Poster

EFFECT OF SOL-GEL CONFINEMENT ON THE BIOPHYSICAL PROPERTIES OF THE ENZYME BOVINE CU, ZN SUPEROXIDE DISMUTASE USED IN BIOSENSORS

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Immobilization of proteins in sol-gel glasses has allowed the development of a new generation of robust and sensitive analytical devices as well as contributes to the investigation of the effect of molecular confinement on the structure of proteins (1). The immobilized protein usually preserves its structural integrity and functionality, while interactions with the matrix and its surface seem contribute to alter its dynamics and stability. With the aim of better understanding the nature of such interactions we have encapsulated the enzyme bovine erythrocyte copper-zinc superoxide dismutase (BSOD) in a sol-gel matrix. This protein has been previously immobilized by our group in sol-gel glasses, to build a fluorescent biosensor for superoxide radical (2), however, an exhaustive characterization of its biophysical properties upon encapsulation has not been carried out to date.

BSOD is a dimeric enzyme of molecular mass 32000 daltons, which catalyses the dismutation of the superoxide free radical to oxygen and hydrogen peroxide, participating in the cellular defense mechanism against oxidative damage. The protein contains a single fluorescent tyrosine residue (Tyr-108) per subunit, which is solvent exposed, and no tryptophan residue. In this study we have encapsulated the protein, at physiological pH, in the porous of silica glass matrixes prepared from tetraethyl ortosilicate through an alcohol-free sol-gel route. Porous walls of sol-gel matrix (pI ~ 2) and protein (pI ~ 4.95) are negatively charged at such conditions. Fluorescence spectra, quenching experiments, fluorescence lifetimes and anisotropy measurements indicate that immobilization does not lead to any major conformational change, at least in the region of protein where the tyrosine residue is located. In addition, time-resolved anisotropy decays, recorded at pH 7.4 and 3.5,

indicate that the internal dynamics of BSOD is preserved upon immobilization, although the protein shows a different rotational behaviour as compared with that in the bulk aqueous solution, especially when the pH is kept below its isoelectric point. Such dynamical behaviour does not alter the protein function, which shows the same catalytic activity upon sol-gel encapsulation.

1. Gupta, R., Chaudhury, N.K., 2007. Biosens. Bioelectron. 22, 2387-2399.
2. Pastor, I., Esquembre, R., Micol, V., Mallavia, R., Mateo, C.R., 2004. Anal. Biochem. 334, 335-343.