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Oponentský posudek doktorské disertační práce

Jana Hladílková: Ion specific Hofmeister effects on proteins and peptides

In her PhD thesis Jana Hladílková tries to understand the theory behind the Hofmeister series, focusing on the interactions between ions and proteins, as the observed effects of ions on proteins cannot be explained purely by the ion-water interaction and the strength of hydration, as Hofmeister had concluded, but hint to an additional key role of the protein and its structure and physical properties. The protein model systems in her study were the betaine-homocysteine S-methyltransferase BHMT and the haloalkane dehalogenase LinB, as both systems are experimentally studied in collaborating groups either in the Loschmidt laboratories in Brno (LinB) or at the IOCB in Prague (BHMT). Both systems represent real-life systems, and are in the respective groups studied with respect to their enzymatic activities. Additionally, an elastin-like polypeptide served as a pure model system to study ion-peptide interactions, especially for studying backbone vs charged side chain interactions. Jana Hladílková in her study applied classical molecular dynamics with highly advanced analytical methods to study the above mentioned systems in aqueous salt solutions, and confronted her results with experimental data gained in the collaborating groups.

The thesis is written in very good English and contains five chapters: A three page introduction that briefly gives the history of the Hofmeister series and explains the thesis' motivation as well as the research focus. So already after the first three pages the reader gets a very good picture what to expect further and can read the next chapter, named "synergy of theory and experiment" with a clear understanding on what she or he should focus on. The 9 pages of this chapter are giving an overview about the used methods, including essentials for understanding the experimental methods used in the collaborating labs. Chapters 3 and 4 then describe the results for the ion-protein interactions and the ion-peptide-interactions, respectively, connecting the individual papers, giving additional information and interpretation and sorting out what was done by the candidate and what by the various collaborators. Both chapters make up nearly 30 pages, and make the thesis a kind of a hybrid between a collection of papers with a short comprehensive introduction and a "full" thesis that gives all results and details in the thesis. I appreciate the way the thesis is balanced between these two extremes, which makes it easy to read. The reader gets fast the most important information, orients himself well in the thesis, and doesn't need to go back forth and back between text and attached papers all the time. Well done!

The thesis then finishes with two pages of conclusions, summing up the main findings of the candidate's scientific work: 1. LinB follows the cationic Hofmeister series, which can be explained by the weak binding far from the active site at the mouth of the entrance tunnel that results in a moderate influence on the enzymatic activity. 2. BHMT does not follow the Hofmeister series, but potassium binding does dramatically enhance the activity, which can be explained with its ability to lose its water molecules easily in the limited space of the active site. 3. Anionic binding with neutral residues is dominated by the backbone and follows the anionic Hofmeister series. 4. For charged residues the sidechains play the key role and a reversed Hofmeister series is observed i.e. the stronger hydrated is the anion, the more it interacts with the charged sidechains.

The thesis is backed up by seven papers, on three of which Jana Hladílková is the first author. It is necessary to note that one of the included papers has been published as a full paper in the Journal of the American Chemical Society with an impact factor of 11.4 and has gained to date already 46 citations, which is a remarkable number of citations just 2 years after publication. Altogether her publications were cited 82 times according to the Web of Science (2.9.2014), which demonstrates that her research is well recognized by the scientific community.

Questions for the defense that should be addressed by the candidate:

1. If I interpret your results correctly, they indicate that in case of the presence of non-specific interactions (non-specific in the sense of not site targeted) the proteins follow the Hofmeister series for both, cations as well as anions. In case there are specific site-targeted interactions in the active site, or that specific interactions with the charged side chains prevail, the Hofmeister series is not valid and each case needs to be studied individually, and the ion order depends on the nature of the specific interaction (for example in case we have only interactions with charged side chains outside the active site the series is reversed). Do you think that this statement would hold generally or could this be specific for your limited number of selected model systems only?
2. In chapter 3.3. you report unpublished QM/MM results that were conducted to determine the most probable time of proton release. However you conclude that you cannot answer the question if the proton is transferred directly to the bulk or whether it moves as an ion pair (though you can answer that it is released when the halide anion is still present and no other molecule occupies the active site). From the information you give it seems that polarization of the MM part is not included in your calculations. Do you think that including MM polarization for example by the method of induced dipoles, where the induced dipole on a particular atom (calculated from atomic polarizability) is approximated by the set of induced charges on the neighboring atoms, could improve your calculations and solve the still open question mentioned above?

Finally, it is my pleasure to state that Jana Hladílková until now conducted internationally recognized high quality science. The well written thesis that tells a thrilling story and the publications that back up the thesis show, without leaving any doubts, that the applicant fulfills all criteria for being awarded a PhD degree. Therefore I can fully recommend Jana Hladílková for being awarded the PhD degree.

(Český doplněk: Jana Hladílková jasně prokázala tvůrčí schopnosti, práce bez sebemenších pochybů splňuje požadavky kládené na disertační práce v oboru fyzikální chemie)



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