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Anisotropic Solute and Solvent Diffusion in Protein Crystals

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1. Introduction

Biocatalytic routes that use proteins (enzymes) as tools for accomplishing industrially important chemical reactions in a stereo-, regio-, and chemoselective way require active proteins, which are stable and easy to handle over extended periods of use in production processes. Crystallization and successive cross-linking of proteins is one of the possible ways to improve the stability of proteins against mechanical, thermal and pH stresses in real processes by retaining their catalytic activity. However, in such protein crystals, the access of reactant molecules to the catalytic active parts of the protein requires diffusion through the internal pore structure of the crystals.

In the present work, we report on recent intracrystalline diffusion studies of small solute molecules and on water as the most common solvent in biocatalysis in lysozyme protein crystals. Solute diffusion was studied by monitoring the uptake of fluorescein molecules using confocal laser scanning microscopy (CLSM). Water diffusion measurements were performed by pulsed field gradient nuclear magnetic resonance (PFG NMR).

2. Materials and Methods

Lysozyme protein crystals of tetragonal (6LYT) and orthorhombic (1AKI) structures were synthesized from chicken egg-white lysozyme following the procedures described in ref. [1]. Cross-linked protein crystals were obtained by using glutaraldehyde as cross-linking agent. After synthesis, the protein crystals were filtered from the synthesis solution and rinsed in water.

Confocal laser scanning microscopy measurements of fluorescein diffusion were preformed using the approach and set-up described in ref. [1]. In such studies, the uptake of sodium fluorescein from the aqueous solution surrounding a single protein crystal is observed in time-resolved 3-dimensional microscopic intensity maps. They are analysed using a 3d diffusion model [2] to yield the components of the diffusion coefficients in the three crystallographic directions (D_a, D_b, D_c). For comparison with the NMR data, an averaged diffusion perpendicular to the crystallographic *c*-axis (D_{ab} =½(D_a + D_b)) and an anisotropy ratio ($\eta = D_{ab}/D_c$) were calculated.

For the pulsed field gradient NMR studies of water self-diffusion, a bed of cross-linked protein crystals immersed in water was introduced in a 7.5 mm sample tube. Inside the NMR spectrometer, the tube was chilled to –10 °C. At this temperature, the intercrystalline water is frozen to ice and does not contribute to the intensity of the stimulated spin echo used for the NMR diffusion studies. Due to freezing point reduction in small pores, the intracrystalline water in the protein crystals is still liquid and mobile, which allows its observation by the stimulated echo NMR sequence. The PFG NMR spin echo attenuations clearly deviated from the pattern expected for single component isotropic diffusion. They were analysed using the model of an axisymmetrical diffusion tensor [3], which yields diffusion coefficients parallel (D_{par}) and perpendicular (D_{perp}) to the main axis. An anisotropy ratio ($\eta = D_{perp}/D_{par}$) was also calculated.

3. Results and Discussion

Table 1 summarizes the results for the diffusion studies of fluorescein and water in tetragonal and orthorhombic lysozyme protein crystals. The largest component of diffusion of water in the micropores (D_{par}) is more than one order of magnitude smaller than that of bulk liquid (super-cooled) water at the same temperature. For the fluorescein molecule, diffusion in cystallographic *c*-direction, which represents the direction with the larger pore diameter [2], is reduced by more than two orders of magnitude compared to the corresponding value in liquid water. These reductions of diffusivities reflect the interaction of the diffusant with the protein and the pore structure. They are larger for the fluorescein because steric hindrances are more pronounced for larger diffusants.

Fluorescein and water diffusion coefficients in lyozyme protein crystals depend on the direction with respect to the crystal orientation. Perpendicular to the largest components it is less than one order of magnitude smaller than D_c and D_{par}, respectively. The anisotropy ratios for fluorescein and water diffusion η are in reasonable agreement with each other. Possible reasons for the observed diffusion anisotropies are differences of the intracrystalline pore geometries and pore diameters in the three crystallographic directions of the protein crystal.

Table 1: Diffusion of fluorescein and water in lysozyme protein crystals.

		fluorescein by CLSM method non-cross-linked crystals			water by PFG NMR method cross-linked crystals		
crystal structure	PDB name	D_c 10^{-13} m²/s	D_{ab} 10^{-13} m²/s	η	D_{par} 10^{-11} m²/s	D_{perb} 10^{-11} m²/s	η
tetragonal	6LYT	3.3 ± 0.2	0.5 ± 0.1	0.15	5.7 ± 0.5	0.9 ± 0.3	0.16
orthorhombic	1AKI	7.0 ± 0.5	1.1 ± 0.2	0.15	5.6 ± 0.5	0.6 ± 0.2	0.11
temperature		(289 ± 1) K			(263 ± 1) K		

References

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