Progress in Nuclear Magnetic Resonance Spectroscopy 92–93 (2016) 54–70

Contents lists available at ScienceDirect

Progress in Nuclear Magnetic Resonance Spectroscopy

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

How to tackle protein structural data from solution and solid state: An integrated approach

Azzurra Carlon [a], Enrico Ravera [a], Witold Andrałojć [a], Giacomo Parigi [a], Garib N. Murshudov [b], Claudio Luchinat [a,*]

[a] Magnetic Resonance Center (CERM) and Department of Chemistry "Ugo Schiff", University of Florence, Italy[1]
[b] MRC Laboratory for Molecular Biology, Francis Crick Ave, Cambridge CB2 0QH, UK

Edited by David Neuhaus and Gareth Morris

ARTICLE INFO

Article history:
Received 15 September 2015
Accepted 13 January 2016
Available online 21 January 2016

Keywords:
Paramagnetic restraints
Residual dipolar couplings
Pseudo-contact shifts
Structural refinement
Integrated structural biology

ABSTRACT

Long-range NMR restraints, such as diamagnetic residual dipolar couplings and paramagnetic data, can be used to determine 3D structures of macromolecules. They are also used to monitor, and potentially to improve, the accuracy of a macromolecular structure in solution by validating or "correcting" a crystal model. Since crystal structures suffer from crystal packing forces they may not be accurate models for the macromolecular structures in solution. However, the presence of real differences should be tested for by simultaneous refinement of the structure using both crystal and solution NMR data. To achieve this, the program REFMAC5 from CCP4 was modified to allow the simultaneous use of X-ray crystallographic and paramagnetic NMR data and/or diamagnetic residual dipolar couplings. Inconsistencies between crystal structures and solution NMR data, if any, may be due either to structural rearrangements occurring on passing from the solution to solid state, or to a greater degree of conformational heterogeneity in solution with respect to the crystal. In the case of multidomain proteins, paramagnetic restraints can provide the correct mutual orientations and positions of domains in solution, as well as information on the conformational variability experienced by the macromolecule.

Contents

* Corresponding author at: Via L. Sacconi 6, 50019 Sesto Fiorentino (FI), Italy. Tel.: +39 0554574296; fax: +39 0554574924.
E-mail addresses: carlon@cerm.unifi.it (A. Carlon), ravera@cerm.unifi.it (E. Ravera), andralojc@cerm.unifi.it (W. Andrałojć), parigi@cerm.unifi.it (G. Parigi), garib@mrc-lmb.cam.ac.uk (G.N. Murshudov), claudioluchinat@cerm.unifi.it (C. Luchinat).
[1] URL: http://www.cerm.unifi.it/.

http://dx.doi.org/10.1016/j.pnmrs.2016.01.001
0079-6565/

1. Introduction

The most widely used techniques for elucidation of molecular structures at atomic resolution are X-ray crystallography and NMR spectroscopy, accounting as of July 2015 for 90% and 9.4% of all deposited protein structures, respectively, and 58% and 41% of all deposited nucleic acid structures. Besides new experimental techniques gaining more and more resounding success (e.g. cryo-EM, with more than 390 new entries in the last 3 years and resolution down to 2.2 Å [1]), X-ray and NMR still play a key role in answering many unresolved questions in the structural biology field. The unique importance of the integration of these two techniques has been recognized, taking advantage of the distinctive features of each. The strength of X-ray crystallography lies in a precise determination of a unique macromolecular structure (or a unique set of different structures present in the asymmetric unit of the crystal), whereas NMR spectroscopy has the power to probe the dynamics experienced in solution. On the other hand, neither X-ray nor NMR, if used as stand-alone tools, can provide a complete, precise and accurate picture of the biological system under investigation and of its interactions with other complexes or biomolecules. It also should be mentioned that X-ray crystallographic diffraction and NMR data are intrinsically different – the former gives information that progresses from the overall shape of the molecule up to individual atom positions as the resolution increases, whereas NMR provides immediate information about short-range inter-atom distances and bond orientations, which progresses to overall shape of the molecule with increasing number and quality of restraints. Therefore, the two techniques are highly complementary, because the combination of the two yields valuable information throughout the spectrum of distance scales, even in the presence of suboptimal X-ray and/or NMR data.

Despite its great success, there are some intrinsic limitations of X-ray crystallography: molecules in crystals experience crystal packing forces that may change their conformation and/or reduce conformational heterogeneity. NMR data are usually very accurate, but the collection of a large number of long-range interatomic distances is often very difficult, resulting in a lower precision of the NMR model with respect to the X-ray structure. Furthermore, NMR restraints are usually too few for solving molecular structures without strongly relying on prior knowledge defined by geometrical constraints based on covalent bonding. Therefore, it has long been known that X-ray and NMR data provide complementary information, which can be profitably analysed together for a more accurate description of biomolecules. Moreover, the complementarity of X-ray and NMR resides in the different types of information provided by these techniques, since X-ray relies mostly on the contribution given by the heavy atoms to the electron diffraction pattern, while for NMR the vast majority of restraints involve the hydrogen nuclei. Even more importantly, as anticipated above, at low and medium resolution, X-ray data contain information on overall shape and long-range structural details, whereas short-range structural details, of the order of the interatomic distances, are accessible only at very high resolutions, which are not always achievable. In contrast, NMR data mainly provide direct information on local details, in the form of interatomic distances or orientations of vectors connecting chemically bound nuclei. Therefore, information from NMR and X-ray data is perfectly complementary.

Among the structural restraints which can be obtained in NMR spectroscopy, pseudo-contact shifts (PCSs) [2] and residual dipolar coupling (RDCs) [3] have attracted increasing interest during the last decades for their intrinsic long-range nature. They can in fact provide structural information on the relative positions or orientations of pairs of atoms throughout the whole macromolecule or a large part of it. When the molecule is paramagnetic, dipolar interactions arise between the nuclei and the residual electron polarization, which is proportional to the magnetic susceptibility. If the magnetic susceptibility is anisotropic, these dipolar interactions do not average to zero upon rotation and PCSs arise (see later). An anisotropic magnetic susceptibility is usually associated with metal ions coordinated to the molecule [4–6] which, if not originally present, can be included by substitution of a diamagnetic metal ion [7–16] or, alternatively, rigidly attached through tags [17–41]. Other paramagnetic centres, such as organic radicals, have too little anisotropy to cause PCS or alignment effects. Magnetic susceptibility anisotropy also causes partial alignment of the molecule. In turn, partial alignment prevents internuclear dipolar interactions to be completely abolished by rotation, causing RDCs. This self-orientation is an alternate way to generate RDCs without using an external alignment medium [4,42–54]. PCSs and paramagnetic RDCs depend on the molecular nuclear coordinates in a common frame defined by the magnetic susceptibility anisotropy tensor associated with the paramagnetic metal. Self-orientation RDCs can also be obtained in the case of molecules for which the diamagnetic susceptibility is anisotropic [3,55–59] although in this case PCSs are not present.

It is interesting to observe that the presence of self-alignment also affects the chemical shifts of the observed species, if the chemical shielding of the nucleus is anisotropic. In the case of paramagnetic systems, the observed shift (not to be confused with the PCS) will be a combination of the effects of chemical shielding anisotropy and of the interaction with the electron average magnetic moment [60–62]; in diamagnetic systems it will reflect the chemical shielding anisotropy [63].

PCSs and RDCs contain structural information that has proved very helpful for solving protein structures [5,10,64–70], and they have therefore been included as structural restraints in the most commonly used programs for protein structure determination from NMR data [65,69,71–75].

PCSs and RDCs are even more precious restraints in the investigation of proteins constituted by multiple domains, and of protein–protein complexes. In the case of rigid systems, in which the structure of each single unit is known, PCSs and RDCs can be