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Absence of γ-Glutamyl Transpeptidase and the Role of GSSG Transport in the Turnover of GSH in Erythrocytes

To the Editor:

In 1964, Hochberg et al.[1] demonstrated the half-life of glutathione in red blood cells to be 2–4 days. Thus even before the confirmation in vivo of the synthesis of this compound[2] the problem of accounting for its rapid turnover had arisen. In the interim, three major hypotheses have been espoused to explain the rapid turnover of this unique tripeptide: (1) the formation of glutathione-protein mixed disulfides; (2) the catabolism of glutathione; and (3) the transport of oxidized glutathione (GSSG) from the red blood cell.

Mixed disulfides. Initially, it was proposed that glutathione may form mixed disulfides with hemoglobin and other proteins of the red blood cell. However, the turnover of glutathione could not be accounted for by this mechanism because glutathione reductase, in the presence of NADPH, was shown to cleave hemoglobin-S-S-G,[3] releasing GSH. Furthermore, even if glutathione reductase did not act in this manner, there was not enough protein in the red blood cell to account for the turnover rate.

Catabolism of glutathione. Glutathione contains a γ-peptide linkage between its glutamic acid and cysteine residues which normal intracellular peptidases, specific for an α-peptide linkage, cannot cleave. The only known enzyme that can cleave this γ-peptide linkage is γ-glutamyl transpeptidase (GGT or γ-glutamyl transferase, E. C. 2.3.2.2). This enzyme transfers the γ-glutamyl moiety of glutathione to an acceptor amino acid and has been proposed to play a role in amino acid transport.[4]

Several investigators have demonstrated the presence of low levels of GGT in red blood cells.[5–7] However, using the identical methods published by these investigators, as well as immunologic techniques, we were unable to demonstrate the presence of GGT or an antigen-like human kidney GGT in human or rabbit erythrocytes.[8,9] Although significant amounts of GGT were present in white blood cells, contamination of the red blood cell preparations by leukocytes could not explain the enzyme levels reported by the previous investigators. Other studies have confirmed the absence of this enzyme in rabbit[10] and now human red blood cells.[11] Thus the catabolism of glutathione by GGT neither accounts for the turnover of this compound nor the transport of amino acids in red blood cells.

Transport of GSSG. In 1969, we[12] demonstrated that GSSG is preferentially and actively transported from the red blood cell under oxidative stress. At that time, however, the substrate kinetics of the transport of GSSG could not be studied due to technical difficulties; therefore no definite conclusion was made as to the role of transport in the turnover of glutathione.

In subsequent investigations, we[13] used red cell ghosts to determine the kinetics of glutathione transport. These studies confirmed the requirement of energy in the form of ATP for the transport of GSSG. In addition, they demonstrated that the transport of GSSG from the red cell ghosts was substantially slower at lower concentrations of GSSG, those closer to physiologic in erythrocytes, as compared to

higher concentrations. Therefore, the transport of GSSG apparently may not account for the rapid turnover of glutathione in red blood cells.

It should be noted, however, that the red cell ghost system is artificial, and definite conclusions concerning the transport kinetics of GSSG cannot be drawn. For example, glucose-6-phosphate dehydrogenase-deficient erythrocytes incubated with 0.14 *M* glucose exhibit a substantial amount of GSSG transport (70 nmoles/ml RBC/hr) when challenged with hydrogen peroxide (unpublished observations), while red blood cell GSSG levels remain closer to physiologic levels during the 4-hr experiment. This finding indicates that GSSG is actively transported from the red cells under oxidative challenge even at physiologic concentrations of GSSG. Indeed, Smith[14] has presented kinetic data on GSSG transport in whole red blood cells, supporting the hypothesis that this mechanism may account for the turnover of glutathione in this tissue.

Although questions still remain, investigations concerning the transport of GSSG from the red blood cell may provide the answer to the turnover of glutathione in this tissue. Certainly, evidence supporting the other two hypotheses has almost been exhausted, and, to our knowledge, no other mechanism has been proposed.

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