Observed 'live': Water is an active team player for enzymes

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In biologically active enzyme substrate compounds, as can be found in medicines, water plays a more decisive role than has been imagined up to now. The surrounding water acts like an "adhesive", in order to keep the substrate at the right place on an enzyme. For this, the dynamism of the water is retarded. Scientists at the RUB under Prof. Dr. Martina Havenith (Physical Chemistry) in close cooperation with the group of Prof. Irit Sagi from the Israeli Weizmann Institute have been able to observe and prove the retardation of the water's dynamism "live" for the first time in close. The researchers are reporting on their results in *Nature Structural & Molecular Biology*.

Which role does the solvent play?

Enzymes are natural substances accelerating and controlling the metabolic processes in the body. They are, for example, of central importance for the immune system, as they control the balance between activating and inhibiting defensive reactions and play an important role in inflammation reactions. It had been known for some time that enzymatic functions take place in various solvents at highly differing speeds. But up to now, the contribution made by the solvent - this is water in biological processes - on a molecular level had not yet been clarified.

Two new techniques combined

Prof. Havenith's group at the RUB and Prof. Irit Sagi's group at the Institute of Structural Biology of the Weizmann Institute have combined two newly developed experimental techniques, in order directly to prove the significance of the water for the enzymatic functions. The object of their study was matrix metalloproteases (MMP). MMPs can be found outside our cells in the so-called extracellular matrix, where they fulfil central tasks as message transmitters, managers or maintenance units on a molecular level. As a result of the decomposition of the extracellular matrix, the MMP are actively and directly involved in the reconstruction of our tissue, e.g. in embryo or tumour growth and in wound healing. The numerous possible fields of use make this family of enzymes an important field of study for the development of medicines. "The mechanism for the enzymatic activity of the matrix metalloproteases is however not yet known on a molecular level, which poses still challenges on any synthetic drug design," says Prof. Havenith.

Precise characterisation of all "players"

For precise understanding of the reaction, the researchers looked at all the "players" involved: the matrix metalloprotease enzyme as the "lock", its activating substrate - the "key" - and the water as a solvent, the reaction environment. In the experiment, the scientists investigated the binding of the substrate to the MMP. With the help of time-resolved X-ray spectroscopy, they were able to characterise precisely the structural changes in the vicinity of the active enzyme centre (here: of the zinc atom) with atomic resolution. With the help of kinetic THz absorption spectroscopy (KITA), they recorded the changes in time of the fast water movements.

The role of water for future drug design

In various MMP-protein combinations, an unambiguous correlation was

found between the fluctuations of the water network, the structure changes and the function. Molecular dynamic simulations provided an explanation for the observations: While the substrate has not found yet the "correct point" of the enzyme - the lock-, the water dynamism, i.e. the opening and reformation of hydrogen bonds between water molecules (the "terahertz dance" of the water), is fast. At the same time as the substrate is docking onto the active centre, the water movement in the environment slows down. Water then acts then more like a kind of adhesive there, which keeps the substrate at this point. This change of the THz dance of the water with the formation of the enzyme-substrate binding is however exclusively observed in biologically active enzyme-substrate combinations. "The retardation of the water dynamism, observed for the first time, thus appears to be an essential part of the functional control", says Prof. Havenith. "Therefore, in future, taking the role of the water into account in the development of medicines, for example for tumour therapy, might become important."

"Solvation Science@RUB"

This work is part of "Solvation Science@RUB", the research topic of the new center of molecular spectroscopy and simulation of solvent controlled processes at the RUB (ZEMOS), and of the excellence cluster application of the RUB "RESOLV", which is now under review at the German council of science. In chemistry, process engineering and biology, there are an enormous number of publications describing solvents as inert (passive) media for molecular processes. Beyond this traditional view, the active role of the solvent is however becoming more and more visible. New experimental and theoretical methods now permit investigation, description and systematic control of the structure, dynamism and kinetics of complex solvation phenomena on a molecular level. "So it is now most timely to develop general models with a predictive power for solvation processes", says Prof. Havenith. Precisely that is the objective of "Solvation Science@RUB".

More information: M. Grossman, B. Born, M. Heyden, D. Tworowski, G. Fields, I. Sagi, M. Havenith: Correlated structural kinetics and retarded solvent dynamics at the metalloprotease active site. *Nature Structural & Molecular Biology*, Advance Online Publication (AOP), doi:10.1038/nsmb.2120

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