Analysis of fulvic acid – protein complexes by radiochemical and spectroscopic techniques

Chernysheva M.G., Shnitko A.V., Konstantinov A.I., Badun G.A.

Lomonosov Moscow State University, Moscow, Russia, chernysheva@radio.chem.msu.ru

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In the present work the interaction between fulvic acid separated from Suwannee River (SRFA) that is IHSS standard and two model globular proteins (lysozyme and albumin) was studied at the liquid-liquid interface as well as in the bulk of an aqueous solution. We used tritium labeled compounds, obtained by means of tritium thermal activation method, for determination direct amount of both SRFA and protein at the aqueous-p-xylene interface and in the solid precipitate. Note that p-xylene was used as model of cellular membrane. Aqueous-air interface was characterized by tensiometry.

It was found that both lysozyme and albumin substitute SRFA from both liquid-liquid and aqueous-air interfaces. Moreover, lysozyme also formed a precipitate with SRFA. Using tritium labeled compounds it was found that molar ratios SRFA:lysozyme in the adsorption layer and in the precipitate were similar, when SRFA concentration was lower than 20 mg/L. At high SRFA concentrations molar ratios SRFA:lysozyme reached 3:1 in the adsorption layer and 2:1 in the solid phase. However, when the mass ratio was higher than 1:2 (SRFA:albumin) albumin loses its secondary structure, while lysozyme preserves its structure as well as an enzymatic activity even increases it being encapsulated by SRFA.

To analyze the interaction in the bulk of an aqueous phase the fluorescent spectra of proteins as well as its mixtures with SRFA was recorded in the range from 290 to 540 nm at excitation wavelength 280 nm to determine the intrinsic fluorescence of tryptophan. Tryptophan fluorescence quenching in the presence of SRFA indicates the interaction between SRFA and both proteins. Formation of complex between albumin and natural organic matter via hydrogen and van der Waals bonds results in the reduction of α -helix of protein, while lysozyme has more stable structure that is stabilized by disulfide bridges. We can suggest that lysozyme preserves its structure being encapsulated by SRFA. Therefore, preservation of the enzymatic activity can be expected.

Enzymatic activity of lysozyme was measured in the relationship to *Micrococcus luteus* cells by means of turbidimetric assay. It was found that SRFA preserves and even increases the enzymatic activity of lysozyme. When SRFA concentration is about 10-20 mg/L, the enzymatic activity reaches the highest value and decreases at SRFA concentration of 50 mg/L, remaining one and a half times that for lysozyme in the absence of fulvic acid. Note that Trp108 is in van der Waals contact with Glu35, one of two catalytic carboxyl groups and hydrophobization of Trp108 enhances the substrate binding ability of lysozyme. The decrease in activity at SRFA concentration of 50 mg/L is probably due to the steric hindrance of the interaction between the enzyme and the substrate because of the possible adsorption of free SRFA on the cell surface. Although, it is important to emphasize that free SRFA did not lead to *Micrococcus luteus* cells lysis.

Therefore, our results suggest that interaction of SRFA with lysozyme does not lead to significant changes in the protein structure as compared with albumin that probably lose its secondary structure being involved in the complex with SRFA.