Application of the $^{19}$F-Waterlogsy Type Experiment for NMR-Based Screening of Fluorinated Compounds

Kazuo Furihata1, Hiroaki Utsumi2, Toshiyo Kato2, Chiseko Sakuma3 and Mitsuru Tashiro*4

1Division of Agriculture and Agricultural Life Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
2JEOL RESONANCE Inc., Musashino, Akishima, Tokyo 196-8558, Japan
3School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Horinouchi, Hachioji, Tokyo 192-0392, Japan
4Department of Chemistry, College of Science and Technology, Meisei University, Hino, Tokyo 191-8506, Japan

Abstract

The WaterLOGSY type experiment with $^{19}$F detection was applied to observe the interaction between a fluorinated compound and a macromolecule. The proposed experiment, which was developed based on the $^{19}$F($^{1}$H) saturation transfer difference experiment, was carried out using the conventional spectrometer equipped with a single high band amplifier and a $^{1}$H/$^{19}$F-double tuned probe. The selective $^{19}$F detection is advantageous in screening the fluorinated compounds, considering that $^{19}$F is a sensitive nucleus in NMR spectroscopy. The effective approach to discriminate binding of the fluorinated compounds to proteins with $^{19}$F detection was demonstrated using the complex of diflunisal and human serum albumin.

Keywords: $^{19}$F-NMR; NMR-based screening; Fluorinated compound; WaterLOGSY

Introduction

NMR spectroscopy has been utilized as a useful method for analyzing macromolecular complexes and screening compounds with affinity to target macromolecules. Various NMR-based screening methods have been developed using chemical shift perturbation [1-3], transferred NOE [4,5] and diffusion and relaxation editing methods [6,7]. In the development of NMR techniques for screening compounds, one of the essential requirements is the selective detection of the ligand signals while suppressing signals from target proteins. It has been shown that NOE-pumping [8], reverse NOE-pumping [9], saturation transfer difference (STD) [10] and water-ligand observed via gradient spectroscopy (WaterLOGSY) [11,12] experiments could directly detect bound ligands. These methods were designed to detect ligands with proton detection, and furthermore, the NMR-based methods have been extended to fluorine detection [13,14]. An incorporation of fluorine into drugs provides simultaneous modulation of electronic, lipophilic and steric parameters, indicating that inclusion of fluorine atoms in a drug molecule can alter its chemical properties and biological activities, and also influence the interaction with its target [15]. In analysis of fluorinated compounds, some useful NMR methods, which are applicable to the NMR-based screening, are required to be developed. Although $^{19}$F NMR spectroscopy is feasible to analyze the fluorinated compounds [16-19], the pervasive NMR spectrometer consoles consist of a single high and a few low band amplifiers, which are incapable of performing $^{1}$H-$^{19}$F heteronuclear experiments. In the recent study, an efficient technique to achieve $^{1}$H-$^{19}$F heteronuclear experiments using a conventional NMR spectrometer equipped with a $^{1}$H/$^{19}$F/$^{13}$C-double tuned probe was developed in our study [20]. Here, we propose an effective approach to discriminate binding of the fluorinated compounds to proteins with $^{19}$F detection, using the complex of diflunisal (Figure 1a) and human serum albumin (HSA).

Materials and Methods

Instrumentation and chemicals

All of the spectra were recorded at 20°C on a Varian 600 MHz NMR system or JEOL ECZ-400S spectrometer. Diflunisal, enoxacin (Figure 1b) and HSA were purchased from Sigma-Aldrich. Two NMR samples were prepared; (i) a 5 mm tube containing a solution of 4.0 mM diflunisal and 4.0 mM enoxacin, (ii) double NMR tubes, which comprised a 3 mm tube used as an inner tube containing 10 mM enoxacin and a 5 mm tube used as an outer tube containing 5.0 mM diflunisal and 0.1 mM HSA. The enoxacin solution, which was prepared in the absence of HSA, was used as a negative control. Each experiment contained 90% $^{1}$H2O and 10% $^{2}$H2O.

NMR spectroscopy

The experimental parameters of $^{19}$F($^{1}$H) STD experiment at $^{1}$H frequency of 600 MHz were as follows; data points=8192, spectral width of $^{19}$F=16026 Hz, number of scans=8192, d1=0.1 s, d2=1.5 ms, on-resonance frequencies of $^{1}$H=0.9 or 4.8 ppm, and off-resonance frequency=-20 ppm. In the reference $^{19}$F experiment, number of scans=8192, data points=8192, spectral width of $^{19}$F=16026 Hz, number of scans=8192, d1=0.1 s, d2=1.5 ms, on-resonance frequencies of $^{1}$H=0.9 or 4.8 ppm, and off-resonance frequency=-20 ppm. In the reference $^{19}$F experiment, number of scans=8192, data points=8192, spectral width of $^{19}$F=16026 Hz, number of scans=8192, d1=0.1 s, d2=1.5 ms, on-resonance frequencies of $^{1}$H=0.9 or 4.8 ppm, and off-resonance frequency=-20 ppm.

Received March 25, 2016; Accepted April 07, 2016; Published April 09, 2016

Citation: Furihata K, Utsumi H, Kato T, Sakuma C, Tashiro M (2016) Application of the $^{19}$F-Waterlogsy Type Experiment for NMR-Based Screening of Fluorinated Compounds. Pharm Anal Chem Open Access 2: 111. doi:10.4172/2471-2698.1000111

Copyright: © 2016 Furihata K, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Results and Discussion

Pulse sequence optimization

Share of one high band amplifier without circuitry changes enabled acquiring the 1H-19F heteronuclear spectra, although the pervasive NMR spectrometer, consisting of a single high and a few low band amplifiers, is incapable of performing 1H-19F experiments. In the present research, the 19F{1H} STD pulse sequence was modified to be applicable to the WaterLOGSY type experiment for screening of fluorinated ligands bound to protein. In comparison with the former type 19F{1H} STD pulse sequence (Figure 2a), the present pulse sequence was rather simplified as shown in Figure 2b. The consecutive 1F and 1H 90° pulses between the gradient pulses G1 were omitted, and a single 1H pulse at the low power was incorporated for the selective excitation of proteins. In the 19F{1H} STD experiment, two type of spectra were acquired depending on the 1H resonance of selective excitation. In the first experiment, the 1H resonance at 0.9 ppm, corresponding to the methyl region of protein, was selectively excited. During the subsequent process, the proton magnetization of the protein was transferred to fluorine of the ligand for detection. This experiment corresponds to the conventional STD experiment with 19F detection, which can also be acquired using a sample solution of 100% 2H2O. The 19F{1H} STD spectra acquired using the above pulse sequences are shown in Figure 3. The 19F chemical shifts of diflunisal were -112.5 and -115.0 ppm, and that of enoxacin was -129.4 ppm. Enoxacin in a 3 mm tube, corresponding to the inner tube, was used as a free ligand in the absence of HSA. Although the 1H resonance of a methyl group of enoxacin (1.4 ppm) was close to that of the selective excitation (0.9 ppm), an intensity of the 19F signal of enoxacin resonating at -129.4 ppm in the 19F{1H} STD experiment was minute (Figure 3b). This result indicated that the 1H selective excitation at 0.9 ppm unexcited 19F of enoxacin and the 19F signals of diflunisal bound to HSA were selectively detected. The present optimization of the pulse sequence resulted in the increase of sensitivity by a factor of 1.7.

19F - WaterLOGSY type experiment

In the second experiment, the 1H resonance at 4.8 ppm, corresponding to the water resonance, was selectively excited. The 1H magnetization of water, which was transferred to the protein, was again transferred to the bound ligand via direct or indirect relay processes. The signals of enoxacin were expected to be observed with the opposite phase with respect to those of the bound ligand. This experiment is conceptually identical to the WaterLOGSY. In a sample solution without HSA, all 19F signals of diflunisal and enoxacin were observed with the same phase as the negative signals (Figure 4a). In the experiments using a sample of double NMR tubes, the 19F signals of diflunisal which bound to HSA and that of enoxacin as a negative control were observed as opposite phase (Figure 4b). The present methods are feasible to distinguish the bound molecules from the unbound molecules. In comparison of the conventional 19F spectra in the presence and absence of HSA, (Figure 4c and 4d), the signals of diflunisal, resonating at -112.8 and -115.0 ppm, were clearly broadened in the presence of HSA (Figure 4c), indicating the complex formation.

Figure 2: Pulse sequences of 19F{1H} STD experiment. (a) The former type pulse sequence and (b) the present 19F{1H} STD pulse sequence. The thin bars represent 90 degree pulses. All pulses were along x unless otherwise shown. The experimental parameters were: d1=0.1 s, d2=1.5 ms, G1=7.2 G/cm, gradient pulse width=2.0 ms, 1H soft pulse width in (b)=2.5 s. Phase cycling: φ1=π, x, -x, -x, x, y, -y, -y, y, φr=x, x, -x, -x, y, y, -y, -y.
Citation: Furihata K, Utsumi H, Kato T, Sakuma C, Tashiro M (2016) Application of the 19F-WaterLOGSY Type Experiment for NMR-Based Screening of Fluorinated Compounds. Pharm Anal Chem Open Access 2: 111. doi:10.4172/2471-2698.1000111

Figure 3: (a), (b) The $^{19}$F/$^1$H STD spectra acquired using the pulse sequences shown in Figure 2a and 2b, respectively. The $^1$H resonance at 0.9 ppm was selectively excited. Double NMR tubes, which comprised a 3 mm tube containing 10 mM enoxacin and a 5 mm tube containing 5.0 mM difflusional and 0.1 mM HSA, were used.

Figure 4: (a) The $^{19}$F/$^1$H STD spectrum and (d) the reference $^{19}$F spectrum acquired using a sample solution of 4.0 mM difflusional (●) and 4.0 mM enoxacin (●). (b) The $^{19}$F/$^1$H STD spectra and (c) the reference $^{19}$F spectrum acquired using double NMR tubes, which comprised a 3 mm tube containing 10 mM enoxacin and a 5mm tube containing 5.0 mM difflusional and 0.1 mM HSA. In (a) and (b), $^1$H resonance at 4.8 ppm was selectively excited.

Conclusion

It was demonstrated that the WaterLOGSY type experiment with $^{19}$F detection was an effective method to selectively detect the fluorinated compounds bound to macromolecules. The $^{19}$F detection was advantageous in setting the NMR experiments, because suppression of the protein signals before acquisition using the $T_2$ filter was unnecessary and sample solutions containing 10% $^2$H$_2$O can be used for the present experiments. Solvent exchange to 100% $^2$H$_2$O often causes loss of the valuable samples. The proposed approach for discrimination of binding is expected to be a useful NMR-based screening method applicable to the fluorinated compounds.

Acknowledgements

This work was supported by two Grant-in-Aids for Scientific Research (No. 20580108 for KF and 15K05550 for MT) from the Ministry of Education, Culture, Sports, Science, and Technology.

References

1. Chen Y, Reizer J, Saier MH, Fairbrother WJ, Wright PE (1993) Mapping of the binding interfaces of the proteins of the bacterial phosphotransferase system, HPf and IAglc. Biochemistry 32: 32-37.
2. Gronenborn AM, Clore GM (1993) Identification of the contact surface of a streptococcal protein G domain complexed with a human Fc fragment. J Mol Biol 233: 331-335.
3. Shuker SB, Hajduk PJ, Meadows RP, Fesik SW (1996) Discovering high-affinity ligands for proteins: SAR by NMR. Science 274: 1531-1534.
4. Meyer B, Weimar T, Peters T (1997) Screening mixtures for biological activity by NMR. Eur J Biochem 246: 705-709.
5. Shimizu H, Field RA, Homans SW, Donohue-Rolle A (1998) Solution structure of the complex between the B-subunit homopentamer of verocytotoxin VT-1 from Escherichia coli and the trisaccharide moiety of globotriaosylceramide. Biochemistry 37: 11079-11082.
6. Lin M, Shapiro MJ, Wareing JR (1997) Diffusion-edited NMR-affinity NMR for direct observation of molecular interactions. J Am Chem Soc 119: 5249-5250.
7. Hajduk PJ, Olejniczak ET, Fesik SW (1997) One-dimensional relaxation- diffusion-Edited NMR methods for screening compounds that bind to macromolecules. J Am Chem Soc 119: 12257-12261.
8. Chen A, Shapiro MJ (1998) NOE Pumping: A novel NMR technique for identification of compounds with binding affinity to macromolecules. J Am Chem Soc 120: 10258-10259.
9. Chen A, Shapiro MJ (2000) NOE Pumping. 2. A high-throughput method to determine compounds with binding affinity to macromolecules by NMR. J Am Chem Soc 122: 414-415.
10. Mayer M, Meyer B (2001) Group epitope mapping by saturation transfer difference NMR to identify segments of a ligand in direct contact with a protein receptor. J Am Chem Soc 123: 6108-6117.
11. Dalvit C, Pevarello P, Talò M, Veronesi M, Vulpetti A, et al. (2000) Identification of compounds with binding affinity to proteins via magnetization transfer from bulk water. J Biomol NMR 18: 65-68.
12. Dalvit C, Fogliatto GP, Stewart A, Veronesi M, Stockman BJ (2001) WaterLOGSY as a method for primary NMR screening: practical aspects and range of applicability. J Biomol NMR 21: 349-359.
13. Dalvit C, Fagerness PE, Hadden STA, Sarver RW, Stockman BJ (2003) Fluorine-NMR experiments for high-throughput screening: theoretical aspects, practical considerations, and range of applicability. J Am Chem Soc 125: 7696-7703.
14. Dalvit C, Flocco M, Stockman BJ, Veronesi M (2002) Competition binding experiments for rapidly ranking lead molecules for their binding affinity to human serum albumin. Comb Chem High Throughput Screen 5: 645-650.
15. Park BK, Kitteringham NR, O’Neill PM (2001) Metabolism of fluorine-containing drugs. Annu Rev Pharmacol Toxicol 41: 443-470.
16. Corcoran O, Lindon JC, Hall R, Ismail IM, Nicholson JK (2001) The potential of 19F NMR spectroscopy for rapid screening of cell cultures for models of mammalian drug metabolism. Analyst 126: 2103-2106.
17. Måsson M, Sigurgeirsdóttir JF, Jónsdóttir S, Loftsson T (2003) Examination of 19F-NMR as a tool for investigation of drug-cyclodextrin complexes. Drug Dev Ind Pharm 29: 107-112.
18. Shikii K, Sakurai S, Utsumi H, Seki H, Tashiro M (2004) Application of the 19F NMR Technique to Observe Binding of the General Anesthetic Halothane to Human Serum Albumin. Anal Sci 20: 1477-1479.
19. Vulpetti A, Hommel U, Landrumb G, Lewis R, Dalvit C (2009) Design and NMR-based screening of LEF, a library of chemical fragments with different local environment of fluorne. J Am Chem Soc 131: 12949-12959.
20. Sakuma C, Kurita J, Furihata K, Tashiro M (2015) Achievement of $^{1}$H-$^{19}$F heteronuclear experiments using the conventional spectrometer with a shared single high band amplifier. Magn Reson Chem 53: 327-329.