# PHYSICOCHEMICAL PROPERTIES OF HUMAN ERYTHROCUPREIN

I. M. Vasilets,* M. M. Shavlovskii, and A. P. Il'in

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The present paper gives preliminary information on the characteristics of a copper-containing protein of erythrocytes – erythrocuprein. Erythrocuprein, which was first isolated from bovine erythrocytes [1], has also been found in other mammals, including man [2]. The protein possesses enzymatic activity, catalyzing the dismutation of peroxide radicals ($O_2^-\cdot + O_2^-\cdot + 2H^+ \rightarrow O_2 + H_2O_2$) [3]. Peroxide radicals are formed in many biological redox reactions and may possess a toxic action because of their high reactivity. Dismutase activity has been found in all the representatives of the animal and vegetable kingdoms investigated [4-6], which alone indicates the important biological role of peroxide dismutases, including erythrocuprein.

We obtained erythrocuprein from washed erythrocytes of human blood by a slight modification of Stansell's method [7]. At all stages of isolation and purification, the protein was identified by the presence of copper, by its specific dismutase activity (inhibition of the reduction of Nitro Blue Tetrazolium in the presence of a system producing peroxide radicals) [8], and by the specific absorption maxima at 264 and 675 nm. The erythrocuprein that we isolated appeared as a homogeneous substance on chromatography on DEAE-cellulose and on Sephadex G-75, and on ultracentrifugation it sedimented in the form of a single symmetrical peak with a sedimentation constant of 3.1 S. Electrophoregrams of native erythrocuprein in polyacrylamide gel at pH 8.7 (tris-HCl system) were characterized by the presence of two closely adjacent zones stained by Coomassie R-250 and possessing specific dismutase activity. However, when the native protein was subjected to isoelectric focusing in a 7.5% polyacrylamide gel containing 2% of ampholines pH 3-6, the erythrocuprein was concentrated in the form of a narrow zone with pH 4.88. The protein isolated after preparative isoelectric focusing in polyacrylamide gel had a reduced dismutase activity and when it was resubjected to electrophoresis two zones appeared. In the tris-HCl system, probably, a change in the molecular organization of part of the protein takes place, as also shown by Stansell and Deutsch [9]. The electrophoregrams in polyacrylamide gel in the presence of 0.1% sodium dodecyl sulfate were characterized by the presence of two closely adjacent protein zones corresponding, from the mobility of the proteins, to a molecular weight of about 20,000. The treatment of the erythrocuprein with 2-mercaptoethanol did not affect its behavior on electrophoresis in the presence of sodium dodecyl sulfate. Consequently, the erythrocuprein molecule contains two subunits of similar molecular weight possibly bound by noncovalent bonds.

We were unable to determine the N-terminal amino acid of erythrocuprein by the DNP method, which is apparently due to the blocking of the terminal amino group of the protein. Titration of the C-terminal amino acid by Matsuo's method [10] followed by acid hydrolysis of the protein showed that the whole of the radioactive label was concentrated in threonine, which is the C-terminal amino acid for both subunits of erythrocuprein.

Peptide maps in a thin layer of cellulose of tryptic hydrolyzates of the reduced protein carboxymethylated with bromoacetate labeled with respect to carbon were characterized by the presence of about 30 ninhydrin-positive spots of which two gave intense blackening of an x-ray film on autoradiography. An amino acid analysis [11] showed that the molecule of human erythrocuprein contains 40 arginine and lysine residues and, thus, on tryptic hydrolysis, taking into account the two identical subunits, should form about 20 peptides. The number of peptides that we found (about 30) shows that the erythrocuprein molecule con-

*Deceased.

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sists of two subunits of not completely identical composition. These results agree well with those of an electrophoretic study of the protein.

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