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DIAMIDE-INDUCED MODIFICATIONS OF PROTEINS IN MEMBRANE-CYTOSKELETON COMPLEX OF CRYOPRESERVED ERYTHROCYTES

N. G. ZEMLIANSKYKH, O. E. MAKASHOVA, O. O. MYKHAILOVA, R. K. MIGUNOVA, L. A. BABIJCHUK

Institute for Problems of Cryobiology and Cryomedicine, National Academy of Sciences of Ukraine, Kharkiv; e-mail: nzemliansky@gmail.com

Space-conformation modifications of the proteins in the membrane-cytoskeleton complex of human erythrocytes under the cryopreservation in the presence of different cryoprotective agents (CPAs) were studied using a protein-cross-linking reagent diamide, which oxidizes the sulfhydryl groups of proteins with the development of disulfide cross-links between individual macromolecules and the formation of high-molecular aggregates. Disturbances in spatial orientation of proteins within the membrane-cytoskeleton network or changes in the structural packing of molecules can change the availability of SH-groups to the oxidizing action of the reagent. Analysis of the protein profile of the erythrocyte ghosts (membrane-cytoskeleton complex) with Ds-Na-PAGE can distinguish the changes in proteins involved in the modification during the cell cryopreservation with different CPAs.

The level of diamide-induced aggregation of proteins in human erythrocytes frozen under the protection of glycerol, dimethylsulfoxide (Me_2SO), sucrose, dextran and polyethylene glycol (PEG), largely reflected the effectiveness of CPA protective action. Low levels of protein aggregates in erythrocytes after freeze-thawing in the presence of glycerol, PEG and $M_{e2}SO$ correlated with high safety cells. Meanwhile, a high hemolysis after freeze-thawing with sucrose and dextran, which gives a measure of a degree of cell destruction, was consistent with the formation of a significant amount of diamide-induced aggregates. Consequently freezing erythrocytes in the presence of sucrose and dextran was accompanied by an increase in availability of reactive sulfhydryl groups due to disturbances in the structure of protein macromolecules. The rise in aggregate amount was patently caused by structural disorders of the two main proteins, spectrin and band 3.

Nevertheless, after removal of CPAs only in erythrocytes frozen under glycerol protection, the distribution of protein fractions and diamide-induced aggregation were close to the control cells. In erythrocytes frozen in the presence of Me_2SO and PEG, after removal of the CPAs, the contents of the spectrin and band 3 were decreased.

These facts confirm the possibility of a nonspecific modification of the proteins in the membrane-cytoskeleton complex under the impact of CPAs and freezing, which can affect the physical properties of membranes and the erythrocytes stability.