THE EFFECT OF HYDRATED FULLERENES ON ERYTHROCYTE MEMBRANE

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Introduction

Recently much attention has been given to the study of the biological activity of disperse nanoparticles because of intense nanotechnology development, fullerenes (spherical molecules C_{60} nearly 0,7 nm in diameter) being of special interest. The technique developed to prepare water-soluble fullerene derivatives has made it possible to study the biochemical, biophysical and medical applications of that compounds. Medical use of fullerenes and the derivatives requires considerable precaution since the mechanism of their interactions with biopolymers and cells is investigated insufficiently. This study has been aimed at the effect of hydrated fullerene molecules on red blood cells (RBC) *in vitro*.

Procedures

In our experiments we employed water-soluble fullerenes [C_{60}/C_{70}FWS] prepared by transfer to water phase during ultrasonic sonolysis of toluene fraction of fullerenes C_{60}/C_{70} [1].

Scanning electron microscopy (SEM) has been used to study the morphology of erythrocytes affected by fullerenes. Stability of the cells has been evaluated by the rate of human erythrocyte lysis in presence of [C_{60}/C_{70}FWS] of varied concentration at physiological (autohemolysis) and elevated temperature (thermohemolysis) [2]. The effect of fullerenes on human and trout RBC membrane protein conformational state has been examined by ESR using maleimide spin label that modify the proteins of erythrocyte cytoskeleton spectrin-actin complex. ESR spin probe technique (5-doxylstearic acid (5-DSA)) has been employed to probe membrane annular lipids, i.e. lipid microenvironment of membrane proteins. Catalysing effect of fullerenes on redox reactions in the erythrocyte membrane induced by Fe^{2+} has been revealed by variation of 5-DSA spin probe paramagnetism. Thermally induced structural transitions of erythrocyte ghost membrane proteins have been registered by differential scanning microcalorimetry (DSC).

Results and discussion

[C_{60}FWS] solution of 216 μm concentration represents a transparent brownish colloid dispersion formed both by hydrated fullerenes and their hydrated clusters of different size the least containing 13 hydrated C_{60} molecules of 3-4 nm total diameter whereas the largest amounts to 60 nm [3]. Hydration and stability of fullerenes in solution is a rather complex issue currently considered according to the hydration model of water-fullerene donor-acceptor complex formation [3].

Water-soluble fullerenes of the 3-10 μm final concentration were shown to intensify erythrocyte hemolysis insufficiently, i.e. by 10-20% at 37, 56, 58 and 60ºC with no effect on the activation energy of the process. By using SEM the number of spherocytes was shown to increase to some extent on adding 5 and 10 μm fullerene as compared to control. Alteration of surface appearance may be observed on incubation of erythrocyte suspension for 24 h at 37°C, that is discocytes with a single and plural protuberances as well as echinocytes occur. Such appearance of the cell surface was more often by 20-40% in presence of fullerene. Hence, membranotropic activity of fullerenes exhibits in the alteration of cytoarchitectonics: the fraction of deformed cells increases that leads to the reduction hemolytic stability.

Hydrated fullerenes of 0,1-50 μM concentrations induced the intensification of intramolecular segmental mobility and weakening of interprotein interaction in spectrin-actin complex both in human and in fish erythrocytes. Saturation of the effect was observed at 1-5 μM. Introduction into membrane structure of 5-DSA that obviously localizes preferentially in annular lipids has made it possible to register changes that can be considered as the rise of 5-DSA concentration in annular parts and even some repartition of the probe to the bilayer. This follows from characteristic changes of ESR spectra caused by the rise of spin-probe dipole-dipole interactions.

The data obtained can be accounted for by thickening of spin-probe molecules in the certain membrane parts. At the same time, DSC data

showed that the parameters of thermoinduced transitions of erythrocyte membrane (transition temperature and enthalpy) remain unaltered. This evidences that the interaction of membrane proteins with surrounding phospholipids are unaffected.

To study the oxidation processes the probe has been introduced into erythrocyte membrane and kinetics of the probe paramagnetism has been controlled on adding $FeSO_4$ of 0,04 to 0,8 mM concentration to the erythrocyte ghost suspension in presence and in absence of 20 μM fullerenes. Immediate reduction of the probe in the system was followed by the growth of ESR signal amplitude within 3 min. Growth rate raised pro rata with $FeSO_4$ concentration and increased in presence of fullerenes when $FeSO_4$ concentration exceeded 0,04 mM. Effect of fullerenes was not registered at lower $FeSO_4$ concentrations that may suggest indirectly that fullerenes have no prooxidant properties and they do not produce formation of active oxygen forms. The dissolved oxygen concentration in the vicinity of fullerenes can be raised although. The probe oxidation rate and ESR signal increase were lower sufficiently in presence of 10 % ethanol. This suggests an involvement of hydroxyl radicals in the process.

Likely mechanism of [C_{60}/C_{70}FWS] activity can be as follows. Hydrated fullerene molecules or their clusters with their low negative net charge are localized in the erythrocyte surface vicinal water affecting its properties by virtue of their hydration specificity. Those may be local surface tension and surface relief nearby membrane proteins. This can influence local redistribution of fatty acids and/or interaction of integral proteins. These suggestions require additional verification.

Conclusions

Membranotropis activity of fullerenes has been revealed. The changes of erythrocyte shape, thermostability and structural dynamics of membrane proteins are likely to result from the influence of [C_{60}/C_{70}FWS] on the state of erythrocyte vicinal water.

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