Lead Hemolytic Action — a Possible Mechanism

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Abstract. Lead-induced changes in the antioxidative properties of the two basic macrostructural erythrocyte components: membrane and cytosol, were studied using a model system of Fe^{2+}-induced peroxidation of phospholipid liposomes, and possible ways of initiation of lipid peroxidation in erythrocyte membranes were suggested. Lead ions incorporate rapidly in the erythrocyte and react with membrane components, diminishing the antioxidative properties of the membrane. Interactions with the cytosol components, most likely with hemoglobin, result in reactive oxygen species and methemoglobin formation. Due to this, and along with the lead-induced decreased level of reduced glutathion and decreased activity of some protective enzymes, the prooxidant activity of the cytosol increases. Both conditions are favourable for lipid peroxidation in the membranes, eventually resulting in hemolysis.

Key words: Lead ions — Erythrocyte membrane — Hemolysis

Introduction

Red blood cell membranes contain rather great amounts of polyunsaturated fatty acids. The high oxygen and hem iron concentrations in the cytosol are favourable conditions for initiating lipid peroxidation with serious consequences for the cell: inhibition of the membrane transport processes, inactivation of the enzyme systems, hemolysis, etc. Under normal conditions, peroxidation is suppressed by the antioxidative systems, localized in the cytosol and membranes (Mead 1979; Gutteridge and Stocks 1976). The processes of lipid peroxidation can be enhanced by external factors which inactivate the antioxidative systems.

In the course of studies on the effects of heavy metals on erythrocytes in vitro, it has been observed that lead ions cause lipid peroxidation (Ribarov and Benov 1980) and have a strong hemolytic action (Passow et al. 1961; Valles and Ulmer 1972). Yet, the mechanism of these effects remains unclear. It is therefore interesting to study lead-induced changes in the antioxidative properties of the two basic macrostructural erythrocyte components: membrane and cytosol, as well as the possible ways of initiation of lipid peroxidation in erythrocyte membranes.

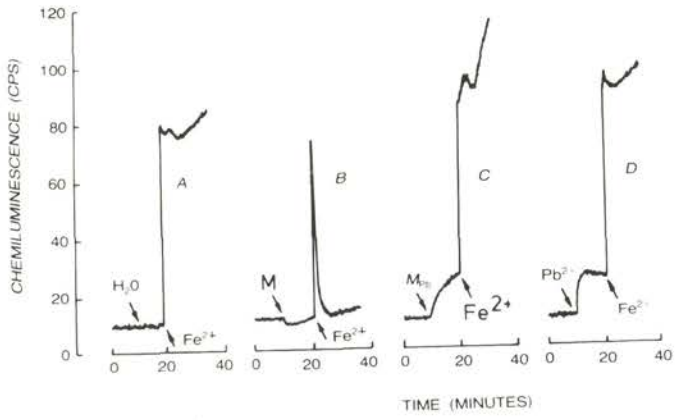

Fig. 1. Chemiluminescence kinetics of Fe^{2+}-initiated peroxidation of phospholipid liposomes. The followings were added to 5 ml of liposome suspension (5 mg phospholipids/ml): *A* — 1 ml distilled water (H_2O); *B* — 1 ml suspension of erythrocyte membranes, 0.21 mg protein/ml (M); *C* — 1 ml suspension of erythrocyte membranes with the same protein concentration preincubated with $PbCl_2$ for 15 min, 10^{-3} mol/l (M_{Pb}); *D* — 1 ml $PbCl_2$, 10^{-3} mol/l (Pb^{2+}). The peroxidation was initiated by the addition of 1 ml 7.10^{-4} mol/l $FeSO_4$.

Material and Methods

The experiments aimed at the determination of antioxidative properties of the erythrocyte membrane and cytosol were performed using a model system of Fe^{2+}-initiated peroxidation of phospholipid liposomes (Vladimirov and Archakov 1972). Lipid peroxidation is accompanied by light emission with an intensity proportional to the square of the concentration of peroxy radicals, which are the main factor of the peroxidation processes. Therefore, recording of the chemiluminescence provides important information on the course of the peroxidation.

The phospholipid liposomes (5 mg phospholipids/ml) prepared according to the method of Johnson et al. (1971) were used. Total phospholipids from egg yolks were obtained as described previously (Clark and Switzer 1977).

Peroxidation was initiated by the addition of $FeSO_4$ (final concentration 10^{-4} mol/l) into the phospholipid suspension.

Fig. 1 illustrates a typical chemiluminescence kinetics recorded after the addition of Fe^{2+} to the phospholipid suspension (*A*). The amplitude of the rapid spike obtained immediately after the introduction of the ferrous ions provides information on the concentration of hydroperoxides in the system. Further changes of the chemiluminescence reflect the development of the peroxidation. The addition of chemical compounds, or biological material to the model system used (e.g. erythrocyte membranes or cytosol), results in changes of the chemiluminenscence kinetics. A decrease in chemiluminescence is indicative of antioxidative properties of the probe added and vice versa.

The erythrocyte membranes were obtained by the method described previously (Dodge et al. 1963), and were resuspended in distilled water to obtain a protein concentration of 0.21 mg/ml. The pH of the suspension was adjusted to 7.4 with 0.01 mol/l NaOH. Cytosol, free of membranes, with a hemoglobin concentration of 10^{-4} mol/l was used. In some of the experiments the cytosol was

deproteined by treatment with an equal volume of chloroform, followed by centrifugation (1000 g, 5 min). In order to study the influence of lead ions on the antioxidative properties of the erythrocyte components, the samples of membranes and cytosol were preincubated with $PbCl_2$, 10^{-3} mol/l for 15 minutes.

Chemiluminescence measurements were carried out using a photometric equipment with high sensitivity and high signal/noise ratio.

The production of reactive oxygen species was measured by the method described previously (Ribarov and Bochev 1982).

Results and Discussion

Fig. 1 illustrates the kinetics of the chemiluminescence associated with Fe^{2+}-catalyzed lipid peroxidation in a liposome suspension containing untreated erythrocyte membranes (curve *B*), and lead-treated membranes (curve *C*). The obtained results showed that the addition of erythrocyte membranes to our phospholipid model system led to a diminution in the amount of the lipid hydroperoxides, and suppressed lipid peroxidation. This observation suggests that the erythrocyte membrane may contain some components with antioxidative properties. A preliminary (15 min) incubation with lead ions led to a strongly diminished antioxidative activity of the membranes. In addition, the drastic rise of chemiluminescence in this case shows that the presence of lead-treated membranes in the liposome suspension contributes to the development of the lipid peroxidation. It may be supposed that this effect is due to co-oxidation processes of the lipids in the model system and those of the erythrocyte membranes. The addition of lead ions to the liposome suspension (curve *D*) had no influence on the chemiluminescence kinetics. The spontaneous chemiluminescence (before the addition of Fe^{2+}) was increased, while the concentration of the hydroperoxides as well as the rate of the peroxidation remained unchanged.

Fig. 2 illustrates the chemiluminescence kinetics obtained for liposome suspension containing untreated (*B*) and lead ions-treated (*C*) cytosol. Curves *A* and *D* illustrate the kinetics of the peroxidation in the control liposome suspension and the suspension containing protein-free cytosol, respectively. Obviously, the addition of cytosol to the liposome suspension greatly diminished the intensity of the spontaneous chemiluminescence. This effect might have been due to the high absorbance of the light emitted by the hemoglobin molecules (Zhuravlev and Zhuravleva 1975). The addition of Fe^{2+} to the suspension preincubated with cytosol resulted in a chemiluminescence spike; its amplitude was nearly identical with that obtained in the control. Taking into account the effect of light absorbance by the hemoglobin, it may be concluded, that in this case significant amounts of hydroperoxides are produced. This is in a good agreement with the data reported previously by Tappel (1955).

As illustrated in Fig. 2 (curve *C*) the addition of lead-treated cytosol to the

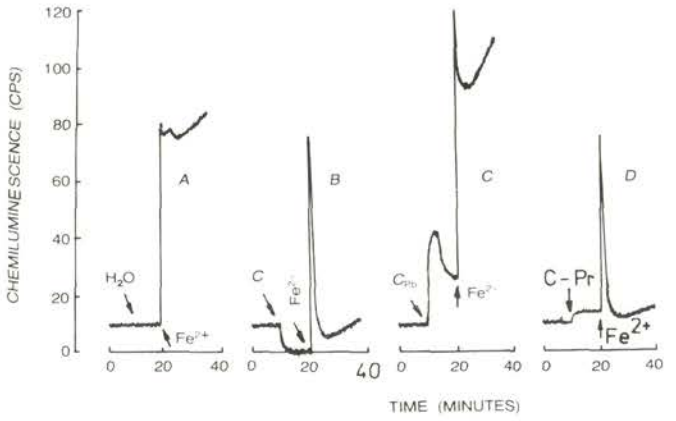

Fig. 2. Chemiluminescence kinetics of Fe^{2+}-initiated peroxidation of phospholipid liposomes. The followings were added to 5 ml of liposome suspension (5 mg phospholipids/ml): *A* — 1 ml distilled water (H_2O); *B* — 1 ml cytosol with a hemoglobin concentration of 10^{-4} mol/l (*C*); *C* — 1 ml cytosol with the same hemoglobin concentration preincubated with $PbCl_2$ for 15 min, 10^{-3} mol/l (C_{Pb}); *D* — 1 ml chlorophorm-deproteinated cytosol (C—Pr).

liposome suspension resulted in the occurrence of a chemiluminescence spike and a comparatively high level of spontaneous chemiluminescence. These effects may be related to the peroxidatitive processes initiated by superoxide radicals produced in the course of the lead-induced hemoglobin oxidation. The addition of Fe^{2+} to the liposome suspension containing lead-treated cytosol led to decomposition of the accumulated lipid hydroperoxides and to development of an expressed peroxidation.

The prooxidative properties of the cytosol, observed in the present experiments, might have been due to its protein components. This suggestion was tested in an experiment using protein-free cytosol (curve *D*). In this case the addition of Fe^{2+} led to the decomposition of the accumulated hydroperoxides, however without a marked development of lipid peroxidation. The inhibition of the peroxidative processes by the protein-free cytosol might have been due to its high content in reduced glutathione: reduced glutathione is known to possess strong antioxidative properties (Kosower and Kosower 1979).

Hemoglobin is the major protein component of the erythrocyte cytosol. It may be suggested that the increased prooxidant activity of the cytosol is at least in part due to the lead-hemoglobin interaction. The ability of the lead-hemoglobin interaction to initiate lipid peroxidation was demonstrated previously (Ribarov et al. 1981; Ribarov and Bochev 1982). It was shown that the addition of lead ions to the hemoglobin solution resulted in methemoglobin formation and production of

reactive oxygen forms able to oxidize the luminol solution. These oxygen forms were identified as being superoxide radicals and hydrogen peroxide.

Based on the results obtained, a possible mechanism for the lead hemolytic action may be proposed.

Lead ions added to erythrocyte suspension are known to incorporate rapidly in the erythrocytes (Passow et al. 1961). Some of the ions react with membrane components diminishing its antioxidative activity. Some others react with the cytosol components, most likely the hemoglobin molecules. As a result of the lead-hemoglobin interaction, reactive oxygen forms and methemoglobin are produced. Along with the lead-induced decreased level of reduced glutathione and the decreased activity of some protective enzymes, as glutathione peroxidase and catalase, this results in an increased prooxidant activity of the cytosol. The decreased antioxidative activity of the membrane and the increased prooxidative activity of the cytosol represent favourable conditions for initiation of lipid peroxidation in the membranes, eventually leading to hemolysis.

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