Comparitive Sensitivity of a pH-sensitive Spin Probe in Natural and Isotopically-Substituted States

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Introduction

The spin probe 2,2,3,4,5,5-hexamethyl imidazolidine-1-yloxy (here called HMI) when examined by ESR is pH sensitive with a pK of 4.6. This arises from the difference in spectral width of the 3-line, •NO-derived spectrum depending upon whether the 3-N is protonated (low pH) or not (high pH). When low-frequency ESR-based techniques are used HMI can be detected *in vivo* in the rat stomach, where it responds to the local pH [1,2]. Although HMI toxicity is low, at high concentrations the ESR spectra are disturbed due to various proton exchanges hence low doses (concentrations) of HMI give better pH evaluation but poorer signal-to-noise. The purpose of this study, therefore, is to explore the use a totally perdeuterated, ^{15}N-substituted (in the 1-position) form of HMI (referred to as HMI-15) for low-frequency *in vivo* ESR pH measurement. The ^{15}N substitution provides a 2-line spectrum with increased peak height and perdeuteration reduces the width of the lines, hence increasing detection efficiency. The *in vivo* ESR-based techniques used in this study are FC-DNP [3] and LODESR [4]. To obtain more detailed information about the low-frequency ESR spectra a home-built RF-ESR system [5] was employed for *in vitro* studies.

Materials and Methods

HMI and HMI-15 were obtained from the Novosibirsk Institute of Organic Chemistry. Solutions of 5 and 2.5 mM concentration were made in water, with 15 ml of solution being used for *in vitro* LODESR and FC-DNP and 1 ml for RF-ESR. pH of the solution was adjusted with HCl or KOH as required. FC-DNP was performed using an ESR frequency of 120 MHz and and NMR frequency of 2.5 MHz. All experiments used a TR of 1200 ms, a 400 ms duration ESR pulse and an average power level of 16.6 W. The FC-DNP spectrum was collected in 64 steps over a field range of 0.002 to 0.006T. LODESR spectra were collected at 296 MHz with a modulation frequency of 280 kHz and an ESR power level of 4 W continuous during the irradiation period. The field sweep was from 0.0075 to 0.0125T. RF-ESR spectra from solutions were collected as 1024 points at 290 MHz with a field sweep from 0.0085 to 0.0120T. Male Sprague Dawley rats of body weight 280 to 300 g were starved overnight prior to intubation with 3.5 ml of a 5 mM solution of one of the spin probes. In some cases the probe was in a 0.1M sodium bicarbonate solution. The rats were then anaesthetised with intraperitoneal ketamine/xylazine prior to placement in the ESR systems.

Results and Discussion

Solutions: Table 1 shows details of spectra for solutions of HMI and HMI-15. Spectra from FC-DNP and LODESR were collected as absorption-type curves and RF-ESR as the first derivative. Values are, therefore, shown as base-to-peak heights and FWHM for the *in vivo* modalities and P/P height and width for RF-ESR. For comparison, the signal height values are normalised to the low-frequency HMI value for each modality (using the pH 7.7 value for RF-ESR). Comparison can be made between the HMI and HMI-15 values for each modality but not between modalities. As expected each modality, using fully unprotonated samples, shows a narrower overall spectral width from HMI-15 than from HMI. A reduction is also seen in the individual line widths from HMI-15, although the extent of this reduction is dependent upon measurement technique, being 33% and 44% for the low and high-field lines respectively for RF-ESR, 28% and 23% for LODESR and 17.5% and 9.%% for FC-DNP. In all cases an in signal height is observed from the ^{15}N-substituted compound although, once again, the effect is less for FC-DNP than the other modalities.

The two probes were examined in fully unprotonated and fully protonated states by RF-ESR. The expected pH-dependent decrease in spectral width, with relatively little effect on individual line width or peak height, was obtained for HMI. HMI-15 showed a similar phenomenon as far as spectrum and line widths were concerned but gave an increase in signal height as the overall spectral width reduced with acidification of the solution. This effect is being investigated.

In Vivo Studies: 3.5 ml of 5 mM HMI-15 solution gavaged into the stomachs of rats gave narrower spectra than those observed in the fully protonated HMI-15 solutions. For LODESR the *in vivo* spectrum had an overall width of 21.1 Gauss, compared with 22.8 G *in vitro* and for FC-DNP the *in vivo* width was 23 G compared with 24.5 G *in vitro*. If these reductions are compared with those seen in Table 1 between the RF-ESR spectra obtained at pH 6.9 (32.1 G) and pH 2.1 (29.6 G) the *in vivo* results indicate that HMI-15 responds to the low stomach pH in a similar way to HMI [1].

When the HMI-15 solution was made up in 0.1M sodium bicarbonate the spectra obtained *in vitro* by FC-DNP and LODESR were identical to those obtained from HMI-15 made up in water. Gavage with HMI-15 plus bicarbonate produced an increased overall spectral width when compared with gavage with the agent in water. The spectral width for LODESR (21.48 G) was less than that obtained *in vitro* from the fully unprotonated solution which may have been due to incomplete neutralizing of the stomach acid by the bicarbonate. The experiment does, however, demonstrate the capacity of HMI-15 to act as an *in vivo* pH meter.

Conclusions

In vitro the differences in spectral shapes and parameters between HMI and HMI-15 are as expected in light of their ^{14}N/^{15}N substitution. The narrowing of the individual lines due to perdeuteration was also observed, ranging 40% for RF-ESR to 12% for FC-DNP. This narrowing makes a significant contribution to the detectability of the agent by FC-DNP and LODESR and it is expected thatlower concentrations of HMI-15 than of HMI would yield results *in vivo*. HMI-15 shows similar pH sensitivity than HMI. The ability to work as a pH meter is improved by having a greater difference of spectral width between the protonated and unprotonated states of the spin probe. Previous experiments with HMI have shown that its range of narrowing is adequate for *in vivo* use and this remains true for HMI-15.

References

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Table 1 (all field widths in Gauss; RF=RF-ESR, DNP=FC-DNP, LOD=LODESR)

	pH	Full width (G)	Low field line width	Low field line signal	High field line width	High field line signal
HMI:						
RF	7.7	32.1	1.35	1.00	1.35	1.02
RF	2.5	29.6	1.30	1.02	1.20	1.01
DNP	7.7	34.0	2.30	1.00	2.10	1.31
LOD	7.7	32.1	2.20	1.00	2.15	0.92
HMI-15:						
RF	6.9	22.6	0.90	3.26	0.75	3.24
RF	2.1	20.7	0.70	4.85	0.70	4.76
DNP	6.9	24.5	1.90	1.81	1.90	2.31
LOD	6.9	22.8	1.58	2.25	1.65	2.25