Broadband ^{1}H decoupling was applied by waltz-16 modulation of the decoupler. All spectra were acquired with a sample temperature of 25 °C. FIDs were multiplied by Gaussian functions and zero-filled prior to Fourier transformation. The digital resolution of the ^{19}F NMR spectra were at least 0.12 Hz (0.00026 ppm) per point. Chemical shifts are shown with respect to 5-fluorouridine (in the lighter isotope solvent) as the internal reference.

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