Progress in Nuclear Magnetic Resonance Spectroscopy 62 (2012) 1–33

Contents lists available at ScienceDirect

Progress in Nuclear Magnetic Resonance Spectroscopy

journal homepage: www.elsevier.com/locate/pnmrs

Current applications of ^{19}F NMR to studies of protein structure and dynamics

Julianne L. Kitevski-LeBlanc [a], R. Scott Prosser [a,b,*]

[a] *Department of Chemistry, University of Toronto, UTM, 3359 Mississauga Rd., North Mississauga, Ontario, Canada L5L 1C6*
[b] *Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A8*

A R T I C L E   I N F O

Article history:
Received 21 February 2011
Accepted 1 July 2011
Available online 13 July 2011

Keywords:
^{19}F NMR
Biosynthetic labeling
Fluorinated tags
Protein conformation
Protein dynamics

Contents

1. Introduction . . . 2
   1.1. Properties of the ^{19}F nucleus and its utility in protein NMR studies . . . 2
2. Experimental aspects . . . 2
   2.1. Labels . . . 2
   2.2. Tags . . . 3
   2.3. Aliphatic amino acid analogs . . . 5
   2.4. Aromatic amino acid analogs . . . 10
   2.5. Labeling protocols . . . 12
      2.5.1. Chemical modification . . . 12
      2.5.2. Peptide synthesis . . . 14
      2.5.3. Uniform biosynthetic incorporation . . . 14
      2.5.4. Site-directed biosynthetic labeling . . . 15
3. Experimental issues . . . 16
   3.1. CSA relaxation . . . 16
   3.2. Structural perturbations . . . 17
   3.3. Assignments. . . . 18
      3.3.1. Mutagenesis strategies . . . 18
      3.3.2. Additional experimental strategies . . . 18
      3.3.3. NMR-based assignments . . . 19
4. Applications . . . 20
   4.1. Inherent properties of the chemical shift . . . 20
   4.2. Solvent exposure . . . 20
      4.2.1. Photo-CIDNP . . . 20

Abbreviations: BFTA, 3-bromo-1,1,1-trifluoroacetone (BTFA); CIDNP, Chemically Induced Dynamic Nuclear Polarization; COSY, correlation spectroscopy; CPMG, Carr Purcell Meiboom Gill; CD, circular dichroism; CSA, chemical shift anisotropy; DAGK, diacyl glycerol kinase; HCN, ^{1}H, ^{13}C, and ^{15}N; HMQC, heteronuclear multiple-quantum correlation; HSQC, heteronuclear single-quantum correlation; NMR, nuclear magnetic resonance; NOE, nuclear overhauser effect; NOESY, nuclear overhauser effect spectroscopy; PFP, 4-(perfluoro-*tert*-butyl)phenyliodoacetamide; 1D, one-dimensional; 2D, two-dimensional; SIIS, solvent induced isotope shifts; TOCSY, total correlation spectroscopy; TFET, 2,2,2-trifluoroethanethiol; TFASAN, trifluoroacetamidosuccinic anhydride; SETFA, S-ethyl-trifluorothioacetate.

* Corresponding author at: Department of Chemistry, University of Toronto, UTM, 3359 Mississauga Rd., North Mississauga, Ontario, Canada L5L 1C6. Tel.: +1 905 828 3802; fax: +1 905 828 5425.

E-mail address: scott.prosser@utoronto.ca (R.S. Prosser).

0079-6565/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.pnmrs.2011.06.003

4.2.2. Solvent Induced Isotope Shift . . . 20
4.2.3. Paramagnetic shifts . . . 22
4.2.4. The combination of shift measurements . . . 24
4.3. Heteronuclear NOEs . . . 26
4.4. Dynamics . . . 27
5. Conclusions and future directions . . . 29
Acknowledgements . . . 31
References . . . 31

1. Introduction

While traditional ^{1}H, ^{13}C, and ^{15}N (HCN) solution state NMR protocols provide a wealth of data on protein structure and dynamics, ^{19}F NMR provides a unique perspective of conformation, topology and dynamics and the changes that ensue under biological conditions. In particular, ^{19}F NMR has provided insight into biologically significant events such as protein folding and unfolding [1–3], enzymatic action [4–6], protein–protein [7], protein–lipid [8,9] or protein ligand [10,11] interactions as well as aggregation and fibrillation [12,13] in both soluble and membrane protein systems [14–16]. ^{19}F NMR applications are also often used to complement other biophysical techniques, such as circular dichroism (CD), fluorescence, X-ray crystallography and HCN NMR. In some cases, topological or dynamic information can be obtained from ^{19}F NMR studies in systems too large or unstable for full structural analysis. In the arena of dynamics, the large chemical shift range associated with the fluorine nucleus provides experimental access to additional motional timescales in line shape and T_2 relaxation studies. Even minor conformational changes, which may be undetectable using HCN two-dimensional (2D) approaches, can be discerned using simple one-dimensional (1D) ^{19}F NMR approaches. The development of ^{19}F reporters and fluorinated amino acid analogs has also experienced a resurgence. The range of probes and labeling strategies now available offer the possibility of selective labeling via site-directed approaches or uniform biosynthetic labeling with high yield and purity. With recent and ongoing advances in NMR cryogenic probe technology, multidimensional NMR assignment, and labeling approaches, it would seem that ^{19}F NMR may offer still greater possibilities in studies of protein structure and dynamics.

In this review, we focus on recent progress in the application of solution state ^{19}F NMR to studies of protein structure, dynamics, and function, building upon the seminal work by others extending from the 1970s to the late 1990s [17–22]. Details regarding the application of fluorine in solid state biomolecular NMR have been covered recently [23]. We will begin by introducing the basic features of fluorine NMR, followed by a survey of the range of fluorinated labels currently in use, and a review of sample preparation procedures. We will then address some of the common ^{19}F NMR approaches used in protein studies and the concomitant difficulties associated with assignment, line broadening, and labeling. Finally we highlight several recent results, which make use of these approaches in studies of protein folding, and ligand binding.

1.1. Properties of the ^{19}F nucleus and its utility in protein NMR studies

The fluorine nucleus is a spin-½ species, which exits in 100% natural abundance and possesses a magnetogyric ratio that is 83% that of the proton. The large magnetogyric ratio translates into both high sensitivity in 1D ^{19}F NMR spectroscopy, and strong dipolar couplings, allowing for the measurement of ^{19}F–^{19}F and ^{19}F–^{1}H NOEs for distance restraints [24] and the study of topology and contact with solvent [25–27]. The high sensitivity coupled with the virtual absence of background fluorine signal is advantageous in studies of protein complexes or *in vivo* applications, where signal intensity can be severely attenuated.

Perhaps the most useful attribute of ^{19}F NMR is the inherent sensitivity of the fluorine chemical shift to its local environment. The fluorine chemical shift is primarily influenced by a large paramagnetic term, making it exquisitely sensitive to local van der Waals interactions and electrostatic fields [17]. The position of a ^{19}F resonance relative to the corresponding denatured chemical shift position correlates well with burial in proteins and even slight differences in the dielectric properties of H_2O and D_2O manifest themselves as different ^{19}F chemical shifts for water exposed ^{19}F probes. The range of chemical shifts in proteins of both fluoroaliphatic and fluoroaromatic probes, as a function of environment, is consequently $\sim$100 times larger than that of the corresponding ^{1}H nuclei, providing a sensitive means of studying conformational change often without the need to resort to 2D NMR approaches to achieve separation of resonances. The 100-fold increase in chemical shift dispersion has another advantage in studies of dynamics, since frequencies are likely to be modulated to a much greater extent, making it easier to monitor weak binding, folding, enzyme kinetics, and conformational exchange, and the related physical and thermodynamic properties [28–30]. Moreover, the inclusion of paramagnetic additives can render the fluorine chemical shift even more sensitive to local environment in some cases. For example, water soluble or hydrophobic paramagnetic shift reagents may be added to determine topological information such as solvent exposed surface area and hydrophobicity [31,32].

Fluorine probes are incorporated into proteins in a variety of ways, as described below. In general, the substitution of a native amino acid with a fluorinated variant, or chemical modification using a fluorinated tag, is weakly perturbing to the global structure and function of the protein [25,33,34], or can be made so by fractional labeling [35]. While the van der Waals radius of a fluorine atom is only 20% larger than hydrogen, fluorine–fluorine interactions are reinforced by the fluoro-stabilization effect and introducing multiple fluorine atoms may substantially perturb water interactions. While these effects can generally be ameliorated by reducing the fraction of fluorine, there are examples where even monofluorinated variants alter native protein characteristics [34,36]. We therefore devote a considerable portion of this review to current ^{19}F labeling schemes in addition to straightforward approaches to both assessing structural and functional perturbations, and minimizing deleterious effects.

2. Experimental aspects

2.1. Labels

Fluorine probes can be incorporated into proteins via one of two ways: (1) biosynthetic, wherein the expression medium is supplemented with a fluorinated amino acid analog, or (2) chemical modification, where a fluorinated moiety is reacted with a given residue or protein site. Progress with unnatural amino acids, most notably fluorinated phenylalanine variants, has made it possible to achieve site-specific fluorine labeling by biosynthetic means with

Download English Version:

https://daneshyari.com/en/article/5419607

Download Persian Version:

https://daneshyari.com/article/5419607

Daneshyari.com