Journal of Physical Chemistry & Biophysics

OPEN ACCESS Freely available online

Perspective

Solid-State NMR Crystallography: From Catalytic Active Complexes to Enzymes

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ABSTRACT

Despite the tremendous importance of catalysis for all types of chemistry and biochemistry, there is still a huge gap in detailed knowledge of the processes and reaction intermediates on the surfaces of the catalysts or inside. The combination of conventional and Dynamic Nuclear Polarization (DNP) enhanced solid-state NMR spectroscopy, x-ray diffraction, electron microscopy, chemical modelling and quantum chemical calculations, often loosely summarized as NMR crystallography, has evolved into one of the most powerful characterization tools to fill this gap and study solid catalysts and chemical processes on their surface or the active center of an enzyme. These techniques give an unprecedented view in the chemistry of immobilized homogeneous transition metal catalysts, supported e.g. on silica or crystalline nanocellulose (CNC) or polymer based core shell structures as carriers or reactants and reaction intermediates on transition metal nanoparticles (MNPs). The contribution presents recent examples from our group about solid-state NMR spectroscopic characterizations of mono- or binuclear Rhodium, Ruthenium and Iridium catalysts and a Nickel containing enzyme. The focus is set to the immobilization of Wilkinsons's type catalyst and the dirhodium-acetate dimer (Rh2ac4). These are linked covalently to high-surface silica or crystalline nanocellulose support materials, employing amine, phosphine, pyridyl or carboxyl functions on the surface of the support materials or mesoporous silica supports. Combinations of 13C-, 15N-, 29Si- and 31P- CP MAS, J-resolved 31P- MAS and HETCOR solid-state NMR techniques are employed to monitor the preparation of the catalyst. Moreover, by DNP enhanced solid-state NMR it is feasible to detect different carboxyl and amine binding sites in natural abundance at a fast time scale.

Keywords: Solid state NMR; REDOR; DNP

INTRODUCTION

With regards to NMR spectroscopy, the expression "crystallography" is utilized from an expansive perspective to mean the investigation of crystalline solids and the plan of particles in precious stones. Obviously, NMR has been occupied with crystallography since the soonest long stretches of strong state NMR. In any case, the expression "NMR crystallography" has as of late been received and is commonly held for examines that are acted related to diffraction techniques or where grid boundaries or precious stone balance are unequivocally determined.1–10 Several ongoing surveys give fantastic acquaintances with this subject and feature the manners by which NMR spectroscopy and X-beam diffraction strategies are complementary.11 specifically, X-beam diffraction techniques are unparalleled in their capacity to decide structure structures for frameworks that have long-run crystalline request, while NMR spectroscopy is unequaled in its capacity to decide neighborhood substance structure. Together, the two can provide chemically-detailed, three-dimensional structures. Over the past 40–50 years, great advances in the understanding of the relationship between protein structure and biological function have been achieved by a combination of bioorganic mechanistic studies, the determination of protein structures at near atomic resolution, and the modification of protein structure using the tools of molecular biology. What has become clear is that much of the chemistry of enzyme catalysis is based on simple organic chemical interactions consisting, for example, of Brønsted acid-base catalysis, Lewis acid catalysis, and nucleophilic and electrophilic catalysis. Yet it remains unclear how the enormous rate accelerations achieved by enzyme active sites occur within the context of transition-state kinetic theory.

To move our understanding to the next level, a more detailed knowledge of the chemical microenvironment of the catalytic site is needed. High-resolution X-ray crystal structures provide part of

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Received: February 52021, **Accepted:** February 19, 2021, **Published:** February 26, 2021

Citation: Buntkowsky G (2021) Solid-State NMR Crystallography: from Catalytic Active Complexes to Enzymes. Phys Chem Biophys. 10:292. .

the answer by identifying the protein residues and cofactors that interact with the reacting substrate. However, the resolution of most protein structures (1.5 to 2.5 Å) generally is insufficient to determine the protonation states of the acid-base functional groups. Thus investigators usually infer protonation states from hydrogen-bonding patterns and intuition based on the aqueous solution pKa values of small molecule models. NMR spectroscopy can give another piece of the appropriate response, as the connections of synthetic move (both isotropic and anisotropic), dipolar coupling, and quadrupolar coupling are amazingly delicate tests of the compound microenvironment and can recognize, for instance, direct protonation of an ionizable gathering or the adjustment in hybridization of a responding substrate

Framework Structure: X-Ray Crystallography

NMR crystallography requires a structural framework as a starting point for building, refining, and testing three-dimensional chemical models of the enzyme active site. Here we review the progression of crystal structures in tryptophan synthase and the pivotal role these advances have played in understanding mechanism and allostery, and how these advances motivate the use of even higher-resolution, mechanistic probes.

Tryptophan synthase catalyzes the last two steps in the biosynthesis of L-tryptophan (L-Trp) (Scheme I). Early work established that the substrates for the bienzyme complex are 3-indole-D-glycerol-3-phosphate (IGP) and L-serine (L-Ser), the bienzyme complex has the subunit composition 2, the -subunits require pyridoxal 5-phosphate (PLP) for catalytic activity, and that indole is a channeled intermediate.

Despite these successes, significant questions regarding the chemical mechanism for the substrates' transformation remain because the resolution of the X-ray structures does not allow for protonation states to be established on the reacting substrate, PLP coenzyme, or catalytic residues. The catalytic activity of the PLP-requiring -site is also dependent upon the protonation states of the ionizable groups on the PLP moiety (the PLP phenolic hydroxyl, pyridine ring nitrogen, and phosphoryl group) and on the reacting substrate (the Schiff base nitrogen linked to the PLP C4 carbon, and the carboxylate of the reacting substrate), which are highlighted in Figure 1(a). Therefore, to fully understand the mechanism requires chemically-detailed structural models of the intermediates in the enzyme active site.

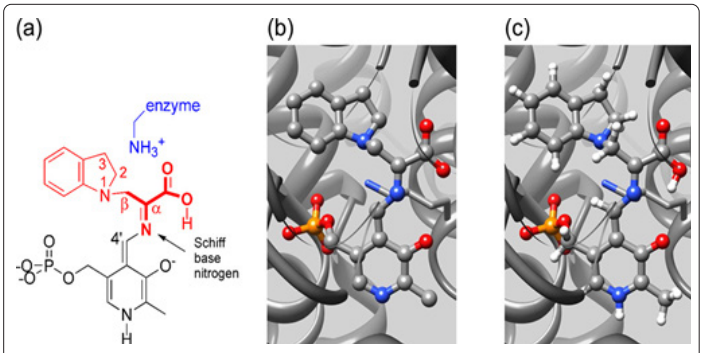

Figure 1: NMR Spectroscopy

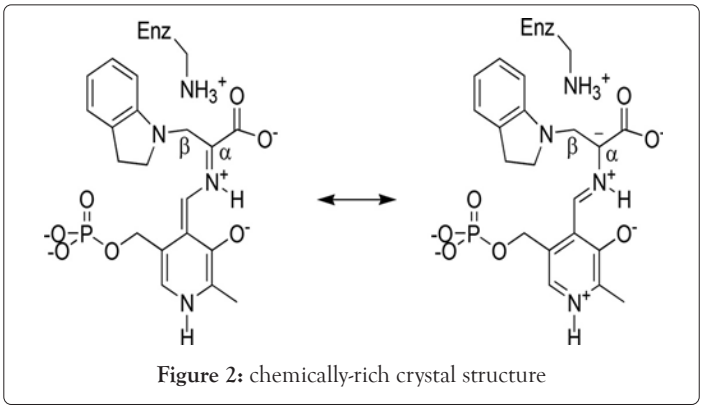

Figure 2: chemically-rich crystal structure

Chemical Structure: NMR Spectroscopy

Our work has focused on the use of isotropic chemical shifts for NMR crystallography in tryptophan synthase. We note that, when available, anisotropic NMR interactions can provide significant additional restraints for refining models. NMR crystallography uses isotropic chemical shifts in two specific ways. First, the chemical shift is used in its analytical role to directly report on the chemical state of a probe atom, often answering chemical questions regarding the direct protonation or hybridization state at that site. For example, McDowell et al. showed that when labeled Ser was supplied as a substrate to tryptophan synthase, solid-state C NMR spectroscopy could identify the change in hybridization at the beta carbon as water is lost to form the E(A-A) intermediate. Second, the chemical shift is used in NMR crystallography to report on the chemical and structural environment surrounding a probe nucleus. This is the manner in which chemical shifts are used in the structural refinement of organic and inorganic molecular crystals using NMR crystallography; the chemical state of the probe atom is known, but its shift depends not only on its chemical structure, but also its three-dimensional molecular conformation and the precise location and chemical state of nearby atoms in the surrounding crystal lattice.

CONCLUSION

Enzymes have evolved to achieve remarkably efficient and specific chemical transformations. Yet atomic-level details of enzyme mechanisms remain elusive: the intermediates are transient and the chemistry that drives the transformation, such as changes in hybridization and protonation states, is difficult to characterize in functioning enzyme systems. NMR crystallography is poised to make a significant contribution to this understanding by the synergistic combination of solid-state NMR spectroscopy, X-ray crystallography, and computational chemistry. This fusion allows specific models of the chemical structure to be built upon the coarse X-ray framework and then tested by comparison of predicted and assigned chemical shifts. The result is a unique and chemically-rich view into functioning enzyme catalysis, which for the case of tryptophan synthase leads to a new acid-form hypothesis for the indoline quinonoid intermediate.