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Solid state NMR studies of molecular recognition at protein–mineral interfaces

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1. Introduction to biomineralization

Nature has evolved sophisticated strategies for engineering hard tissues through the interaction of proteins, and ultimately cells, with inorganic mineral phases. The remarkable material properties of bone and teeth thus result from the activities of proteins that function at the organic-inorganic interface. The underlying molecular mechanisms that control biomineralization are of significant interest to both medicine and dentistry, as disruption of biomineralization processes can lead to bone and tooth demineralization, atherosclerotic plaque formation, artificial heart valve calcification, kidney and gallstone build-up, dental calculus formation, and arthritis [1–3]. A better understanding of the biomolecular mechanisms used to promote or retard crystal growth could provide important design principles for the development of calcification inhibitors and promoters in orthopedics, cardiology, urology, and dentistry. Similarly, a better understanding of how these proteins recognize and assemble in bioactive form on inorganic mineral phases could also aid in the development of surface coatings to improve the biocompatibility of implantable biomaterials and for hard tissue engineering and regeneration technologies.

At the level of fundamental science, it is important to note the lack of molecular structure information available

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for biomineralization proteins in general, and in particular for mammalian proteins that directly control calcification processes in hard tissue. Even the most fundamental questions about how the proteins interact at the biomineral surface, such as their general structure and orientation on the calcium phosphate surfaces, or whether the acidic residues are truly interacting directly with the crystal surface, remain largely uncharacterized at the experimental level. In order to develop a better structure-function level understanding of protein-crystal molecular recognition, we have begun to utilize solid-state NMR techniques to determine the molecular structure of proteins and peptides on calcium phosphate surfaces. In addition, these same techniques have provided interesting molecular dynamics information for the proteins on the biomineral surface. In this review, we will highlight recent work that is providing insight into the structure and crystal recognition mechanisms of an exemplary salivary protein model system, but which also provides a general approach to studying protein-crystal interactions in molecular detail.

Understanding the function of a biomineralization protein requires that the secondary and tertiary structure of the molecule be defined within its biological context, i.e. the protein in contact with the crystal surface. In addition, the precise nature of the interactions between the protein and the crystal which underlie the recognition process must be understood. This requires knowledge of the contacts formed between the amino acid side chains of the protein and the ions in the crystal faces. The involvement of water molecules in these interactions must be understood as well.

Current investigations of protein–mineral interactions are frequently conducted with techniques that characterize the macroscopic behavior of proteins in the presence of mineral crystals. Equilibrium properties such as protein-crystal binding constants are derived via adsorption isotherm measurements, where data are usually analyzed by assuming a simple Langmuir model of protein adsorption onto the crystal faces. But the most commonly-used approach for determining protein-crystal interactions in vitro are kinetic experiments in which a small amount of protein is dissolved in a saturated solution of a particular inorganic salt and the time required for crystals to form is compared to a control solution in which no protein is present. Assays also exist for determining selective binding of a particular crystal face by a protein as well as oriented nucleation of crystals in the presence of acidic proteins [4]. Recently, isothermal titration calorimetry has been used to determine binding enthalpies and binding affinities for proteins to mineral surfaces [5].

However, to extend beyond macroscopic aspects of protein-crystal interactions, high resolution spectroscopic methods must be used to provide information about the atomic level structure of the protein on the crystal face, under physical conditions that are biologically relevant (physiological levels of hydration and pH). Information about the secondary structural motifs and tertiary folding that characterize the adsorbed protein, together with information on the exposure of protein side chains to the crystal face, may lead to an understanding of how particular proteins promote or inhibit nucleation.

The lack of high resolution structural data for proteins on surfaces is the result of a lack of high resolution structural methods that can be brought to bear on relevant problems. The conventional methods of high resolution structural biology, i.e. X-ray crystallography and solution nuclear magnetic resonance (NMR) spectroscopy, have provided information on a few biomineralization proteins in the pure crystalline and solution states [6–9], but both techniques are severely limited in their abilities to elucidate the structures of proteins on biomineral surfaces. Although traditional surface science methods like photo-electron spectroscopy and NEXAFS have provided important information on proteins adsorbed onto planar surfaces, and in particular may be used to characterize the degree of long range ordering in systems of adsorbed proteins on polymer surfaces as well as average structural properties, these techniques have yet to provide detailed atomic-level structural information for surface-adsorbed proteins. In addition, surface diffraction methods and many optical techniques are not applicable to proteins adsorbed onto surfaces of porous materials (e.g. porous plastics) or to other surfaces lacking long-range ordering.

To fully appreciate the utility of solid state NMR in the study of protein structure at biomaterial interfaces, it is important to recognize the complex nature of the protein–surface problem. There is first the familiar structural aspect, alluded to briefly above, which includes defining the secondary and tertiary structures of the adsorbed protein and by implication any structural changes which occur upon binding to the surface. Second, the structure and chemical composition of the crystal surface in contact with the protein side chains must also be understood. The dynamics of the adsorbed protein are a less familiar but no less important aspect. It is desirable that the protein be observed on the surface under biologically relevant conditions, i.e. fully hydrated. Dehydration of the sample may alter not only the structure of the protein from its biologically relevant form but may quench the dynamics of the protein on the surface. Here we refer to both whole-molecule dynamics describing the protein's rigid body kinematics on the surface and to internal dynamics wherein the protein's conformation may be labile on the NMR time scale.

2. NMR methods for the study of protein structure at biomaterial interfaces

Solid state NMR has long been used to identify and characterize the structures of small organic molecules adsorbed onto catalytic surfaces [10–12], argon matrices [13], silica and alumina surfaces [14–16]. There similarly exist a number of solid state NMR pulse techniques capable in principle of reporting the structures of surface-adsorbed