Lysis of Normal and Reduced Glutathione-Deficient Sheep Erythrocytes by Tellurite and Selenite

C. CROWLEY, J. D. YOUNG and E. M. TUCKER


Mammalian erythrocytes normally contain high concentrations of the tripeptide GSH.* The major role of GSH in these cells is to protect against oxidative damage, the GSH–GSSG couple acting as a redox buffering system. Sheep exhibit two distinct types of inherited erythrocyte GSH deficiency. One type is associated with a diminished activity of γ-glutamyl-cysteine synthetase, the first enzyme of GSH biosynthesis (Young & Nimmo, 1975). The second type of GSH deficiency is due to the lack of availability of cysteine, a component amino acid of glutathione, resulting from the absence of a specific amino acid-transport system (Young et al., 1975, 1976; Young & Ellory, 1977). Both lesions can be found in the same animal, and erythrocytes from such 'double-low' GSH sheep have a lower GSH concentration than that found with either type of deficiency alone (Tucker et al., 1976).

Lysis of erythrocytes has been shown to occur in the presence of tellurite (Blais et al., 1972), and intracellular GSH appears to be involved in the lytic process (DeMeio & Onischuk, 1974). We have investigated this phenomenon further by studying the effects of tellurite and the closely related selenite on normal and GSH-deficient erythrocytes from sheep.

* Abbreviations: GSSG and GSH, oxidized and reduced glutathione respectively.

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Sheep erythrocytes, washed with 0.9 % NaCl (haematocrit 0.5 %), were incubated with 1 mM-potassium tellurite at 37°C in a medium containing 140 mM-NaCl, 5 mM-KCl, 2 mM-MgCl₂, 0.1 mM-EDTA and 20 mM-Tris/HCl, pH 7.4. Cell lysis was determined by centrifuging the erythrocyte suspension for 20 s at 10000 g in an Eppendorf 3200 microcentrifuge and measuring the A₅₄₀ of the supernatant. Animals were classified as to GSH type on the basis of erythrocyte GSH and amino acid concentrations (Tucker et al., 1976). The curves are the means ± S.E.M. for three animals of each type. ○, Normal cells [2.98 ± 0.28 mmol of GSH/litre of cells (mean ± S.E.M.)]; △, γ-glutamyl-cysteine synthetase-deficient cells (1.56 ± 0.08 mmol/litre of cells); □, amino acid-transport-deficient cells (1.21 ± 0.33 mmol/litre of cells); ●, 'double-low' GSH cells (0.82 ± 0.19 mmol/litre of cells).

Fig. 1 shows the time course of lysis of normal and GSH-deficient erythrocytes from sheep in the presence of 1 mM-potassium tellurite. Cells with one or both lesions lysed considerably less readily than normal cells and the rate of lysis was related to the intracellular GSH concentration. Further experiments showed that before lysis of normal cells there was a decrease of 40 % in the intracellular GSH concentration. Lysis of normal erythrocytes was completely inhibited by pretreatment of the cells with sufficient diamide [diazenedicarboxylic acid bis-(NN-dimethylamide)] (Kosower et al., 1969) to oxidize 95 % of the intracellular GSH. The rate of lysis of 'double-low' GSH cells was increased 8-fold when glucose (5 mM) was included in the incubation medium. Glucose had little effect on the rate of lysis of normal erythrocytes. Incubation of normal erythrocytes with 1 mM-potassium selenite resulted in lysis. The haemolysis, however, was less than that observed with the same concentration of tellurite (40 % and 93 % lysis after 4 h with selenite and tellurite respectively). Selenite (1 mM) did not cause significant lysis (< 5 %) of the three types of GSH-deficient erythrocyte.

Incubation of 'double-low' GSH cells with extracellular GSH and tellurite or selenite resulted in rapid lysis (Fig. 2). Maximum haemolysis occurred at GSH/tellurite (selenite) ratios of 5–10. The shape of the selenite curve suggested the presence of two separate components: one, similar to that seen with tellurite, with a maximum at a GSH/selenite ratio of 5–10 and the other increasing over the entire range of GSH/selenite ratios. The latter component was not observed when the experiment was performed at 0°C.

These results indicate that intracellular GSH is involved in the lysis of erythrocytes by both tellurite and selenite and that at least at high GSH/tellurite (selenite) ratios both compounds act in a similar way. An important intermediate in the complex series of
Fig. 2. Lysis of 'double-low' GSH erythrocytes by tellurite and selenite in the presence of extracellular GSH

'Double-low' GSH erythrocytes were incubated in medium containing 1 mM-GSH and various concentrations of potassium tellurite or potassium selenite. Other experimental details are given in the legend to Fig. 1. ○, Tellurite (10 min at 37°C); △, selenite (3.5 min at 37°C); ▲, selenite (41 min at 0°C).

Reactions between GSH and selenite is 2-seleno-1,3-diglutathione, GS-Se-SG (Painter, 1941; Ganther, 1968, 1971; Sandholm & Sipponen, 1973):

$$4GSH + K_2SeO_3 \rightarrow GS-Se-SG + GSSG + 2KOH + H_2O$$

In the presence of excess of thiol (GSH/selenite ratio >4), GS-Se-SG is rapidly reduced to the highly reactive nucleophile glutathione selenopersulphide, GS-SeH, which may undergo further reduction to form other reactive species (Rhead & Schrauzer, 1974). GS-SeH can also be formed from GS-Se-SG enzymically by the NADPH-dependent glutathione reductase (Ganther, 1971). Our findings suggest that erythrocyte lysis by selenite and tellurite may be mediated by one or more of the reactive intermediates. The results further indicate that lysis by tellurite or selenite could be used as a means of detecting GSH-deficient erythrocytes. The lytic action of selenite explains the haematological symptoms associated with selenium poisoning (see review by Moxon & Rhian, 1943).

This work was supported by a project grant from the Medical Research Council.

Ganther, H. E. (1968) *Biochemistry* 8, 2898-2905

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