A PHOSPHORESCENCE METHOD TO PROBE PROTEIN DYNAMICS

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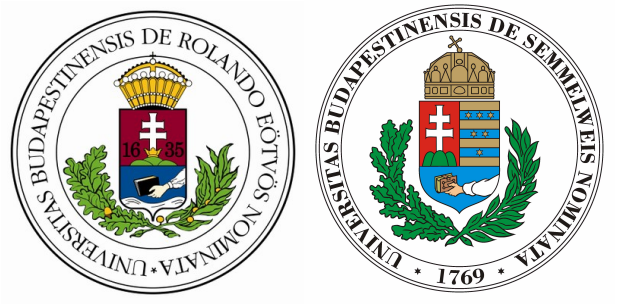

INTRODUCTION

In the recent years the importance of dynamics in the understanding of protein function is gaining additional importance. Several methods are available, however each of them can only cover a slice of the extremely wide time-scales of dynamical events. Many enzyme-cycles have a characteristic time of about a few ms, thus this timescale is of special importance. However, it is exactly this timescale, which is hardly accessible, especially in large systems. during my PhD work I have developed a new phosphorescence based method, which is independent of the size of the system, to study the activation of ms timescale, collective dynamics in proteins. I present the method in the case of human hemoglobin,together with results on the dissociation constant, and isothermal compressibility. Since it is important to know, if the new method is generally applicable, I also show results on two other proteins: phosphoglycerate kinase, and dUTP-ase. In both cases relevant information can be gathered by the new method.

The method is briefly the following: the protein is fast-frozen to 10 K temperature, and then heated in 5 - 10 K steps. During the heating phase the average phosphorescence lifetime is measured. It is important that no glass-forming substances, such as glycerol, are added to the sample. In such an experiment, the average lifetime does not change significantly up to about 180 K, then, around 190 - 210 K a sharp, 50 - 90 % lifetime decrease can be observed. This reduced lifetime can be measured in the next few temperature steps, and then in a second step the phosphorescence signal decreases below the detection limit of our instrument. The two above steps conform to two consecutive transitions of the system. I will show the results of my PhD work in the form of questions, and then answers.

QUESTIONS

1. What is the cause of the transitions?
   - Is it specific to the protein, or is it caused by a phase-transition of the solvent? Can the two steps be related to two different transitions? What can the two steps be assigned to?
   - Can the transition originate from the chromophore itself? Does it depend on the type of the chromophore?
   - If one of the transitions is characteristic of the protein, then is it in a native state? Does the freezing process influence the structure of the protein?
2. What type of thermodynamic model can be set-up to describe the transition?
   - What kind of parameters can be calculated?
   - How realistic are these parameters?
   - Do these parameters relate to the function of the protein?
   - If the function of the protein is changes, does it reflect in the parameters?
3. How can the parameters, and their changes be related to other experimental results?

   Two experiments are considered:

   a) Tetramer $\rightarrow$ dimer dissociation constant in hemoglobin.

      I have determined the tetramer $\rightarrow$ dimer dissociation constant of hemoglobin by a pressure-perturbation method, under various conditions. In this study the following questions were raised:

      i. Does only the quaternary structure change in the experiment?
      ii. Can pressure-induced denaturation cause the observed effects?
      iii. How can we interpret the pressure-induced changes?
      iv. What type of model can be formulated?
      v. How do the allosteric effectors influence the results?
      vi. How can the results be related to the results of the phosphorescence experiment?

b) Changes in the isothermal compressibility by the presence of allosteric effectors:

With a fluorescence line narrowing experiment the inhomogeneous population density function (IDF) can be calculated. From the IDF with the help of a model the isothermal compressibility can be calculated, or estimated. We have answered the following questions:

i. Does the IDF shift along the energy scale in a linear way by pressure perturbation?

ii. Since the compressibility is proportional to the slope of the shift, does this slope depend on the presence of allosteric effectors?

iii. How can the results be related to the results of the other experiments?

4. What general conclusions can be drawn from the results obtained on hemoglobin?

5. Can the above conclusions be generalized? How can we apply the phosphorescence method in other proteins?

a) Is there an transition in phosphoglycerate kinase (PGK) ?

i. How can we interpret the results?

ii. Are the new results relevant for the understanding of PGK function?

b) What do we learn from the dynamics of dUTPase by the application of the new method?

ANSWERS - RESULTS

1. We have shown that the first transition around 180 - 210 K is specific for a dynamical transition of the protein.

- The second step above 250 K can be related to a phase-transition of the solvent. We have confirmed this by a direct experiment, in which only a chromophore was embedded in the solvent, without the protein: in this case only the second transition could be observed, without the first. The temperature of the second transition is close to the melting-point of the solvent, which further supports this assignment.

- We have shown that the chromophore used in hemoglobin does not have any transition itself in the temperature range used in the experiments. We have also shown, that if the tryptophan was used as a chromophore, then the same transition can be observed, thus the transition is independent of the type and position of the chromophore.
- Comparing several freezing protocols, we have elaborated a method by which the structure of the protein is maintained during the process, without any significant structural changes or denaturation. We have confirmed this by FTIR and UV-VIS spectroscopy.

2. We have developed a simple model to describe the first transition corresponding to the protein.

   We assume that the system can be in two distinct states: at low temperature in the dynamically inactive state, in which the diffusion of quencher molecules is effectively inhibited, thus the quenching rate is negligible, and the average lifetime is long. When the temperature is raised, the dynamics of the protein matrix becomes activated, which results in an increased diffusion, and thus in an increased quenching, which corresponds to a drop of the average lifetime. Since the transition is independent of the type and position of the chromophore, it can be concluded that the activated dynamics is of collective, global nature. Since the phosphorescence lifetime is sensitive to events in the ms timescale, thus in this type of experiment the ms timescale dynamics can be monitored.

   - The activation energy and entropy of the global dynamics can be calculated from the model.
   - The calculated parameters correspond to a transient cavity of the size of a few amino acids. Such cavities are hypothesized, e.g., in myoglobin.
   - The parameters are sensitive to the presence of allosteric effectors, thus to the alteration of protein function.

3. Results of the additional experiments:

   a) We have developed a model to describe the dissociation-related spectroscopic changes.

- We have shown that there is no denaturation, or significant tertiary structural change of the structure during the pressure-perturbation experiment.
- We have shown that the pressure-related spectral changes can be related to a tetramer to dimer dissociation.
- Treating the shape of the spectra as a linear combination of the spectrum of the tetramer and the dimer, from the pressure dependence it is possible to determine the dissociation constant.
- We have shown that on both R and T states the dissociation constant of hemoglobin is susceptible to the presence of allosteric effectors.
- Global, collective dynamics may have a significant influence on the dissociation constant.

b) We have shown that the IDF shifts linearly with pressure.

- The allosteric effectors influence the slope of this shift in accordance with their efficiency, however the effect is smaller than in other experiments.
- It can be assumed, that a change in the collective dynamics is responsible for the observed changes in the isothermal compressibility.

4. It can be assumed, that collective, ms timescale dynamics plays an important role in the function of allosteric effectors. They partly exert their action by modifying the collective dynamics. It can be assumed, that the role of collective, ms timescale dynamics in the regulation of protein function is a general phenomenon. A possible interpretation can be, that the collective dynamics encodes functional information, which can be read-out simultaneously by distinct remote parts of the structure.

5. We have observed a similar transition in both PGK and dUTPase as seen in hemoglobin. This supports the conclusion that it is a general phenomenon.

   a) In the case of PGK we have determined the activation energy and entropy of four different constructs.

      i. We have shown that the two domains of PGK can be activated in an asymmetric way, however in both domains the activation is sensitive to the presence of the other domain.

ii. We have shown that in PGK there is an alternative dynamical communication between the two domains besides the already known hinge-bending motion.

b) In dUTPase, it can be concluded from the preliminary data that the C terminal arm, which plays an important role in the catalysis, is strongly coupled to the solvent in the apo-enzyme, but its dynamics is determined by the rest of the protein in the substrate-bound form.

PUBLICATIONS

1. I. K\"{o}vesi, G. Schay, T. Yonetani, M. Laberge, and J. Fidy. High pressure reveals that the stability of interdimeric contacts in the r- and t-state of HbA is influenced by allosteric effectors: Insights from computational simulations. *Biochim Biophys Acta*, 1764(3):516 - 521, 2006.

2. G. Schay, L. Smeller, A. Tsuneshige, T. Yonetani, and J. Fidy. Allosteric effectors influence the tetramer stability of both r-and t-states of hemoglobin A. *Journal of Biological Chemistry*, 281(36):25972 - 25983, 2006.

3. G. Schay, L. Herényi, M. Kellermayer, K. Módos, T. Yonetani, and J. Fidy. Millisecond time-scale protein dynamics exists prior to the activation of the bulk solvent matrix. *The Journal of Physical Chemistry B*, 115 (19):5707 - 5715, 2011.

OTHER PUBLICATIONS

1. G. Schay, L. Herényi, A. Kaposi, L. Smeller, and J. Fidy. Collective, ms timescale dynamics may play a signifcant role in the allosteric regulation of hemoglobin. (under submission).

2. G. Schay, L. Herényi, K. Szigeti, A. Kaposi, L. Smeller, and J. Fidy. Allosteric effectors have an asymmetric influence on the compressibility of hemoglobin subunits - a fluorescence line narrowing study. (under submission).

3. G.Schay, L. Herényi, J. Fidy, and Sz. Osváth. Role of Domain Interactions in the Collective Motion of the Phosphoglycerate Kinase *Biophysical Journal*, submitted.

4. G. Schay, R. Galántai, M. Laberge, and J. Fidy. Protein matrix local fluctuations and substrate binding in HRPC: A proposed dynamic electrostatic sampling method. *International Journal of Quantum Chemistry*, 84 (2):290 - 301, 2001.

CONFERENCES

1. Laberge M., Galantai R., Schay G., and Fidy J. Dynamic electrostatic selectivity in the binding of HRPC to small aromatic substrates. *Biophysical Society Annual Meeting 2001,* feb.18-21, Boston. [poster]

2. Schay G., Laberge M., Szigeti K., Kövesi I., Smeller L., Tsuneshige A., Yonetani T., and Fidy J. Role of dynamics in the influence of allosteric effectors on the oxygen binding of Human Hemoglobin. *World Biophysics Congress,* 2005.sep.1-4, Montpelier. [poster]

3. Schay G., Kövesi I., Smeller L., Laberge M., Yonetani T., and Fidy J. The interdimeric interface of Human HbA is influenced by the binding of allosteric effectors in both R and T quaternary states. *Semmelweis University Faculty of Pharmacy 50 years anniversary int. symposium* 2005, oct.12-15, Budapest. [poster]

4. Schay G., Smeller L., Yonetani T., and Fidy J. Allosteric effectors influence the tertiary structure of R and T states of Hemoglobin A : evidence for conformational changes at the interdimeric interface. *Biophysical Society Annual Meeting 2006,* feb.18-23 Salt Lake City, (int.student travel grant) [poster]

5. Schay G., Smeller L., Yonetani T., and Fidy J. Changes at the intersubunit interface of human Hemoglobin A upon effector binding does not result in a (largeb[1]) compressibility change at the heme pocket. *XLIV. EHPRG Meeting*, 2006, sept. 4-8, Prague. [poster]

6. Schay G., Smeller L., Yonetani T., and Fidy J. Changes at the intersubunit interface of human Hemoglobin A upon effector binding does not result in a (large) compressibility change at the heme pocket. *9th International Summer School on Biophysics SUPRAMOLECULAR STRUCTURE AND FUNCTION 2006*, sept. 16-28,Rovinj, Red Island. [presentation]

7. Schay G., Smeller L., Szigeti K., Kaposi A.D., Yonetani T., and Fidy J. Changes at the interdimeric interfaces of Human Hemoglobin

1 Az eredeti címből ez kimaradt

A upon effector binding shows the importance of global tertiary structural changes in the regulation of Hb function. *Joint meeting of the Hungarian and German Biophysical Societies 2007*, may. 17-20, Hünfeld. [presentation]

8. Schay G., Smeller L., Yonetani T., and Fidy J. Changes at the intersubunit interface of human Hemoglobin A upon effector binding does not result in a (large) compressibility change at the heme pocket. *COST D30 Meeting* 2007, oct. 26-27, Bordeaux. [poster]

9. Schay G., Osváth Sz. , Yonetani T., and Fidy J. The role of collective, ms timescale dynamics in the regulation of multi-domain proteins. A phosphorescence approach. *Biophysical Society Annual Meeting 2008,* feb.17-22, Long Beach. (int.student travel grant) [presentation]

10. Schay G., Osvath Sz., Smeller L., Kaposi A.D., and Fidy J. Method to characterize the global dynamics of proteins by temperature dependent phosphorescence lifetime measurements. Application for the allosteric effect in Hemoglobin. *Biophysical Society Annual Meeting 2009,* feb 28-mar.4, Boston. [presentation]

11. Schay G., Osvath Sz., Herényi L., and Fidy J. Kollektív, ms idõskálájú mozgások vizsgálata PGK esetében - domain kölcsönhatások szerepe a dinamika aktivációjában. *A Magyar Biofizikai Társaság XXIII. Kongresszusa* 2009, aug. 23-26, Pécs. [presentation]

12. Schay G., Fekete M., Kardos J., Kellermayer M., Pongor Cs., and Fidy J. Self-association of the hsRad51 recombinas on ssDNA. Studies by combined higy pressure and fluorescence methods. *12th International Conference on Methods and Applications of Fluorescence* 2011, sept. 11-14, Strasbourg. [poster]

13. Fekete M., Schay G., Kardos J., Pongor Cs., Kellermayer M., and Fidy J. Effect of DNA binding on the self-association of the human recombinase protein Rad51. *8*$^{\text{th}}$ *EBSA European Biophysics Congress* 2011, aug. 23-27, Budapest.

14. Schay G., Fekete M., Kardos J., Kellermayer M., Pongor Cs., and Fidy J. Effects on the self-association of the human recombinase Rad51. *49th European high pressure research group conference* 2011, aug. 28 - sept. 2, Budapest. [poster]

15. Schay G., Fekete M., Kardos J., Kellermayer M., and Fidy J. Self-Association and DNA Binding of hsRad51 Studied by Pressure Perturbation Spectroscopy *Biophysical Society Annual Meeting 2012,* feb.24-29, San Diego [poster]

16. Fidy J., Fekete M., Kardos J., Schay G., Vass K., and Wesolowska M. Conditions inducing the helical filament formation of the recombinase hsRad51 in solution and bound to ssDNA. *Prague protein spring meeting* 2012 may 3 - 6, Prague [poster]