X-ray Absorption Spectroscopy and Coherent X-ray Diffraction Imaging for Time-Resolved Investigation of the Biological Complexes: Computer Modelling towards the XFEL Experiment

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X-ray Absorption Spectroscopy and Coherent X-ray Diffraction Imaging for Time-Resolved Investigation of the Biological Complexes: Computer Modelling towards the XFEL Experiment

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Abstract. The development of the next generation synchrotron radiation sources – free electron lasers – is approaching to become an effective tool for the time-resolved experiments aimed to solve actual problems in various fields such as chemistry, biology, medicine, etc. In order to demonstrate, how these experiments may be performed for the real systems to obtain information at the atomic and macromolecular levels, we have performed a molecular dynamics computer simulation combined with quantum chemistry calculations for the human phosphoglycerate kinase enzyme with Mg containing substrate. The simulated structures were used to calculate coherent X-ray diffraction patterns, reflecting the conformational state of the enzyme, and Mg K-edge X-ray absorption spectra, which depend on the local structure of the substrate. These two techniques give complementary information making such an approach highly effective for time-resolved investigation of various biological complexes, such as metalloproteins or enzymes with metal-containing substrate, to obtain information about both metal-containing active site or substrate and the atomic structure of each conformation.

1. Introduction

Experimental investigation of protein dynamics is a problem of great importance because the conformational changes of the biological complexes in most cases determine their functionality. The solution of this problem becomes possible with the development of next generation synchrotron radiation sources – free electron lasers, providing extremely short pulses with ultrahigh brilliance sufficient for a single molecule imaging. A technique for collecting and evaluating scattering data from such kind of X-ray sources is called Coherent X-ray Diffraction Imaging (CXDI). CXDI

measurements can provide information on the 3D structure of the sample [1-3], e.g. virus, protein or enzyme, however the detailed information about a substrate or an active site cannot be obtained. These tiny changes in the active site may be a key point in understanding of the chemical processes, as in case of iron oxidation in hemoglobin [4] and changes in Fe-porphyrin geometry [5]. For metal containing proteins, such as hemoglobin, X-ray absorption spectroscopy can be applied to investigate atomic and electronic structures of the local environment around the absorbing atom. Being successfully applied to metalloproteins [6] X-ray absorption methods were recognized as an important tool in biophysics to investigate metal binding sites in macromolecules. In this work we suggest a complex technique which combines CXDI and XANES study to show how the complementary structural information can be obtained.

2. Materials and methods

A human phosphoglycerate kinase (PGK) [7], an enzyme which plays an important role in the glycolysis, was taken as a sample object. The initial structure of human PGK (Figure 1a) was extracted from the RSBS Protein Data Bank, reference number 2XE7 [8]. The two domains of this enzyme host 1,3-phospoglyceric acid (3PG) and Mg containing adenosine diphosphate (MgADP) and are associated with large scale conformational changes.

Molecular dynamics simulations were performed in Fujitsu Scigress Explorer Ultra version 7.6 using Alligner's MM3 augmented force field [9]. Further refinement of the enzyme active site was carried out using the PM7 Hamiltonian of the Molecular Orbitals Package [10].

CXDI patterns were calculated by means of the MOLTRANS program developed by Dr. E. Weckert in DESY. The Mg K-edge XANES spectra were simulated using the full multiple scattering approach of FEFF8.4 code [11] with the Hedin-Lundqvist exchange correlation potential. All calculations were performed on the Intel Core i7 personal computer with 8 GB RAM.

3. Results and discussion

3.1. Molecular dynamics simulations

The total energy of the system was constructed from a number of terms corresponding to bond stretches, bond angles, dihedral angles, improper torsions, van der Waals interactions, electrostatic potentials, and hydrogen bonding energies. The van der Waals cut-off distance was set to 9 Å. The enzyme was surrounded by a 9 nm sphere of water. Prior to the molecular dynamics simulation, the initial geometry had been optimized by molecular mechanics using the same force fields with the parameters described above. The time step was set to 1 fs with data recording every 50 fs.

Calculations were performed at different simulated temperatures from 200 to 450 ºK, affecting the kinetic energy per degree of freedom, and indicated that the process is temperature-dependent with PGK being more stable in close conformation at low temperatures, and remaining in the open conformation when heated above 370 ºK. The resulting structure obtained by MD simulation at 300 ºK is shown in the Figure 1b.

3.2. CXDI

CXDI patterns were calculated for the wavelength $\lambda = 1.24$ Å and the range of the scattering angles 2θ from 0 to 7.125° which resulted in the resolution of 10 Å which was shown to be sufficient for determining different conformations of the enzyme (see figure 2). If one does a reconstruction of such a pattern the projection of the electron density in the chosen direction can be obtained. In order to focus on the process of the enzyme transformation from open to close state, the plane of the enzyme closure angle was oriented perpendicularly to the incoming beam. The projections of the enzyme shown in figure 2b are obtained by Fast Fourier Transformation of the calculated patterns with given intensities and phases.

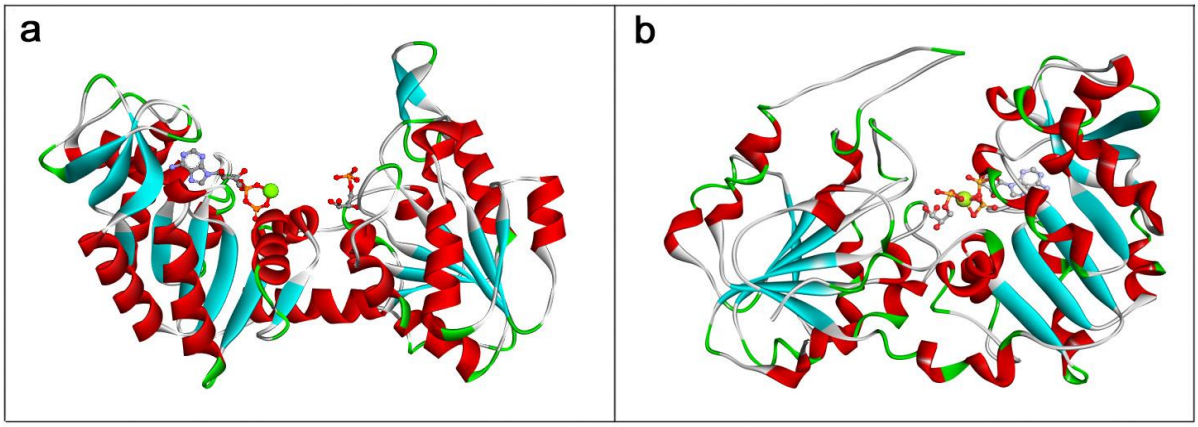

Figure 1. Stable open (a) and close (b) conformations of PGK enzyme obtained from MD. Protein structure shown as a solid ribbon and substrate molecules shown in ball and stick style with green Mg atom scaled for better visualization. Water molecules and hydrogen atoms are not shown.

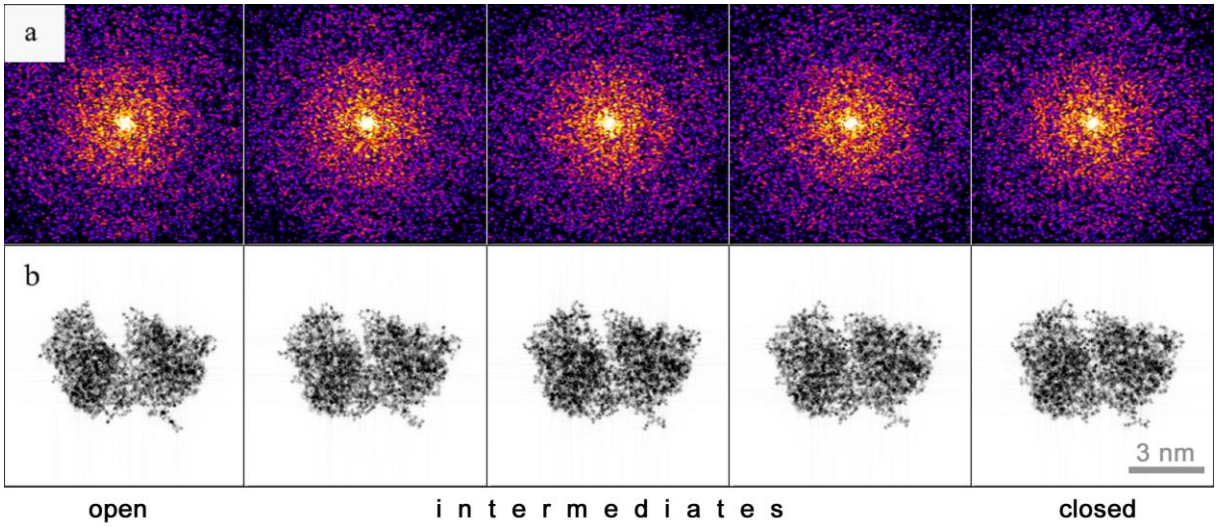

Figure 2. (a) Evolution of CXDI patterns in logarithmic scale for different conformation stages of the enzyme starting from the open stage (left pattern) and finishing with the closed stage (right pattern) and (b) corresponding projections in real space obtained by Fourier Transform of the patterns.

3.3. XANES

In order to obtain accurate structures for XANES calculations, the geometry of active site with MgADP and 3PG and nearest water molecules was optimized using the molecular orbital package MOPAC2012 [10]. According to the optimization results the Mg atom always remains in the octahedral coordination with water and phosphate group's oxygen atoms in the first coordination shell, which is consistent with previous studies of Mn^{2+}ATP complexes [12], analogous to Mg^{2+}ATP.

It is important to note that while Mg atom remains to be octo-coordinated by 6 oxygen atoms, these atoms may belong either to the phosphate groups of the substrate or to the surrounding water molecules. Due to the fact that the displacement of the Mg-O distance in case of Mg-water bonds will be much higher than that of rigid Mg-ADP (or Mg-ATP) bonds, leading to larger broadening of the experimental spectra. As obtained from the MOPAC optimization, in the open conformation Mg atom has 4 water oxygens in the first coordination shell and 2 oxygens of phosphate groups, while in the close conformation 2 water molecules are superseded by additional phosphate group, coming from 1,3-phospoglyceric acid. These results correlate with the experimental studies of ATP complexes with Mn^{2+} ion, having similar to Mg^{2+} chemical properties [12].

XANES spectra (Figure 3) were calculated for the obtained structures using FEFF9 code for spherical clusters with a radius of 13 Å around the absorbing Mg atom extracted from the entire enzyme structure. Full multiple scattering calculations were performed within a sphere of 8 Å, the radius of self-consistent field calculation was set to 5 Å.

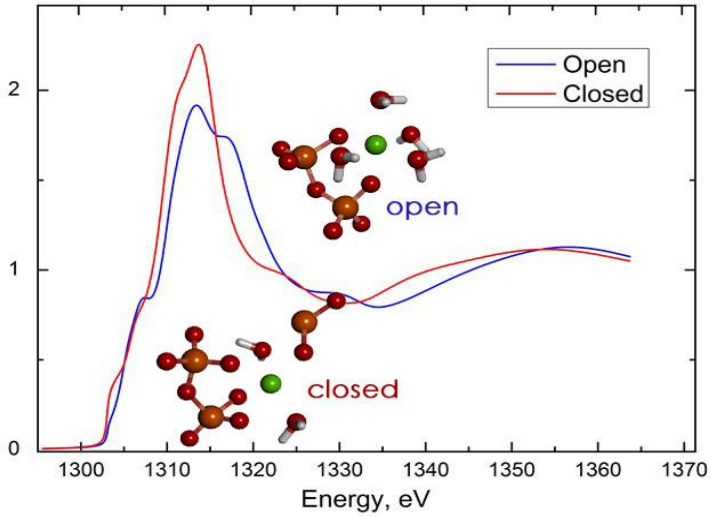

Figure 3. XANES spectra calculated for open (blue) and close (red) conformations of PGK. Atomic structures represent the surrounding of Mg atom (green) by oxygens (red), of phosphates (phosphorus atoms are orange) and water (hydrogens are white).

4. Conclusions

We suggest an approach of measuring combined CXDI and XANES, to follow both the overall state of an enzyme and evolution of local structure around Mg atom. This approach might be applied to various biological samples, like metal containing proteins, which are associated with conformational changes. Simulations of conformational processes may give additional knowledge about the probability of different conformational structures, aimed to help in experimental data evaluation.

5. Acknowledgments

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6. References

[1] Nugent K 2010 *Adv. Phys.* **59**(1) 1–99
[2] Mancuso A, Yefanov O and Vartanyants I 2010 *Journal of Biotechnology* **149**(4) 229-237
[3] Vartanyants I and Yefanov O 2015 *X-ray Diffraction. Modern Experimental Techniques* O H Seeck and B M Murphy (Singapore: Pan Stanford Publishing) pp 341-384
[4] Bianconi A, Congiu-Castellano A, Dell'Ariccia M, Giovannelli A, Burattini E and Durham P 1985 *Biochem. and biophys. research commun.* **131** 98-102
[5] Bianconi A, Congiu-Castellano A, Dell'Ariccia M, Giovannelli A, Burattini E, Castagnola M and Durham P 1985 *Biochimica et Biophysica Acta* **831** 120-124
[6] Eisenberger P, Shulman R, Kincaid B, Brown G and Ogawa S 1978 *Nature* **274** 30-34
[7] Auerbach G, Huber R, Grättinger M, Zaiss K, Schurig H, Jaenicke R and Jacob 1997 *Structure* **5** 1475-1483
[8] Zerrad L, Merli A, Schröder G F, Varga A, Gráczer E, Pernot P, Round A, Vas M and Bowler M W 2011 *J. of Biol. Chem.* **286** 14040-14048
[9] Allinger N, Yuh Y and Lii J H 1989 *JACS* **111** (23) 8551–8582
[10] Stewart J P 2007 *J. of Mol. Mod.* **13** 1173-1213
[11] Rehr J and Albers R 2000 *Rev. Mod. Phys* **72** 621-654
[12] Belli M, Scafati A, Bianconi A, Burattini E, Mobilio S, Natoli C R, Palladino L and Reale A 1983 Il Nuovo Cimento 5 1281-1304