Mechanisms of Anionic Detergent-Induced Hemolysis

E Chernitsky and O Senkovich

Institute of Photobiology, National Academy of Sciences of Belarus, Minsk, Belarus

Abstract. The effect of osmotic protectors (sucrose and polyethylene glycols) and of a decrease in the detergent concentration at different points of hemolysis of human erythrocytes by sodium dodecyl sulphate on the shape of kinetic curves of hemolysis were studied It is shown that slow detergent-induced hemolysis follows the colloid-osmotic mechanism Evidence is provided that rapid hemolysis by sodium dodecyl sulphate is caused by opening of large pores sufficient for the release of hemoglobin molecules rather than by the colloid-osmotic mechanism, and that the kinetics of hemolysis is mainly determined by time dependence of the opening probability of these pores

Key words: Erythrocytes — Hemolysis — Detergent — Sodium dodecyl sulphate

Introduction

The existing ideas concerning the mechanisms of hemolysis by anionic detergents, in particular, by sodium dodecyl sulphate, have changed but slightly in recent 40 years since the time of the publication of a remarkable work by Rideal and Taylor (1957) They have shown that submicellar concentrations of anionic detergents can cause two different kinds of hemolysis at low concentrations they induce only complete slow hemolysis, while at elevated concentrations they also cause rapid hemolysis which, depending on the concentration of the hemolytics, can result in the lysis of various portions of cells (from 0 to 100%) The slow hemolysis, subsequently described mathematically (Rideal and Taylor 1958) was similar in its properties to saponin-induced hemolysis, and was attributed to the colloid-osmotic mechanism

The rapid hemolysis was attributed to an unknown mechanism which involves free phospholipid of the membrane, presumably lecithin (Rideal and Taylor 1957) Subsequently, these works were probably forgotten since researchers who studied detergent-induced hemolysis did not even specify the type of hemolysis they worked

Correspondence to Prof Dr Eugene A Chernitsky, Institute of Photobiology, National Academy of Sciences of Belarus, Academicheskaya St 27, 220072 Minsk, Belarus E-mail sei@biobel.bas-net by

with (Bonsall and Hunt 1971; Kaler et al. 1986), and these two types of hemolysis were as if newly disclosed recently (Bielawski 1990).

Previously we described properties of both types of hemolysis by sodium dodecyl sulphate and nonionic detergent Triton X-100 with respect to the formation of pores in various lipid areas of the membrane by these detergents (Chernitsky at al. 1996; Senkovich and Chernitsky 1996, 1997; Chernitsky and Senkovich 1997). In the present article we provide evidences that, while in the case of slow hemolysis induced by sodium dodecyl sulphate, prehemolytic pores are formed and the hemolysis follows the colloid-osmotic mechanism, rapid hemolysis follows a different mechanism, involving the opening of large pores, sufficient for the release of hemoglobin molecules. The kinetics of this type of hemolysis can mainly be determined by time dependence of the probability of opening of these pores rather than by the duration of processes of the pores formation or the release of hemoglobin from a cell.

Materials and Methods

Donor erythrocytes were washed three times from plasma with an isotonic NaCl solution, and were added as 5% suspension in buffered solution A, containing 150 mmol/l NaCl, 8.1 mmol/l Na_2HPO_4 and 1.9 mmol/l NaH_2PO_4 (pH 7.2), in a thermostated cell, containing a certain amount of the detergent in solution A. The time course of hemolysis was recorded with a Specord UV-VIS spectrophotometer at $\lambda = 670$ nm (Chernitsky and Senkovich 1997). The concentrations of cations K^+ and Na^+ in the erythrocytes were measured using Flapo flame photometer, measurements were done after hemolysis of cells in distilled water. The temperature of the medium and the detergent concentrations to induce rapid and slow hemolysis were chosen so as to yield a convenient rate of the processes.

Results and Discussion

Fig. 1 illustrates the kinetic curves for slow hemolysis induced by sodium dodecyl sulphate. If at time $t = 0$ the osmotic protector sucrose (150 mmol/l) was added to the suspension, kinetic curve K was transformed into curve 1, which corresponded to a decrease in the hemolysis rate. If sucrose was added to the suspension at the lytic stage, e.g. at the moment shown by arrow 3 in Fig. 1, light absorption (D_{670}) increased; this corresponded to cell compression, and the hemolysis rate slowed down at a later time due to dynamic inhibition of hemolysis (Deuticke et al. 1986), i.e. to the slow penetration of sucrose inside the cell. These findings indicate that slow hemolysis by sodium dodecyl sulphate follows the colloid-osmotic mechanism. Using some osmotic protectors of various molecular size (sucrose, polyethylene glycols 600, 1500 and 4000) and measuring the hemolysis rate in their presence

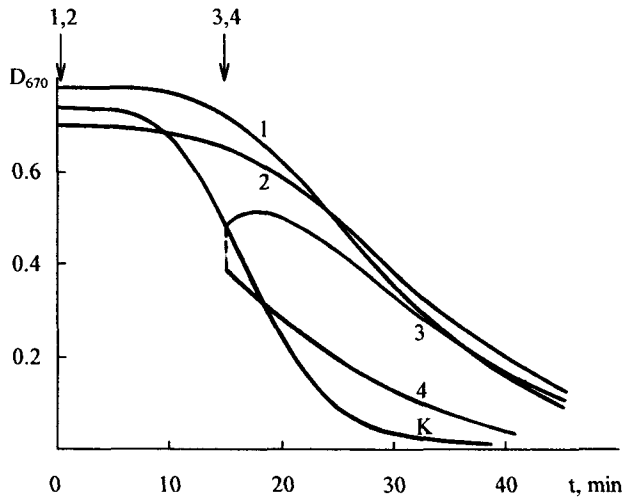

Figure 1. Kinetic curves of slow hemolysis induced by sodium dodecyl sulphate ($c = 100$ μmol/l) for control sample (K), and for samples with the addition of sucrose (150 mmol/l) at times indicated by arrows 1 and 3 (curves 1 and 3) and for samples diluted 1.25 times with solution A at times indicated by arrows 2 and 4 (curves 2 and 4). Temperature at measurement: 37°C, hematocrite: 0.062%.

(30 mmol/l), it could be found that the diameter of the pores responsible for slow detergent-induced hemolysis is about 4 nm (Senkovich and Chernitsky 1997).

Osmotic protectors were used to estimate the size of the pores responsible for rapid hemolysis by sodium dodecyl sulphate, and similar results were obtained. This seemed rather strange as the rate of rapid and slow hemolysis components differ by about two orders of magnitude. When no osmotic protector (polyethylene glycol 4000) was added before the start of hemolysis, and it was only added at the stage of cell lysis almost no inhibition was observed in contrast to what is shown in Fig. 1. Consequently, the osmotic protectors used did not decrease the rate of rapid hemolysis by changing the rate of cell swelling (even polyethylene glycol 4000 freely passes through the pores) but due to the inhibition of pore formation; thus, the pore size cannot be estimated using the standard procedure (Deuticke et al. 1986).

We suggested that this inhibition of pore formation is due to detergent-induced vesiculation of erythrocytes (Chernitsky and Senkovich 1997). Some results support this suggestion (Senkovich and Chernitsky 1997). Vesiculation appears to be a process that competes with rapid hemolysis: first, the rates of both processes are similar; second, compounds that accelerate detergent-induced vesiculation (as

suggested by the activity of acetylcholinesterase in supernatants) inhibit hemolysis.

Thus, rapid hemolysis by sodium dodecyl sulphate appeared to be a vesiculation-dependent process, and the corresponding pores the formation of which involves membrane phosphatidylcholine and the detergent (four molecules per pore) (Chernitsky and Senkovich 1997), appeared rather large, exceeding 4 nm.

To study the mechanism that governs sodium dodecyl sulphate-induced rapid hemolysis, the behaviour of kinetic curves of hemolysis was studied upon decreasing the concentration of the detergent by diluting the reaction mixture with solution A after leaving exposed the erythrocytes to the action of the detergent. It is convenient to choose a dilution at which the detergent concentration in the suspension drops below the threshold concentration of rapid hemolysis. Then, if the reaction mixture is diluted until pores are formed in erythrocytes, no hemolysis will be observed; but if pores have already formed (in case of colloid-osmotic hemolysis) lysis of the corresponding portion of erythrocytes is expected to occur.

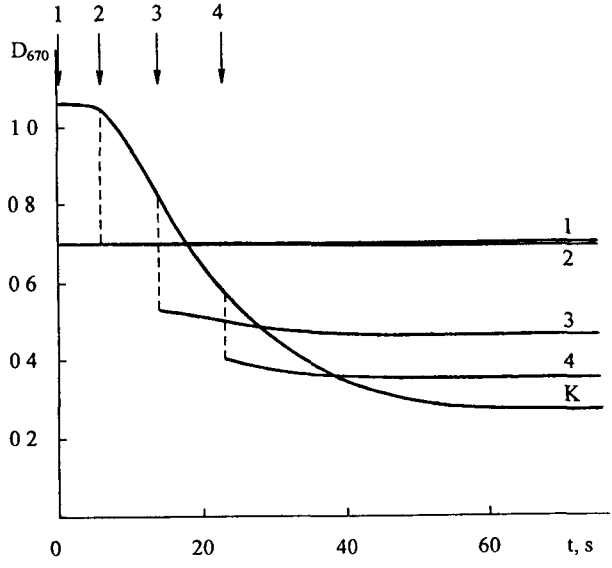

Figure 2. Kinetic curves of rapid hemolysis induced by sodium dodecyl sulphate ($c = 75$ μmol/l) for control sample (K), and for samples diluted 1 7 times with solution A at times indicated by arrows 1–4 (curves 1–4) Temperature at measurement 15 °C, hematocrite 0 124%

As shown in Fig. 2, if the mixture is diluted immediately after the addition of erythrocytes to the detergent-containing solution the kinetic curve is a horizontal

straight line (1). The same straight line (2) is obtained for dilution at the end of the lag-phase; this could be ascribed to the fact that prehemolytic pores (if the mechanism is colloid-osmotic) have not yet formed. If the detergent concentration in the suspension is decreased at the stage of lysis of the erythrocytes, some relative changes in the time-course of hemolysis are observed, but they are insignificant and are probably due to the kinetics of hemoglobin release from that portion of the cells which have been lysed upon the dilution of the suspension (curves 3 and 4).

Thus, the present data indicate that the contribution of the colloid-osmotic mechanism to rapid hemolysis is insignificant, if any. We arrived at the same conclusion earlier when studying the effect of NaCl concentration on parameters of rapid hemolysis (Senkovich and Chernitsky 1997). It is likely that pores responsible for rapid detergent-induced hemolysis are sufficiently large for hemoglobin to leave the cell through them, i.e. they are hemolytic pores rather than prehemolytic ones. It should be noted that the opening probability of hemolytic pores depends on the detergent concentration and on the time of interaction of the detergent with the cell.

It seemed interesting to find out how a decrease in the detergent concentration affects slow hemolysis. Dilution of the mixture at the lytic stage did not reduce the numbers of hemolysed cells, but decreased the rate of hemolysis (Fig. 1, curve 4). This decrease in the rate can be explained by a decrease in the size of prehemolytic pores, or by a decrease in the probability of formation of hemolytic breaks upon the decreasing detergent concentration.

In conclusion, it should be noted that we failed to explain the mechanism of sodium dodecyl sulphate-induced rapid hemolysis by control of the concentration of K^+ and Na^+ ions in the cells and in extracellular medium. Hemolysis proceeds too fast even at low temperatures (4°C) so that the time of centrifugation of the samples (2 min) is comparable with the time of hemolysis. Therefore, the kinetics of the release of K^+ ions from the cells could only be studied at low (< 50 μmol/l) prehemolytic concentrations of the detergent. The experiments have shown that K^+ starts leaving the cells at early vesiculation stages, which complicates the picture of the release of K^+ from the cells at hemolytic concentration of the detergent. Measurements of concentrations of univalent cations that remained in the cells after partial rapid hemolysis revealed a strong dependence of the intracellular concentrations of K^+ and Na^+ on the concentration of the anion detergent in the suspension which was probably caused by the charge effect.

References

Bielawski J. (1990): Two types of haemolytic activity of detergents. Biochim. Biophys. Acta **1035**, 214—217

Bonsall R. W., Hunt S. (1971): Characteristics of interaction between surfactants and the human erythrocyte membrane. Biochim. Biophys. Acta **249**, 266—288

Chernitsky E A , Senkovich O A (1997) Erythrocyte hemolysis by detergents Biol Membrany **14,** 385—393 (in Russian)

Chernitsky E A , Senkovich O A , Kozlova N M (1996) Heterogeneity of pores formed in erythrocyte membrane by lipophilic hemolysins Biofizika **41,** 1270—1274 (in Russian)

Deuticke B , Heller K B , Haest C W M (1986) Leak formation in human erythrocytes by the radical-forming oxidant butylhydroperoxide Biochim Biophys Acta **854,** 169—183

Kaler G V , Rachkovskii L I , Okun I M (1986) Hemolysis by detergents A mathematical model and analysis of concentration and kinetic curves Tsitologiya **28,** 954—963 (in Russian)

Rideal E K , Taylor F H (1957) On haemolysis by anionic detergents Proc Roy Soc (London) Ser B **146,** 225—241

Rideal E K , Taylor F H (1958) On haemolysis and haemolytic acceleration Proc Roy Soc (London) Ser B **148,** 450— 464

Senkovich O A , Chernitsky E A (1996) About heterogeneity of pores formed in erythrocytes by Triton X-100 Izvestiya Akad Nauk Belarusi Ser Biol **2,** 120—122 (in Russian)

Senkovich O A , Chernitsky E A (1997) About the size of pores arising in erythrocytes under the effect of detergents Biol Membrany **14,** 549—556 (in Russian)

Final version accepted August 5, 1998