Solid State Nuclear Magnetic Resonance ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Solid State Nuclear Magnetic Resonance

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

Trends

Solid-state NMR studies of proteins immobilized on inorganic surfaces

Wendy J. Shaw *

Pacific Northwest National Laboratory, PO Box 999, MS K2-57, Richland, WA 99352, USA

ARTICLE INFO

Article history:
Received 1 August 2014
Received in revised form
14 October 2014

Keywords:
Biomineralization
Immobilized proteins
Dipolar recoupling
Protein structure
Protein dynamics
Protein orientation
Multi-dimensional solid state NMR
Amelogenin
Statherin
Silaffin

ABSTRACT

Solid state NMR is the primary tool for studying the quantitative, site-specific structure, orientation, and dynamics of biomineralization proteins under biologically relevant conditions. Two calcium phosphate proteins, statherin (43 amino acids) and leucine rich amelogenin protein (LRAP; 59 amino acids), have been studied in depth and have different dynamic properties and 2D- and 3D-structural features. These differences make it difficult to extract design principles used in nature for building materials with properties such as high strength, unusual morphologies, or uncommon phases. Consequently, design principles needed for developing synthetic materials controlled by proteins are not clear. Many biomineralization proteins are much larger than statherin and LRAP, necessitating the study of larger biomineralization proteins. More recent studies of the significantly larger full-length amelogenin (180 residues) represent a significant step forward to ultimately investigate the full diversity of biomineralization proteins. Interactions of amino acids, a silaffin derived peptide, and the model LK peptide with silica are also being studied, along with qualitative studies of the organic matrices interacting with calcium carbonate. Dipolar recoupling techniques have formed the core of the quantitative studies, yet the need for isolated spin pairs makes this approach costly and time intensive. The use of multi-dimensional techniques to study biomineralization proteins is becoming more common, methodology which, despite its challenges with these difficult-to-study proteins, will continue to drive future advancements in this area.

Contents

1. Introduction . . . 1
 1.1. Studying the interaction mechanism of biomineralization proteins with solid state NMR (SSNMR) . . . 2
 1.2. Dipolar recoupling methods . . . 3
 1.3. Sample preparation . . . 4
2. Studies with hydroxyapatite . . . 4
 2.1. Salivary statherin . . . 4
 2.2. Amelogenin . . . 5
 2.3. Two-dimensional approaches for HAP binding proteins . . . 8
 2.4. Comparisons of HAP binding proteins . . . 9
3. Studies with silicates . . . 10
4. Studies with calcium carbonates . . . 12
5. Outlook . . . 12
Acknowledgments . . . 13
References . . . 13

1. Introduction

Biomineralization proteins direct the construction of hard tissues in the natural world, such as bones, teeth, pearl, nacre and egg shells [1]. The properties of the resulting tissues range significantly, even within a given mineral type, with the proteins

* Fax: +1 509 375 2644.
E-mail address: wendy.shaw@pnnl.gov

http://dx.doi.org/10.1016/j.ssnmr.2014.10.003
0926-2040/

present during construction allowing properties such as strength, hardness, morphology and/or phases that are not realized in their absence. This impressive control over inorganic minerals with the addition of what is effectively a polymer has incredible potential in aiding the development of new materials, if only the mechanisms underlying the formation of biominerals were understood.

Key to the study of any protein is an understanding of its structure, including primary, secondary, tertiary and quaternary structures. The primary structures of the identified proteins are relatively easily determined using biochemical methods, and quaternary structures (protein–protein interactions), if present, are studied with macroscopic techniques such as dynamic light scattering (DLS) and yeast-two hybrid systems as has been demonstrated for the biomineralization protein amelogenin [2,3], although these techniques don't address questions about the molecular level description driving the interactions. Secondary and tertiary structures are critical components of a proteins function, and are typically studied with solution state Nuclear Magnetic Resonance (NMR) or X-ray crystallography. These techniques have been used to provide insight into potential protein function during biomineralization, however, the functional form of biomineralization proteins is immobilized on a surface. Therefore the best mechanistic insight into the biomineralization protein's function will be gleaned from structural studies of the protein bound to its biologically relevant surface, a regime where solution state NMR and X-ray crystallography are not applicable. An additional challenge biomineralization proteins is that many of them are intrinsically disordered. IDP's are thought to be structurally labile to accommodate multiple functions, and adopt a specific structure when performing that function [4]. Therefore, solution state NMR studies of biomineralization proteins which suggest a lack of structure are misleading or oversimplified since the protein is being evaluated outside of its functional environment; consequently, studying them bound to their biologically relevant surface becomes even more essential to extract physiologically relevant data.

1.1. *Studying the interaction mechanism of biomineralization proteins with solid state NMR (SSNMR)*

There are three aspects of a surface immobilized protein that define its interaction: structure, orientation relative to the surface, and dynamics. SSNMR is well suited to address each of these aspects of surface interaction. Secondary, tertiary, and quaternary structure can all be studied by SSNMR, though studies of secondary structure, and when achievable, tertiary structure have been the focus of biomineralization studies to date. The orientation refers to how a particular residue or region within a protein is positioned relative to the surface, i.e. the protein may lay with the backbone parallel to the surface, perpendicular to the surface, or some combination of parallel and perpendicular, achieved by the

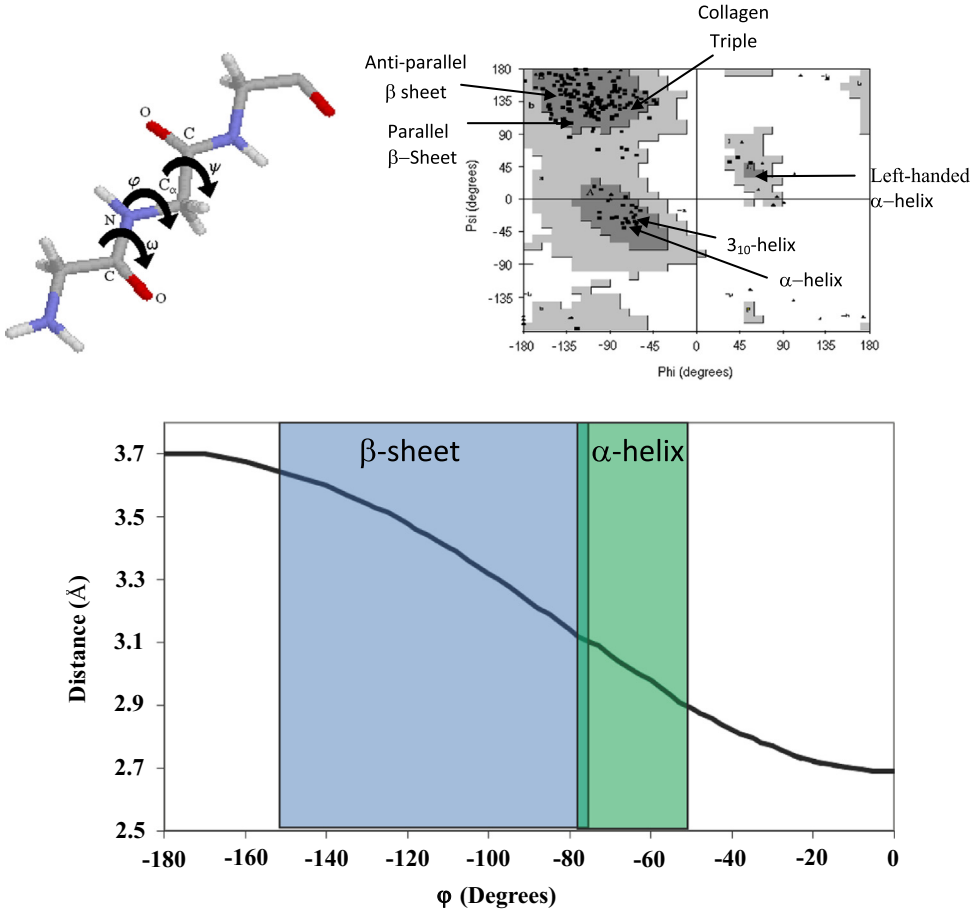

Fig. 1. Left: Torsion angles of a protein fragment are shown. In most cases, ω is restricted to 180° due to the delocalized electron density of the O=C–N bond, leaving φ and ψ as the only variables in determining the secondary structure of the protein. Right: The Ramachandran plot maps φ and ψ combinations, illustrating the energetically favored dihedral angles found in common protein secondary structure. The dark gray areas are the most energetically favored, the light gray, less so and the white areas are energetically unallowed. The black markers represent experimentally determined φ and ψ angles, clustered in the dark gray regions. The special secondary structures (α-helix, β-sheet (parallel and anti-parallel), and the collagen triple helix) have also been indicated, according to their classically defined values. Bottom: The φ angles map out to distances between adjacent carbonyl carbons, as shown, allowing a direct correlation between the distance, or dipolar coupling, and the structure.

Please cite this article as: W.J. Shaw, Solid State Nucl. Magn. Reson. (2014), http://dx.doi.org/10.1016/j.ssnmr.2014.10.003