Journal of Thermodynamics & Catalysis
ISSN: 2157-7544

Davydov, J Thermodynam Cat 2012, 3:4
DOI: 10.4172/2157-7544.1000e110

Editorial Open Access

Merging Thermodynamics and Evolution: How the Studies of High-Pressure Adaptation may Help to Understand Enzymatic Mechanisms

Dmitri R. Davydov*

Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California, USA

Increasing interest of researchers to the use of high hydrostatic pressure has marked recent trends in structural biology [1-4]. It became clear that, along with the exploration of the effects of temperature, the use of pressure as a variable parameter is indispensable for detailed understanding of the mechanisms of protein-protein interactions, protein folding and conformational transitions.

The basis of pressure effects is the change in system volume that accompanies biochemical processes [1,5]. According to the Le Chatellier's principle, increased pressure enhances those processes that are accompanied by a decrease in system volume, and conversely, inhibits processes occurring with a volume increase. The most important part of the volume changes in protein transitions is attributable to protein interactions with solvent [6-9]. These include electrostriction of water on solvent-exposed charges, changes in the solvent packing around exposed nonpolar groups, and water penetration into the protein cavities [1,6-8]. Pressure increase results in enhancement of protein hydration, which therefore constitutes the core of pressure-induced protein transitions. Displacement of equilibria of protein interactions with solvent is the major factor that determines the impact of pressures experienced by deep-sea organisms, deep-living organisms (≤ 1 kbar), where pressure-induced denaturation may be neglected (most proteins are structurally stable up to 3-4 kbar [1]).

Alongside with that, the pressure sensitivity of protein conformational equilibria creates an important challenge for structural adaptation of the proteins of deep-living, pressure-tolerant species. Although the importance of studying the biology of extremophiles, such as organisms living at extreme temperatures or salinity, has been increasingly recognized, the potential of the studies of evolutionary adaptation to high hydrostatic pressures remains underappreciated. The aim of this article is to draw attention of researchers in the fields of structural biology and mechanistic enzymology to the studies of high-pressure adaptation as a powerful approach to explore the conformational landscape of proteins and uncover pivotal conformational transitions in enzymatic mechanisms.

Studies of deep-living organisms (piezophiles) have revealed a profound adaptation of their biochemical and physiological systems for function at elevated hydrostatic pressure [5,9-12]. The most extensive studies have been devoted to the search for pressure-sensing systems and pressure-regulated genes, as well as exploring the basics of high pressure adaptation in biological membranes. However, the adaptation of the enzymes of piezophiles to their function at elevated pressure has received less attention and the mechanisms of this adaptation remain largely unexplored.

Due to the fundamental role that the changes in protein hydration play in enzymatic mechanisms [6,7,13-18], the sensitivity of protein-solvent interactions to hydrostatic pressure constitutes the most important challenge for evolutionary adaptation of piezophilic enzymes. In particular, this structural adaptation must prevent excessive protein hydration at critical steps of the catalytic cycle, while maintaining the functionality of water channeling to the active site (when required). The evolutionary adaptation to high hydrostatic pressures thus changes in the energy landscape of the protein and redistributes the populations of the enzyme conformers. Accordingly, the conformations that are only transient in the catalytic mechanisms of mesophilic enzymes may become stabilized in their piezophilic orthologs placed at ambient pressure. Therefore, comparative studies of the enzymes with different degree of high-pressure adaptation may provide a clue to functionally important conformational dynamics that are common to both piezozymes and their mesophilic orthologs. This premise justifies the value of studies of pressure adaptation for molecular enzymology.

We recently probed the utility of this new strategy in a comparative study with a series of homologous enzymes with different degrees of piezophilic adaptation. Selection of a proper target enzyme system is of vital importance for this pilot study. According to our basic hypothesis, the role of structural adaptation to high pressure is especially important in the enzymes whose functional mechanisms involve hydration-dehydration dynamics. In particular, this is relevant to enzymes with catalytic cycles that involve controlled expulsion and/or penetration of water molecule(s) from/into the active site. Perhaps the most illustrative example of such an enzyme system is represented by cytochromes P450.

Cytochromes P450 are the heme-thiolate enzymes that catalyze oxidation of a wide variety of endogenous and exogenous lipophilic compounds. These versatile monooxygenases, which are present in all domains of life, play diverse physiological functions varying from the synthesis of steroid hormones, prostaglandins and second messengers to oxidation of exogenous compounds, such as drugs and other xenobiotics. Monooxygenation reactions require the input of two electrons (supplied by the flavoprotein or ferredoxin partners) used to activate the oxygen molecule, one oxygen atom of which is then inserted into the substrate molecule, while the second atom is released in the form of a water molecule. However, an important fraction of the activated oxygen may be released without substrate oxidation through the "leaky" shortcuts in the catalytic cycle. This leakage, which results in a futile cycling and generation of reactive oxygen species, such as the superoxide anion-radical and hydrogen peroxide, is closely

***Corresponding author:** Dmitri R. Davydov, Skaggs School of Pharmacy and Pharmaceutical Sciences , University of California San Diego, La Jolla, California, USA, Tel: 185-824-602-71; Fax: 185-824-600-89; E-mail: ddavydov@ucsd.edu

Received August 30, 2012; **Accepted** August 31, 2012; **Published** September 03, 2012

Citation: Davydov DR (2012) Merging Thermodynamics and Evolution: How the Studies of High-Pressure Adaptation may Help to Understand Enzymatic Mechanisms. J Thermodynam Cat 3:e110. doi:10.4172/2157-7544.1000e110

related to the degree of the active site hydration. The mechanisms of conformational gating of the heme pocket that minimizes futile cycling through regulation of its solvent accessibility, prevention of aberrant proton delivery and efficient expulsion of the water molecule formed in catalysis represents one of the most important challenges for the current mechanistic enzymology of cytochromes P450. In our first study with the proposed strategy we sought to uncover these yet elusive mechanisms through a study of piezophilic adaptation in a series of P450 from bacteria with different degree of high-pressure adaptation [19,20].

A search through approximately 20 available either complete or partial genomes of deep-sea bacteria (see https://moore.jcvi.org/moore/ and http://genamics.com/genomes/index.htm for partial lists) revealed the sequences encoding cytochrome P450 proteins in *Moritella sp.* strain PE36, and *Photobacterium profundum* strain SS9. Both strains are piezophilic and evolved to live at pressures of 250–500 bar [21]. The proteins from *Moritella* and *Photobacterium* display a 46% sequence identity and, according to conventional nomenclature, belong to the same P450 family. This comparative study also requires highly similar mezophilic (not pressure-adapted) proteins as reference points. The genome of the shallow water strain of *Photobacterium*, *P. profundum* 3TCK encodes a P450 protein, which is 97% identical to the piezozyme from the strain SS9. Similarly, the genome of the marine bacteria *Moritella viscosa*, a causative agent of winter ulcer in Atlantic salmon [22], also encodes a P450 enzyme, which is 87% similar to the P450 from *Moritella sp.* PE36. These four proteins, which are now classified as the members of the P450 family 261, represent an outstanding target for a comparative research focused on the mechanism of structural adaptation to high hydrostatic pressures.

Three of these four enzymes, namely the two *Photobacterium* enzymes and the piezophilic enzyme from *Moritella,* have been cloned, expressed in *E. coli* and purified in our laboratory. Even the very first studies with these proteins revealed a well-pronounced effect of high-pressure adaptation. Using pressure-perturbation absorbance spectroscopy we were able to demonstrate that the piezophilic enzyme from *P. profundum* SS9 (P450-SS9 or CYP261C1) possesses a pressure-actuated conformational toggle, which controls the solvent accessibility of the heme pocket and prevents the pressure-induced hydration of the active site. Importantly, the characteristic pressure ($P_{½}$) of the transition between the putative "relaxed" and "tense" (pressure-promoted) states of the enzyme lies in the range of 400-600 bar, which is commensurate with the physiological pressure of habitation of *P. profundum* [23,24]. Our very recent experiments with the P450 protein from *Moritella sp.* PE36 (P450-PE36 or CYP261D1), another piezozyme, revealed very similar behavior. In contrast, this pressure-dependent conformational transition was not observed in the mesophilic enzyme from *P. profundum* 3TCK (P450-3TCK or CYP261C2). This is especially striking in view of a very close sequence similarity of P450-SS9 and P450-3TCK, which have only 11 dissimilar amino acid residues (out of 479). This comparison strongly suggests that the unique pressure-related behavior of CYP261C1 and CYP261D1 represent a consequence of the high-pressure adaptation in these enzymes.

Further studies with these proteins revealed another very unusual peculiarity of the pressure-adapted enzymes: both piezozymes are represented with two different stable states, which may be separated with ion-exchange chromatography. These two forms of the enzymes are not inter-convertible and exhibit very different thermodynamic properties (Davydov et al., unpublished). This unusual heterogeneity was characteristic of both piezophilic enzymes (CYP261D1 and CYP261C1), but absent in mesophilic CYP261C2. The contrast between CYP261C1 and CYP261C2 is especially stunning in view of the 97% sequence identity between these two proteins. In our view, this unusual conformational splitting reveals a consequence of high-pressure adaptation in piezophilic CYP261C1 and CYP261D1, which have evolved to fold and function at elevated pressure. Transfer of these proteins from the physiological 250-500 bar to ambient pressure results in an important change in their energetic landscapes and therefore causes an ambiguity in folding when the proteins are expressed in *E. coli* grown at ambient pressure. Besides, its importance for exploration of P450 conformational dynamics, stabilization of two dissimilar conformers observed in piezozymes may provide an important insight into the role of protein-solvent interactions in the mechanisms of the protein folding.

The results discussed above demonstrate an exceptional utility of the studies of conformational adaptation in piezozymes. Merging the molecular evolution with biochemical thermodynamics allows creating a new strategy for exploration of protein conformational mobility through pressure-perturbation studies in a series of enzymes with different degrees of evolutionary adaptation to high pressure.

Besides their importance for molecular enzymology and protein biophysics, the studies of high-pressure adaptation have a high value for biotechnology and bioengineering. The concept of changing the direction of enzymatic reactions and increasing the efficiency of catalysis by high hydrostatic pressure is widely discussed in modern literature [25-27]. Increase in hydrostatic pressure often provides a potent means to change the substrate specificity and stereoselectivity of enzymatic catalysis [28,29]. Elevated pressures are known to increase temperature stability of proteins [27,30], and the combination of high-pressures with increased temperatures enables to increase enzyme turnover by over an order of magnitude [30,31]. However, at present the application of these pressure-based approaches in biotechnology is restricted to a limited number of enzymes, the function of which is not obstructed by increased hydration. Functional and structural comparison of piezozymes with their mesophilic counterparts will approach elaboration of the universal principles of engineering piezotolerant enzymes, thus extending the applicability of pressure-based approaches in biotechnology.

Acknowledgement

This work was supported in part by NIH grant GM054995.

References

1. Mozhaev VV, Heremans K, Frank J, Masson P, Balny C (1996) High pressure effects on protein structure and function. Proteins 24: 81-91.
2. Hui Bon Hoa G, McLean MA, Sligar SG (2002) High pressure, a tool for exploring heme protein active sites. Biochim Biophys Acta 1595: 297-308.
3. Rivalain N, Roquain J, Demazeau G (2010) Development of high hydrostatic pressure in biosciences: Pressure effect on biological structures and potential applications in Biotechnologies. Biotechnol Adv 28: 659-672.
4. Akasaka K (2003) Exploring the entire conformational space of proteins by high-pressure NMR. Pure Appl Chem 75: 927-936.
5. Somero GN (1992) Adaptations to high hydrostatic pressure. Ann Rev Physiol 54: 557-577.
6. Low PS, Somero GN (1975) Activation volumes in enzymic catalysis: their sources and modification by low-molecular-weight solutes. Proc Natl Acad Sci 72: 3014-3018.
7. Weber G, Drickamer HG (1983) The effect of high pressure upon proteins and other biomolecules. Q Rev Biophys 16: 89-112.

8. Shimizu S (2004) Estimating hydration changes upon biomolecular reactions from osmotic stress, high pressure, and preferential hydration experiments. Proc Natl Acad Sci 101: 1195-1199.

9. Imai T (2009) Roles of water in protein structure and function studied by molecular liquid theory. Front Biosci 14: 1387-1402.

10. Royer CA (2002) Revisiting volume changes in pressure-induced protein unfolding. Biochim Biophys Acta 1595: 201-209.

11. Abe F, Horikoshi K (2001) The biotechnological potential of piezophiles. Trends Biotechnol 19: 102-108.

12. Prieur D, Jebbar M, Bartlett D, Kato C, Oger P (2009) Piezophilic prokaryotes. In Comparative high pressure biology. Sebert P, (eds). CRC Science Publishers, Enfield, New Hampshire. 281-318.

13. Somero GN (2003) Protein adaptations to temperature and pressure: complementary roles of adaptive changes in amino acid sequence and internal milieu. Comp Biochem Physiol B Biochem Mol Biol 136: 577-591.

14. Bartlett DH (1992) Microbial life at high pressures. Sci Progress 76: 479-496.

15. Loftfield RB, Eigner EA, Pastuszyn A, Lovgren TNE, Jakubowski H (1980) Conformational changes during enzyme catalysis : role of water in the transition-state. Proc Natl Acad Sci 77: 3374-3378.

16. Pavlic MR (1987) The role of hydration in an enzyme reaction. Arch Biochem Biophys 253: 446-452.

17. Rupley JA, Careri G (1991) Protein hydration and function. Adv Protein Chem 41:37-172.

18. Pocker Y (2000) Water in enzyme reactions: biophysical aspects of hydration-dehydration processes. Cell Mol Life Sci 57: 1008-1017.

19. Pal SK, Zewail AH (2004) Dynamics of water in biological recognition. Chem Rev 104: 2099-2123.

20. Rand RP (2004) Probing the role of water in protein conformation and function. Philos Trans R Soc Lond B Biol Sci 359: 1277-1285.

21. Yayanos AA (1986) Evolutional and ecological implications of the properties of deep-sea barophilic bacteria. Proc Natl Acad Sci 83: 9542-9546.

22. Bjornsdottir B, Hjerde E, Bragason BT, Gudmundsdottir T, Willassen NP, et al. (2012) Identification of type VI secretion systems in Moritella viscosa. Vet Microbiol 158: 436-442.

23. Sineva EV, Davydov DR (2010) Cytochrome P450 from Photobacterium profundum SS9, a piezophilic bacterium, exhibits a tightened control of water access to the active site. Biochemistry 49: 10636-10646.

24. Sineva EV, Davydov DR (2010) Constrained water access to the active site of cytochrome P450 from the piezophilic bacterium Photobacterium profundum. High Pressure Res 30: 466-474.

25. Kunugi S, Tanaka N (2002) Cold denaturation of proteins under high pressure. Biochim Biophys Acta 1595: 329-344.

26. Masson P, Tonello C (2000) Potential applications of high pressures in pharmaceutical science and medicine. High Pressure Res 19: 223-231.

27. Eisenmenger MJ, Reyes-de-Corcuera JI (2009) High pressure enhancement of enzymes: A review. Enzyme Microbial Technol 45: 331-347.

28. Mombelli E, Shehi E, Fusi P, Tortora P (2002) Exploring hyperthermophilic proteins under pressure: theoretical aspects and experimental findings. Biochim Biophys Acta 1595: 392-396.

29. Bruins ME, Meersman F, Janssen AEM, Heremans K, Boom RM (2009) Increased susceptibility of beta-glucosidase from the hyperthermophile Pyrococcus furiosus to thermal inactivation at higher pressures. FEBS J 276: 109-117

30. Mozhaev VV, Lange R, Kudryashova EV, Balny C (1996) Application of high hydrostatic pressure for increasing activity and stability of enzymes. Biotechnol Bioeng 52: 320-331.

31. Aertsen A, Meersman F, Hendrickx MEG, Vogel RF, Michiels CW (2009) Biotechnology under high pressure: applications and implications. Trends Biotechnol 27: 434-441.