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Review

Structure and function in organometallic•protein complexes

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ABSTRACT

Bioorganometallic chemistry is a rapidly growing subfield of organometallic chemistry. One important facet is the study of **organometallic•protein** complexes that contain a covalent bond between the protein and an organometallic prosthetic group. Structural elucidation of these complexes is being used with increasing frequency to determine exactly where metal binding takes place and to obtain accurate structural information. This review summarizes the structures in this field, highlighting how this information has driven the frontier of this research.

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1. Introduction

Organometallic chemists and protein biochemists have long worked independently in fields that share no apparent common ground. Even the ultimate goals of their research are different as organometallic chemists typically focus on catalytic processes and generating new materials while biochemists focus on life sciences and medical applications. This seemed to be a pragmatic separation based on the nature of the compounds in each discipline. Organometallic compounds are frequently sensitive to oxygen and water, and their makers routinely use Schlenk lines or glove boxes for preparation, reaction, and storage. Proteins are studied in buffered aqueous solutions, often with additional stabilizing compounds—including

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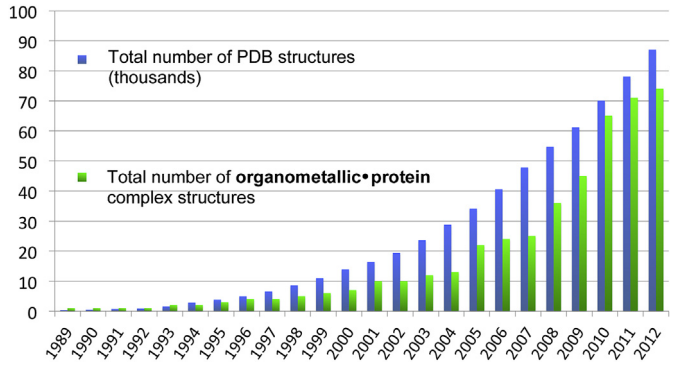

Fig. 1. The number of protein databank structures (www.rcsb.org/pdb) for all biomolecules (blue, in thousands) or **organometallic•protein** complexes (green) graphed according to deposition date. The number of total PDB structures is expected to reach 100,000 structures sometime in late 2013 or early 2014. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compounds that are nucleophiles and/or strong reductants that would readily react with most organometallic compounds [1].

Nevertheless, there is a natural connection between the two fields. In 1954, Dorothy Crowfoot Hodgkin and co-workers published the crystal structure of cyanocobalamin [2] followed by the structure of adenosine cobalamin in 1960 [3]. Cyanocobalamin had been known for years [4]. Scientists were now astonished to find that cyanocobalamin was an artifact of the isolation technique and that the enzyme cofactor encountered *in vivo* was a complex organometallic compound. After a pause of a few decades, multiple structures of a variety of enzymes including carbon monoxide dehydrogenases [5–7], hydrogenases [8,9] and cobalamins [10,11] were published, highlighting enzymes that contain organometallic groups or that perform organometallic transformations—methyl migration, oxidative addition and more—at active sites [12]. Scientists took note.

In recent decades organometallic chemists and biochemists have each found ways to join their fields and prepare synthetic **organometallic•protein** hybrid compounds with a covalent attachment. Initial complexes—mercury organometallic compounds used as phasing agents [13] in the X-ray structural elucidation of carbonic anhydrase crystals—were not appreciated at the time. The first structure of an **organometallic•protein** complex structure, carbonic anhydrase with an organo-mercury inhibitor in its catalytic site, was reported in a series of publications beginning in 1967 [14,15], although it was not deposited until 1989 with PDB code 3CA2 [16]. **Organometallic•protein** complexes have more recently been prepared for potential use as redox, luminescent or infrared spectroscopy probes, enzyme inhibitors, anti-cancer drugs, and reaction vessels containing bound organometallic groups to catalyze stereospecific reactions [12,17,18].

Until a few years ago there were relatively few crystallographic reports of **organometallic•protein** hybrid complexes. Since then, the pace of depositions of these complexes has kept at about one structure for every couple of thousand total PDB structures deposited (Fig. 1).

This exponential growth in the number of reported protein crystal structures is largely driven by technological advances in data collection, e.g. area detectors and synchrotrons, but also driven by advances in protein production, e.g. PCR-based cloning and gene synthesis, heterologous expression with codon optimization, and fast affinity purification strategies.

The use of NMR spectroscopy for structure elucidation has not contributed as extensively to **organometallic•protein** complex structures as directly as it has in other fields. This is probably due to several factors: 1) these complexes are often extensions of research in the field of small molecule organometallic chemistry, which is populated by scientists with X-ray crystallography experience; 2) protein–metal complexes in general can be more difficult to study by NMR than other protein–ligand complexes, because most metals themselves are not amenable to direct NMR observation, either because they are not spin-½ nuclei or because they are difficult to detect; 3) NMR methods often require isotopically enriched proteins that are not readily available for all protein systems. Bertini and Wüthrich in particular have made good use of NMR spectroscopy to study such metal–protein complexes [19], and the field of NMR has made significant contributions to the work in this review by validating that ligation of several **organometallic•protein** adducts occurs in solution at the same sites as in crystal structures. However, the pace of deposited NMR structures has not kept up with recent trends in deposited crystal structures. As Kurt Wüthrich pointed out, "Solution NMR has not yet found its synchrotron" [20]. Should NMR ever obtain the kind of technological enhancement that crystallography has had due to synchrotrons, improved copper sources and X-ray area detectors, then perhaps more **organometallic•protein** complexes will be studied in solution.

In this review we focus on structurally elucidated transition metal **organometallic•protein** complexes where the metal group is bound either by direct metal binding to the protein, or by direct binding of an ancillary ligand to the protein. Hybrid complexes that are considered in this review are listed in Table 1. The review covers the literature from 1967, with the first carbonic anhydrase structure with an organo-mercury inhibitor in its catalytic site, until early 2013.

There are many other structures of proteins where non-covalent interactions with organometallic groups drive structural outcomes. These will not be discussed here. For example organometallic compounds have been prepared that inhibit the activity of enzymes such as kinases because their shape and polarity characteristics are optimal for placement in the binding pocket of the target enzyme [21]; organometallic complexes have been linked to biotin to participate in biotin–avidin technology [22,23]; and, organometallic groups have been inserted in heme proteins in place of the heme prosthetic group [24]. There has also been vigorous research in recent years on the preparation and study of organometallic complexes with amino acid or protein ligands. Many crystal structures of the resulting small molecules have been reported. General reviews have appeared that summarize this area [25,26], and recent work shows that research is continuing [27,28]. Ferrocenyl amino acid complexes, especially 1,1′-disubstituted compounds designed as peptidomimetics, are a closely studied sub-area of this field. It has been reviewed [29] and also continues to evolve [30].

2. Carbonic anhydrase

Carbonic anhydrases comprise an evolutionarily ancient and virtually ubiquitous family of enzymes that catalyze the equilibrium between carbon dioxide and water and carbonic acid [31]. These proteins are found in various regions of the cell: in mitochondria, in the cytosol, in membranes and as secreted proteins. In most organisms, this protein has a tetrahedral zinc ion at the catalytic site that is bound by three histidine side chains and one water molecule. Carbonic anhydrases function by changing the pKa of the bound water molecule, making the water more susceptible to deprotonation, producing a metal-bound hydroxide. This catalytically perfect enzyme operates at the diffusion limit, suggesting that its function is as evolutionarily optimized as possible. The catalysis of the equilibrium between carbon dioxide and carbonate is key to mammalian respiration, maintenance of the buffer system in the bloodstream, and for the synthesis of sugars in green plants [32]. Over the past few decades, many studies into carbonic anhydrase