PRELIMINARY NOTES

BBA 71004

Repair of radiation damage to membrane sulfhydryl groups of human erythrocytes

Evidence has recently accumulated that radiation damage to erythrocyte membrane function involves membrane sulfhydryl groups. Thus, incubation of erythrocytes with sulfhydryl-blocking agents results in similar changes in the passive permeability to cations as does exposure to X-rays[1-3]. Furthermore, irradiation of erythrocytes with doses producing potassium leakage leads to a disappearance of titratable membrane –SH groups which can partly be accounted for by the formation of disulfide groups[2].

It is the purpose of the present communication to demonstrate that the radiation-induced loss of titratable membrane –SH groups is partly reversed when the erythrocytes are allowed to metabolize after exposure under conditions known to give partial repair of radiation-induced permeability changes.

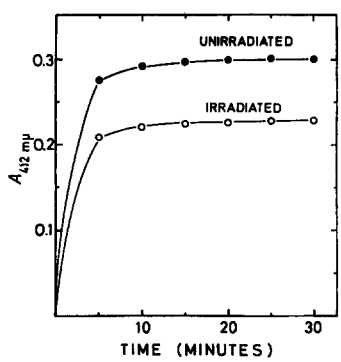

Fig. 1. Titration of sulfhydryl content of membranes isolated from irradiated erythrocytes. Fresh human erythrocytes were washed 3 times with buffered saline (0.01 M sodium phosphate buffer, pH 7.2, in 0.9% sodium chloride), suspended at a hematocrit of 15%, and irradiated at 4° in the presence of air with 196 kR of X-rays. The dose rate was 5.2 kR/min. Immediately after exposure hemoglobin-free membranes were isolated[2,6]. The unirradiated control cells were similarly treated. The protein concentration[7] was adjusted to the same level in all samples (0.86 mg/ml) and sulfhydryl titrations were carried out at pH 8, according to the method of ELLMAN[8], using 5',5-dithiobis-(2-nitrobenzoic acid) (DTNB) in the presence of 1% sodium lauryl sulfate. The abscissa represents the time of color development after addition of DTNB. The maximum absorbance values, recorded after 30 min, were used to calculate the –SH content. Taking into account the dilutions, and using a molar extinction coefficient of 13 600 (ref. 8), an A value of 0.1 corresponds, in the present experiments, to $1.8 \cdot 10^{-8}$ moles of –SH per mg of membrane protein.

When fresh human erythrocytes were irradiated at 4° with 196 kR of X-rays, the –SH content of the membranes isolated immediately after the exposure was 25% lower than in those isolated from unirradiated control cells (Fig. 1). The effect

Abbreviation: DTNB, 5,5-dithiobis-(2-nitrobenzoic acid).

TABLE I

EFFECT OF POST-IRRADIATION TREATMENT OF ERYTHROCYTES ON THE –SH CONTENT OF THEIR MEMBRANES

The cells were irradiated at 4° as described in the legend to Fig. 1. After exposure the cells were kept for 1 h, either at 4° or at 37° in the absence or presence of 0.03 M glucose. Hemoglobin-free membranes were isolated and the sulfhydryl groups titrated as in Fig. 1. Unirradiated controls were similarly treated. In each case the percentage loss of membrane –SH groups was calculated from the maximum absorbances of samples from irradiated and unirradiated cells.

Conditions	*Loss of –SH groups*		*Repair (%)*
	No glucose (%)	*Glucose (%)*	
Incubation temperature 4°	25.7 ± 1.56*	24.4 ± 3.52	—
Incubation temperature 37°	24.0 ± 3.53	10.0 ± 0.65	61

* Standard deviation of the mean.

of various post-irradiation treatments of the cells is shown in Table I. It can be seen that when the irradiated cells were incubated for 1 h at 4°, in the presence or absence of glucose, the radiation-induced loss of membrane –SH groups was the same (about 25%) as immediately after the exposure. This was also the case in cells kept at 37° in the absence of glucose. However, when the cells were incubated at 37° in the presence of glucose, the –SH content of their membranes was only 10% lower than in the unirradiated control cells. Since the membrane –SH content of the unirradiated cells was found to remain constant during the incubation, the results demonstrate that in the irradiated cells kept at 37° in the presence of glucose the concentration of membrane –SH groups increased during the incubation. Apparently, under these conditions repair processes were operating which resulted in the recovery of about 60% of the –SH groups that had disappeared upon irradiation.

Previously it has been found that the radiation-induced changes in passive cation permeability of erythrocytes can be partly reversed when the cells are kept at 37° in the presence of glucose, while no such reversal occurs at low temperature[2,4,5]. The repair in membrane function under such conditions could be explained by the recovery of membrane –SH groups here observed. The recent demonstration of SHAPIRO, KOLLMAN AND ASNEN[1] that radiation-induced permeability damage to erythrocytes may be partially repaired by addition of a thiol, mercaptoethylguanidine, after the exposure, is also consistent with such a mechanism. It should be emphasized that blocking of merely a small fraction (4–18%) of the membrane –SH groups may lead to marked changes in the permeability to cations[3].

In view of the finding that irradiation of erythrocytes leads to oxidation of some of the membrane –SH groups to disulfide groups[2], is seems likely that the observed recovery of –SH groups after irradiation was due to a partial reduction of disulfide groups formed by radiochemical oxidation. Such a reduction could possibly involve the glutathione reductase system. The finding that little or no repair occurred when the cells were incubated in the absence of glucose is consistent with such a mechanism. Work is in progress to test this possibility.

Whatever the mechanism responsible for the recovery of membrane –SH groups after irradiation, the phenomenon as such seems to be of considerable interest.

In view of the established role of –SH groups in the maintenance of the structure and function of the erythrocyte membrane, it seems possible that the repair process involving membrane –SH groups may be of importance to the erythrocyte under various physiological and pathological conditions.

This work was supported by the National Center for Radiological Health, U.S. Public Health Service. R.M.S. is a Fellow in Radiological Research of the James Picker Foundation.

Norsk Hydro's Institute for Cancer Research,
The Norwegian Radium Hospital,
Montebello, Oslo (Norway)

ROBERT M. SUTHERLAND
ALEXANDER PIHL

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Received March 14th, 1967

Biochim. Biophys. Acta, 135 (1967) 568–570

BBA 71003

The inhibition of (Na^+-K^+)-activated ATPase by beryllium

It has been reported that beryllium inhibits alkaline phosphatase (EC 3.1.3.1) by competing with Mg^{2+} (refs. 1–4). Phosphoglucomutase (EC 2.7.5.1) is found to be irreversibly inhibited by binding a mole of beryllium per mole of enzyme, presumably to Mg^{2+} site[5].

Since many divalent cations are known to be inhibitory to Na^+-K^+ ATPase[6], we planned to study the effect of beryllium on this enzyme. During the course of our study, THOMAS AND ALDRIDGE[7] reported on the inhibition of brain microsomal ATPase by rather high concentration of $BeSO_4$ (0.64 mM). They described the inhibition to be due to a combination of beryllium with ATP, thereby depleting the enzyme of its usual Mg^{2+}–ATP complex. He did not consider the inhibition to be due to the direct action of beryllium on the enzyme. We found, however, beryllium inhibition of Na^+-K^+ ATPase prepared from microsomal fraction of guinea-pig kidney cortex to be additionally dependent on the presence of cations such as Na^+, K^+, and Mg^{2+}. The data described below suggest that the inhibition is due to the direct action of beryllium on the enzyme and that Na^+, K^+ and Mg^{2+} change the state of the enzyme in the absence of ATP.

Microsomal ATPase was prepared from guinea-pig kidney cortex[8] and care-