Use of High Pressure in Drug Design

Hans Robert Kalbitzer (University of Regensburg, Germany)

Abstract

High-pressure NMR spectroscopy (HPNMR) is a powerful technique for characterizing conformational equilibria of biological macromolecules in solution [1]. High-pressure X-ray crystallography (HPMX) provides a complementary and orthogonal approach, enabling the direct visualization of pressure-induced conformational changes with atomic resolution under well-defined thermodynamic conditions [2]. From a physical perspective, hydrostatic pressure selectively stabilizes low-volume conformational states and thus shifts equilibria toward sparsely populated substates that are often inaccessible under ambient conditions. The application of high pressure has therefore emerged as a unique and non-invasive tool for detecting rare, low-population "excited" conformational states of proteins that are particularly relevant for functional regulation and drug discovery.

These excited states constitute attractive targets for intrinsic allosteric inhibition, in which small molecules selectively bind to a non-dominant conformational state and thereby redistribute the conformational equilibrium away from the catalytically competent form toward a less active state of the catalytic cycle [3]. Here, we demonstrate how the combined application of HPNMR and HPMX allows not only the identification and thermodynamic characterization of such rare states, but also their direct structural characterization in complex with small-molecule ligands.

As a proof of principle, we study Ras, a key regulator of cellular signal transduction involved in proliferation and differentiation. Oncogenic Ras mutants are constitutively active due to impaired regulatory control. Ras activation is mediated by guanine nucleotide exchange factors (GEFs), which promote the exchange of GDP for GTP, whereas inactivation is driven by GTPase-activating proteins (GAPs), which stimulate GTP hydrolysis.

Using high-pressure NMR spectroscopy in the pressure range from 0.1 to 300 MPa, four conformational states of activated Ras were detected and thermodynamically characterized: state 1(0), the nucleotide-releasing state; state 1(T), the GEF-binding state; state 2(T), the effector-binding state; and state 3(T), the GAP-interacting state. One of the earliest Ras inhibitors identified was Zn^{2+}–cyclen. The three-dimensional structure of its complex with Ras was previously inferred from chemical shift perturbation and paramagnetic relaxation enhancement experiments on wild-type Ras and engineered state 1(T) mutants, followed by docking calculations [4]. However, crystallographic characterization of this cmplex had remained elusive because the ligand-binding site is closed in conventional crystal structures.

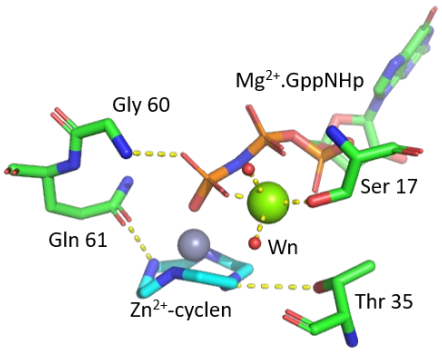

Fig. 1. Coordination of Zn^{2+}-cyclen in a single crystal of Ras.Mg^{2+}.GppNHp in state 1(T) induced by high pressure (500 MPa) [5].

We show that the application of high pressure to Ras crystals induces a conformational opening of the binding site, enabling high-resolution visualization of the Ras–Zn^{2+}–cyclen complex (Fig. 1) by HPMX [5]. These results highlight the methodological complementarity of solution- and crystal-based high-pressure techniques and illustrate the broader potential of high pressure as a controllable thermodynamic variable in structure-based drug discovery. Based on these findings, we propose a general strategy for integrating high-pressure methods into drug development workflows targeting rare, functionally relevant conformational states.

[1] Kalbitzer, H. R. (2015) in "*High Pressure Bioscience - Basic Concepts, Applications and Frontiers*" (K. Akasaka and H. Matsuki, eds, pp. 179-198), Springer, Heidelberg, Germany

[2] Fourme, R., Girard, E., Akasaka, K. (2012) *Curr Opin Struct Biol* **22**:636–642.

[3] Kalbitzer, H. R., Rosnizeck, I. C., Munte, C. E., Puthenpurackal Narayanan, S., Kropf, V., Spoerner, M. (2013). *Angew. Chem. Int. Ed*. **52**, 14242 –14246.

[4] Rosnizeck, I. C., Graf, T., Spoerner, M., Tränkle, J., Filchtinski, D., Herrmann, C., Gremer, L., Vetter, I. R, Wittinghofer, A., König, B., Kalbitzer, H. R. (2010) *Angew. Chem. Int. Ed.* **49**, 3830 –3833.

[5] Girard, E., Lopes, P., Spoerner, M., Dhaussy, A.-C., Prangé, T., Kalbitzer, H. R., Colloc'h, N. (2024) *Chem. Eur. J.* **2024**, e202400304.