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ROLE OF DISULFIDE BONDS IN THE ATTACHMENT AND FUNCTION OF LARGE, EXTERNAL, TRANSFORMATION-SENSITIVE GLYCOPROTEIN AT THE CELL SURFACE

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Summary

Reduction of disulfide linkages by dithiothreitol removes LETS (large, external, transformation-sensitive) protein from the cell surface. This process is dependent upon the concentration of dithiothreitol and the time and temperature of reaction. At 0°C the release of LETS protein by dithiothreitol is completely blocked, but this is apparently not due to a requirement for metabolic energy. At this temperature, reduction of LETS protein is incomplete. These results suggest that intact disulfide bonds are involved in the retention of this protein on the cell surface. Furthermore, reduction of purified LETS protein interferes with its ability to confer flattened morphology and increased adhesivity when added to transformed cells. It appears, therefore, that disulfide bonds are functionally important at the cell surface.

Introduction

Knowledge of the molecular architecture of the cell surface is important for gaining insight into its possible role in cellular behavior. The arrangement of proteins in the plasma membrane has been studied to some extent in erythrocytes. Studies using a variety of cross-linking reagents have indicated the existence of some specific oligomeric complexes between various polypeptides of erythrocyte membranes [1—4].

Because of the greater complexity of other cell membranes, organization of proteins and their interactions with other components within the membrane are poorly understood. Recently, evidence has been presented suggesting that disulfide bonds are involved in the organization of surface proteins in fibroblasts. In particular, LETS glycoprotein (large, external, transformation-sensi-

Abbreviations: SDS, Sodium dodecyl sulfate; LETS, large, external, transformation-sensitive.

tive), a major surface component of fibroblasts (reviewed in ref. 5), appears to be extensively disulfide bonded to itself and possibly also to other proteins. It is present either in the form of dimers or as high molecular weight complexes [6]. The involvement of disulfide linkages in the organization of fibroblast membrane proteins is also suggested by the fact that presence of the reducing agent dithiothreitol enhances the extraction of membrane proteins, particularly LETS glycoprotein [6,7]. Disulfide bonding has also been reported in platelet membranes [8,9], in transplantation antigens [10] and in surface immunoglobulins [11].

The significance of these disulfide bonds is unknown. In this communication we report that reduction of disulfide bonds removes most of the LETS protein from the cell surface. Also, pretreatment of LETS protein, isolated from normal cells, with dithiothreitol interferes with its ability to bring about alterations in the morphology and adhesiveness of transformed cells [12,13]. These results suggest a role for disulfide bonds in the attachment and function of this protein at the cell surface.

Materials and Methods

Cells. Cells of the hamster fibroblast line NIL8 and its transformed derivative NIL8-HSV were grown in Dulbecco's modified Eagle's medium plus 5% fetal calf serum at 37° C in an atmosphere of 95% air/5% CO_2. NIL8 cells were seeded at $5 \cdot 10^4$ cells/35 mm dish in 2.5 ml of medium and grown for 5 days. NIL8-HSV cells were seeded at $2 \cdot 10^5$ cells/35 mm dish and were grown for 2 days.

Treatment with dithiothreitol. Confluent monolayers of NIL8 cells were iodinated using the enzyme lactoperoxidase according to the procedure described previously [14]. To the iodinated cells 2 ml of fresh medium plus serum containing the desired concentration of dithiothreitol (see figure legends) was added and the cells were incubated for different time periods at 37° C.

At the end of the various treatments cells were rinsed with phosphate-buffered saline and solubilized in lysis buffer containing sodium dodecyl sulfate (SDS) and analyzed by electrophoresis on SDS-polyacrylamide gels under non-reducing or reducing conditions using 100 mM dithiothreitol [6,14]. Cells which were to be analyzed under non-reducing conditions were washed with phosphate-buffered saline containing 10 mM iodoacetamide and 10 mM *N*-ethylmalemide to inactivate residual amounts of dithiothreitol before solubilizing them with SDS. Protein concentration of the cell samples was determined according to the method of Lowry et al. [15].

To determine the effect of metabolic inhibitors, iodinated cells were pretreated with 2.5 mM dinitrophenol, 10 mM NaF or 10 mM NaN_3 in 2 ml of medium plus serum for 15 min at 37° C. 2 mM dithiothreitol was then added to the cultures, which were then incubated for 2 h at 37° C.

When the dithiothreitol treatment was carried out at 0° C, dishes of preiodinated monolayers were kept at 0° C for about 5 min and precooled medium plus serum containing 2 mM dithiothreitol was added to the dishes.

Adhesion assay and test of biological activity. LETS protein was isolated

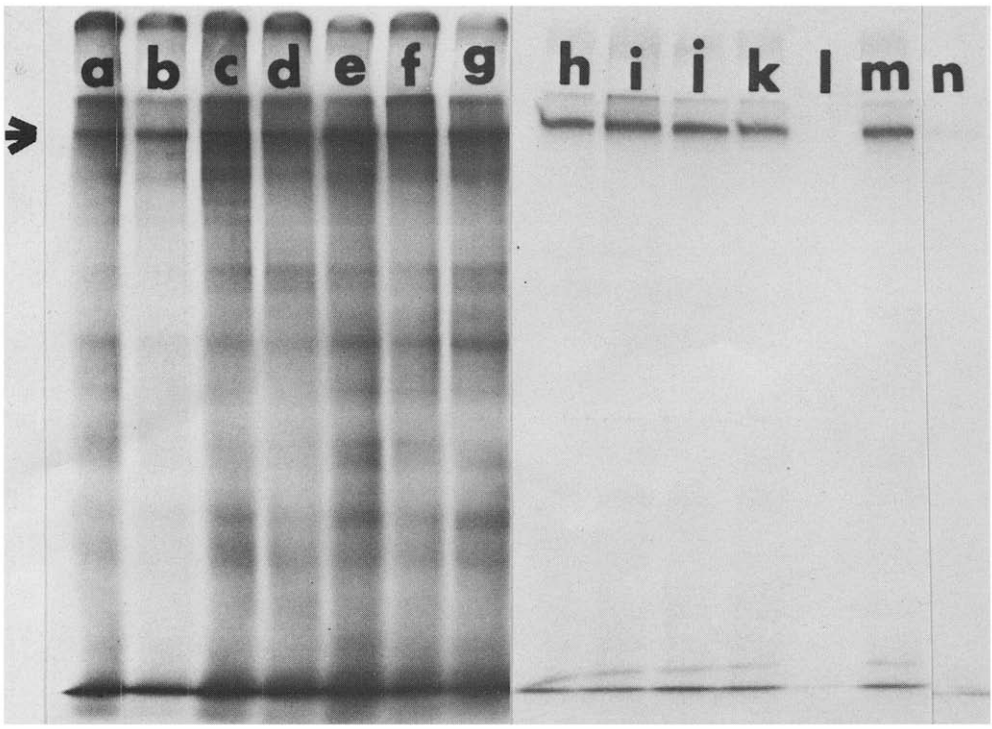

Fig. 2. Effects of metabolic inhibitors and low temperature (0°C) on the release of surface LETS protein from cells treated with 2 mM dithiothreitol: autoradiograms of SDS-polyacrylamide slab gels. Iodinated cells were pretreated with the inhibitors for 15 min at 37°C in complete culture medium. 2 mM dithiothreitol was then added and incubation at 37°C continued for 2 h in the presence of inhibitors. a—g, cell lysates: a, no inhibitor; b, 5 mM dinitrophenol; c, 10 mM NaF; d, 10 mM NaN_3; e, 2 h incubation with 2 mM dithiothreitol at 0°C; f, 2 h incubation with 2 mM dithiothreitol at 0°C followed by temperature shift-up to 37°C for 2 h; g, control NIL cells, no dithiothreitol added. h—n, media: h, no inhibitor was added; i, 5 mM dinitrophenol; j, 10 mM NaF; k, 10 mM NaN_3; l, 2 h incubation with 2 mM dithiothreitol at 0°C; m, 2 h incubation with 2 mM dithiothreitol at 0°C followed by 2 h incubation at 37°C; n, medium from control NIL cells. a—g and h—n are from separate gels. Arrow marks the position of reduced LETS protein.

TABLE I

EFFECTS OF DITHIOTHREITOL ON RELEASE OF LETS PROTEIN AND CELL VIABILITY

After incubation with or without dithiothreitol cells were washed three times with phosphate-buffered saline, trypsinized, plated at 100 cells per 50 or 10 cm dish, and cultured for 10 days in 5% fetal calf serum before staining with Giemsa and counting. Colonies were counted blind by two independent observers. Two separate experiments are shown.

Pretreatment	Percent release of LETS protein *	Plating efficiency	
None (control)	19	53	51
0.5 mM dithiothreitol (2 h)	52	50	51
2 mM dithiothreitol (2 h)	78	59	48
10 mM dithiothreitol (2 h)	90	53	40

* Compare Fig. 1.

treatment lowers the ATP pools even further [18]. This failure to inhibit release of LETS protein with inhibitors of ATP production suggested that the inhibition of release of LETS protein at 0°C was not due to an effect on metabolic rate.

The possibility that at 0°C LETS protein might not be reduced by dithiothreitol was tested by analyzing cells treated with 0.5 and 2 mM dithiothreitol at 37 and 0°C. Fig. 3, a—e shows analysis under reducing conditions. Incubation of cells with dithiothreitol for 30 min at 37°C removed substantial amounts of LETS protein from the surface (Fig. 3, b and c), whereas cells incubated at 0°C had levels of LETS protein comparable to that of control cells (Fig. 3, a, d and e). When the same samples were analyzed under non-reducing conditions, much of the LETS protein from cells treated with dithiothreitol at 0°C and from the control cells was found trapped on top of the gels. This behavior is characteristic of disulfide-bonded complexes of LETS protein [6]. Some LETS protein was also present in the form of dimers (Fig. 3, f, i and j). Treatment with 2 mM dithiothreitol at 37°C reduced most of the prelabeled

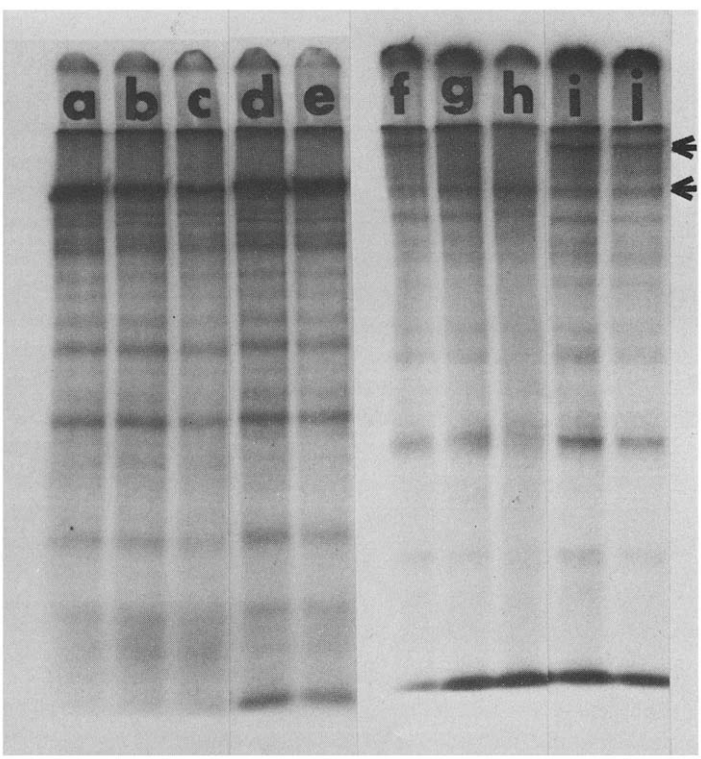

Fig. 3. Treatment of cells with dithiothreitol at 37 or 0°C for 30 min: autoradiography of cell-samples analyzed on SDS-polyacrylamide gels under reducing and non-reducing conditions. a—e, reduced: a, control; b, 0.5 mM dithiothreitol at 37°C; c, 2 mM dithiothreitol at 37°C; d, 0.5 mM dithiothreitol at 0°C; e, 2 mM dithiothreitol at 0°C. f—j, non-reduced: f, control; g, 0.5 mM dithiothreitol at 37°C; h, 2 mM dithiothreitol at 37°C; i, 0.5 mM dithiothreitol at 0°C; j, 2 mM dithiothreitol at 0°C. Arrows show monomer and dimer positions of LETS protein. Note the loss of LETS protein at 37°C (b and c) but not at 0°C (d and e) and reduction in amounts of LETS protein dimer and higher aggregates on top of gel at 37°C (g and h) but not at 0°C (i and j).

greatly diminished. Controls receiving equivalent amounts of dithiothreitol (final concentration 0.1 mM) were essentially as untreated control dishes. At higher final concentrations of reducing agent (0.25—0.5 mM) dithiothreitol itself caused a reduction in numbers of floaters for unknown reasons, but LETS protein preincubated with 5—10 mM dithiothreitol at 37° C produced no further decrease. That is, its biological activity was destroyed by this treatment. However, preincubation of LETS protein with dithiothreitol at 0° C was without measurable effect on its activity up to 2 mM and had only a small inhibitory effect at 5 or 10 mM.

Fig. 5, a and e show NIL8-HSV cells before and after the addition of 50 μg/ml LETS protein; cells became more flattened and aligned after the addition of LETS protein. The morphology of cells treated with 50 μg/ml LETS protein, which was preincubated with dithiothreitol at 37° C was similar to control NIL-HSV cells (compare Fig. 5, b—d with 5a). Cells treated with LETS protein, which was preincubated with 0.5 mM dithiothreitol at 0° C, showed flattened morphology closely resembling that from cells which received untreated LETS protein (compare Fig. 5, f and e). Cultures treated with LETS protein, which was preincubated with 2 mM dithiothreitol at 0° C all attached but did not flatten so extensively and at 5 mM there were a few floating cells in the medium (Fig. 5, g and h).

These results show that the biological activity of LETS protein is sensitive to reduction by dithiothreitol at 37° C and much less so at 0° C. In fact, the small effect seen after incubations at 0° C could well be due to the low final concentrations of dithiothreitol (0.25—0.5 mM) which are present after addition of the treated LETS protein to the cultures at 37° C.

Discussion

It has previously been demonstrated that the intermolecular disulfide bonds linking the LETS monomers in the form of dimers and high molecular weight aggregates are naturally occurring and not experimental artefacts created by lactoperoxidase mediated iodination [6]. The present results indicate that reduction of these disulfide bonds by dithiothreitol brings about release of LETS protein from the cell surface. Although the possibility that the loss of LETS protein is an indirect consequence of general toxic effects of dithiothreitol on cells cannot be ruled out, several lines of evidence suggest that the reduction of disulfide bridges in LETS protein is responsible for the release of LETS protein from the cell surface. First, control experiments with a non-reducing analogue, threitol, failed to show any effect on the turnover of LETS protein. Second, LETS protein is specifically released by dithiothreitol (0.5—2 mM) fairly rapidly (50—80% in 2 h), whereas the levels of other surface iodinatable proteins are not significantly affected (Fig. 2). Third, the release of LETS protein at different temperatures correlates with the degree of reduction of LETS protein (Fig. 3). Finally, treatments which produced near-maximal effects on LETS protein were without effect on cell viability (Table I).

The results reported here also indicate that the biological activity of LETS protein added to transformed cells depends upon the presence of intact disulfide bonds. Pretreatment of purified LETS protein with dithiothreitol at 37° C

destroys its ability to cause alterations in morphology and attachment of floating cells to the substratum in cultures of HSV-transformed NIL cells. In other words, these results imply that the reduced form of LETS protein is non-functional. This is consistent with the observation that the LETS protein secreted into the medium, which is active when added to transformed cells [13], is disulfide bonded [6].

The release of LETS protein from cells by dithiothreitol is strongly dependent on temperature. At 20° C (room temperature) the rate of release is significantly slower than at 37° C (Fig. 1b) and lowering the temperature to 0° C inhibits the removal completely (Fig. 2). This temperature dependence does not appear to reflect a dependence on energy since depletion of the ATP pool of the cells did not affect the release of LETS protein. The release appears to correlate with the ability of the reducing agent to cleave the disulfide bonds of LETS protein (Fig. 3). The fact that inhibition of the biological effects of LETS protein by dithiothreitol is also dependent on the temperature of preincubation in the absence of cells or serum (Figs. 4 and 5) suggests that the temperature effect is on the reaction of dithiothreitol with LETS protein itself. There are precedents for disulfide bonds in proteins which are resistant to reduction [19,20]. Denaturation of the molecules or alteration of the reaction conditions (e.g. pH) can render these bonds sensitive to reduction. It is conceivable that there is a temperature-induced conformational change in LETS protein (or in its associations) which renders some or all of the disulfide bonds resistant to reduction at 0° C.

Whatever the explanation for the temperature dependence, it seems clear that release and inactivation of LETS protein are dependent on reduction of disulfide bonds. This suggests that other covalent intermolecular bonds are not found under the conditions used and that binding of LETS protein to the cell surface occurs either through disulfide bonds or through non-covalent interactions which in turn depend on a native conformation of the LETS protein dimer maintained by disulfide bonding.

These results suggest that disulfide-bonding plays a significant role in the organization and function of a major cell surface protein. This raises the possibility that alterations in the sulfhydryl-disulfide balance at the cell surface could affect cell surface structure and, thus, cellular behavior. Czech and his co-workers have presented evidence suggesting that sulfhydryl oxidation is involved in the action of insulin on fat cells [21—23]. It has been reported that an enzymatic activity capable of reducing protein disulfides is elevated in some tumor cells and in ascitic fluid [24] and that there are apparently fewer disulfide bonded complexes at the surfaces of some transformed cells [6]. The exact significance of these observations remains to be established but the present results indicate some of the possible consequences arising from reduction of disulfide bonds in cell surface proteins.

Aknowledgements

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understood [1,5]. Usually, in order to obtain a better understanding of the mechanisms of the lytic action of chemical compounds the interest is centered on the basic features of the rupture of the cell membrane and comparison of the results with some physicochemical properties of the compounds [1,5,12—15]. When surfactants are used as the lytic agents the key physicochemical properties of these are the critical micelle concentration and the hydrophile-lipophile balance as well as the chemical structure, the nature of the charged head group and the length of the surfactant alkyl tail, etc.

Earlier we studied the hemolytic action of sodium alkyl sulfates [16] and polyoxyethylene derivatives of fatty acids, mercaptans and alkylphenols [17] on human erythrocytes. Hemolytic effects of different surfactants on red blood cells have been reported by many authors [5—12], but due to discrepancies in experimental conditions, the lack of data on the quality of the lytic compounds used etc., the results are very difficult to compare. In this paper the hemolytic action of a number of homologous series of cationic surfactants is studied and the lytic effects of anionic, nonionic and cationic amphiphiles are compared.

Materials and Methods

Red cell suspensions were prepared from human blood as described earlier [16,17]. The determination of the degree of hemolysis under surfactant treatment for 10 min at 20°C at a concentration of $3 \cdot 10^8$ cell/ml was made by estimating spectrophotometrically the amount of haemoglobin released from the cells as in refs. 16 and 17. The concentration of the cells in the experiments with varied cell concentration are indicated below in Fig. 1. The experiments were repeated 3—4 times and the measured deviation from the average C^{50}-value (see below) did not exceed 5—7% for all the agents studied.

Alkyltrimethylammonium bromides $[R_nN(CH_3)_3]Br$ ($R_n = C_nH_{2n+1}$; n = 10,12,14,16) were prepared and purified according to the method of Scott et al. [18], acyl choline chlorides $[R_nCOOCH_2CH_2N(CH_3)_3]Cl$ (n = 9,15,16,17) were obtained by the method described in ref. 19. The cationic surfactants represented by the formula $[R_nCOOCH_2N(CH_3)_3]Cl$ (n = 10,12,14,16) as well as those represented by the formula $[R_nN(CH_3)_2CH_2$  $]Br$ (n = 10,12,14, 16) were kindly supplied by A. Epstein (All-Union Research Institute for Disinfectants and Antiseptics) and were used without further purification.

The critical micelle concentration values of the above surfactants in water and in phosphate-buffered isotonic saline (pH 7.2) were determined by the eosin solubilization technique as in ref. 20.

Results and Discussion

The minimum concentrations of the cationic surfactants C^{50} required to cause 50% lysis of human erythrocytes and the critical micelle concentration values of the agents in the lysis medium are listed in Table I.

The first point to emerge from the results of our experiments is the dependence of the compounds' hemolytic activities on their alkyl chain lengths and

TABLE I

HEMOLYTIC ACTIVITY (EXPRESSED AS C^{50}) AND SOME PHYSICOCHEMICAL CONSTANTS OF HOMOLOGOUS SERIES OF CATIONIC SURFACTANTS

Surfactant	C^{50} * (mol/l)	C^{50} ** (mol/l)	Critical micelle concentration *** (mol/l)
$[R_{10}N(CH_3)_3]Br$	$2.8 \cdot 10^{-2}$	$2.4 \cdot 10^{-2}$	$5.6 \cdot 10^{-2}$
$[R_{10}COOCH_2CH_2N(CH_3)_3]Cl$	$4.2 \cdot 10^{-3}$	—	$2.6 \cdot 10^{-2}$
$[R_{10}N(CH_3)_2CH_2$  $]Br$	$3.3 \cdot 10^{-3}$	—	$1.3 \cdot 10^{-3}$
$[R_{10}COOCH_2N(CH_3)_3]Cl$	$2.1 \cdot 10^{-3}$	$3.0 \cdot 10^{-3}$	$1.8 \cdot 10^{-3}$
$[R_{12}N(CH_3)_3]Br$	$1.8 \cdot 10^{-3}$	$2.0 \cdot 10^{-3}$	$9.0 \cdot 10^{-3}$
$[R_{12}COOCH_2CH_2N(CH_3)_3]Cl$	$1.5 \cdot 10^{-3}$ †	—	$1.6 \cdot 10^{-3}$ †
$[R_{12}N(CH_3)_2CH_2$  $]Br$	$5.3 \cdot 10^{-4}$	—	$2.9 \cdot 10^{-4}$
$[R_{12}COOCH_2N(CH_3)_3]Cl$	$2.7 \cdot 10^{-4}$	$2.3 \cdot 10^{-4}$	$3.5 \cdot 10^{-4}$
$[R_{14}N(CH_3)_3]Br$	$2.0 \cdot 10^{-4}$	$2.5 \cdot 10^{-4}$	$1.3 \cdot 10^{-3}$
$[R_{14}COOCH_2CH_2N(CH_3)_3]Cl$	$6.7 \cdot 10^{-5}$ †	—	$1.0 \cdot 10^{-4}$ †
$[R_{14}N(CH_3)_2CH_2$  $]Br$	$2.7 \cdot 10^{-4}$	—	$9.0 \cdot 10^{-5}$
$[R_{14}COOCH_2N(CH_3)_3]Cl$	$6.0 \cdot 10^{-5}$	$5.5 \cdot 10^{-5}$	$7.3 \cdot 10^{-5}$
$[R_{16}N(CH_3)_3]Br$	$4.0 \cdot 10^{-5}$	$4.5 \cdot 10^{-5}$	$2.8 \cdot 10^{-4}$
$[R_{16}COOCH_2CH_2N(CH_3)_3]Cl$	$4.7 \cdot 10^{-5}$	—	$5.3 \cdot 10^{-6}$
$[R_{16}N(CH_3)_2CH_2$  $]Br$	$9.9 \cdot 10^{-5}$	—	$1.8 \cdot 10^{-5}$
$[R_{16}COOCH_2N(CH_3)_3]Cl$	$7.0 \cdot 10^{-5}$	$5.5 \cdot 10^{-5}$	$1.4 \cdot 10^{-5}$
	Increment per CH_2 group, ΔG_{CH_2} (kcal/mol)		
$[R_nN/CH_3)_3]Br$			0.542
$[R_nCOOCH_2CH_2N(CH_3)_3]Cl$			0.821
$[R_nN(CH_3)_2CH_2$ $]Br$			0.409
$[R_nCOOCH_2N(CH_3)_3]Cl$			0.483

* Human red cell concentration, $3 \cdot 10^8$ cell/ml.

** Neuraminidase-treated human red cells at concentration $3 \cdot 10^8$ cell/ml.

*** Critical micelle concentration-values determined in the lysis medium, phosphate buffered isotonic saline, pH 7.2.

† The values obtained by graphical extrapolation.

the structures of the hydrophilic groups. The critical micellar concentrations of the agents are quite different and it should be noted that the basicity of the agents seems to decrease in the following order: $[R_nN(CH_3)_3]Br >$ $[R_nCOOCH_2CH_2N(CH_3)_3]Cl >$ $[R_nN(CH_3)_2CH_2$ $]Br >$ $[R_nCOOCH_2N(CH_3)_3]Cl$. The decrease in the surfactant basicity in the above order may affect the interaction of the ionic head group of the agent with the cell surface.

To estimate the hydrophobic properties of the lipophilic regions of the surfactants under study it is possible to use the common approach [21] developed in the studies of the contributions of the hydrophilic and hydrophobic portions of an amphiphile to the free energy of micellization. This approach, which con-

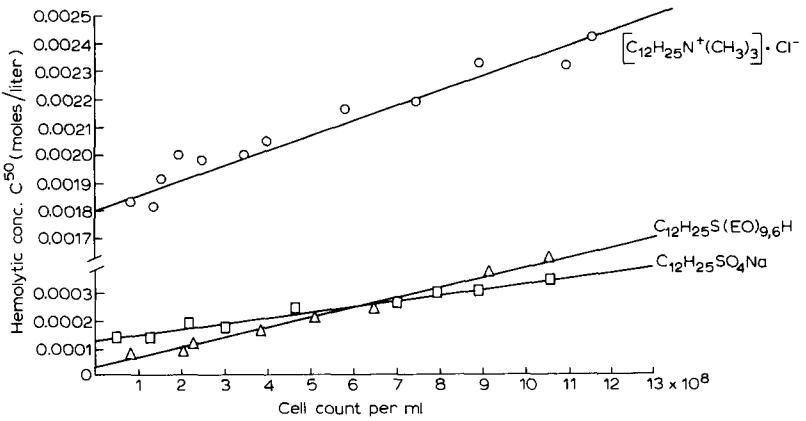

Fig. 1. Concentration of surfactants with dodecyl alkyl chain required for 50% hemolysis as a function of human red cell concentration in phosphate-buffered isotonic saline at pH 7.2 and 20°C.

because some of the cationic agents under study manifest a hemolytic power exceeding that of sodium alkyl sulfate with the same alkyl chain length.

The comparison of the lytic action of different agents on different membranes is complicated by the relationship which exists between the lytic concentration C^* chosen as a measure of the agent lytic power and the number of the cells per unit volume in the experiment. Several authors [5,8,9] have shown that the concentration C^{50} of the surfactant required to produce 50% hemolysis is linearly related to the cell count and that the lines representing these relationships may cross depending on the agents under study [9].

Fig. 1 shows the hemolytic concentration C^{50} versus cell concentration curves for the hemolysis of human erythrocytes by $R_{12}SO_4Na$, $[R_{12}N(CH_3)_3]Br$ and $R_{12}S(CH_2CH_2O)_{9.6}H$. The observed relations can be expressed by: $C^{50} = a + b \cdot N$ /1/, where N is the red cell count, a and b are constants.

It was emphasized in the literature [9] that the hemolytic "potency" should be considered as the result of two distinct and independent properties of the lytic agent: the degree to which the agent is taken up from solution by the cells, i.e., the affinity for the cell membrane, and the uptake required to cause lysis. Surfactants and other soluble amphiphiles are known to bind to biological membranes even at very low concentrations [1,24]. The mechanisms of the amphiphiles binding to membranes are still obscure but the partition model proposed in the literature [24—27] is believed at present to be the most valid one.

The partition model for the process of the agent binding to the membrane is very similar to that used in the phase separation approach to the thermodynamics of micelle formation [22]. There are extensive data [28] indicating that the transfer of an amphiphile from water to the micelle interior is similar to a transfer from aqueous solution to a hydrophobic membrane environment, at least when considered in terms of the free energy of transfer. On the base of this analogy it seems reasonable to assume that, as in the case of surfactant micelle formation where the free concentration of monomers is believed to remain constant above the critical micelle concentration [22], the free concen-

tration value "a" of the agent remains constant at any concentration of cells in the system. It means that when the total lytic agent concentration is increased above the critical value "a" it involves an increase in the amount of bound agent but not in the free one. The free energy of transfer of the agent molecules from water to the membrane, ΔG_b, can be expressed as $\Delta G_b = RT\ln a$ and it provides a measure of the agent affinity for the membrane. Since the parameter "b" seems to include the total uptake of the agent monomers by the cells in the system, it can be used as a measure of the agent's lytic power.

It should be noted that both constants a and b seem to depend on the hemolytic end-point chosen in the experiments. When the agent concentrations needed for various hemolysis degrees (C^*) are determined, the "b" values appear to be equal up until approximately 70% hemolysis, while the "a" values are different, but when C^{80}, C^{90} or C^{100} are measured [5] both a and b values differ greatly. Reasons for this are not completely understood [5,6,9] and they may include the low experimental precision as well as the suggested [29] difference in individual red cell's resistance to lysis or an extremely large contribution of the broken-down membrane fragments and released hemoglobin to the agent uptake at the high hemolysis degrees.

Both a and b constants characterize membrane properties as well as those of the lytic agent. The affinity of the agent for the membrane must depend on its relative lipophility [2,3] and to a much lower degree on the features of the hydrophobic and hydrophilic groups of the agent molecule.

The "a" and "b" values for some of the compounds studied here are listed in Table III together with the affinity for the human red cell membrane values (calculated as above from the a values) and the data on the ΔG_{CH_2} increments and on the octanol-water partition coefficients to account for differences in relative hydrophobic character of the agents.

From the data given in Table III it appears that the affinity for the erythrocyte membrane decreases in an order similar to that observed for the decrease in the relative lipophility of the compounds. Therefore, it appears reasonable to conclude that the affinity is essentially determined by the agent's relative hydrophobic character. Experimental data obtained by Thron [9] seem to sup-

TABLE III

VALUES OF a AND b FOR SURFACTANTS WITH DODECYL ALKYL CHAIN, THE AFFINITY OF THE SURFACTANTS FOR THE HUMAN RED CELL MEMBRANE AND SOME PHYSICOCHEMICAL CONSTANTS OF THE SURFACTANTS

Surfactant	a (mol/l)	b (mol/cell)	ΔG_b * (kcal/mol)	ΔG_{CH_2} (kcal/mol)	log P **
$R_{12}SO_4Na$	$1.19 \cdot 10^{-4}$	$2.35 \cdot 10^{-13}$	5.36	0.63	1.60
$[R_{12}N(CH_3)_3]Br$	$1.80 \cdot 10^{-3}$	$3.53 \cdot 10^{-13}$	3.75	0.54	0.84
$R_{12}S(CH_2CH_2O)_{9.6}H$	$2.87 \cdot 10^{-5}$	$3.24 \cdot 10^{-13}$	6.20	0.69 ***	—

* Values are calculated on the base of the relation $\Delta G_b = +RT \ln a$.

** P is the octanol/water partition coefficient, the data are taken from ref. 3.

*** The value obtained for the homologous series of compounds represented by the formula $R_nO(CH_2CH_2O)_6H$.

port this conclusion since the saponins he studied for their hemolytic action display affinities, the values of which, when estimated as above, are of a similar order of magnitude to those shown in Table III, namely in the range of 5.8—7.8 kcal/mol. It should be noted that digitonin manifests an affinity for the human red cell membrane of 5.755 kcal/mol, very close to that of sodium dodecyl sulfate, 5.358 kcal/mol. At the same time, the lytic activity measures for these agents are very different, the "*b*" values amounting to $25.8 \cdot 10^{-18}$ mol/cell and $2.35 \cdot 10^{-13}$ mol/cell, respectively. It implies that the affinity and the lytic activity in relation to a given membrane are determined by different properties of the lytic agent.

It should be emphasized that the lack of a correlation between the hemolytic power of surfactants and their critical micelle concentrations [16] and the hydrophile-lipophile balance values can be due to the lack of a direct relationship between the lytic activity and the overall affinity of the agent for the membrane. The relationships between the membrane-perturbing effects and the above characteristics of the surfactants found in refs. 13—15 are likely to be indications only that the effects studied in refs. 13—15 could be provided by the action of any compound with the required magnitude of the overall relative hydrophobic character. It was also noted above that this factor is widely known to be important in determining the agent membrane activity [2,3,24].

The question of the general and specific features of the amphiphile-membrane interaction is especially important for the understanding of the selective membrane action of surfactants. Lanyi [30] showed that the effectiveness of Triton X-100 in solubilizing the membranes of *Halobacterium cutirubrum* depends on the respiratory state of the organism. The selective citotoxicity of the nonionic surfactant Triton WR-1339 to cell cultures of malignant origin was demonstrated by Miller et al. [31]. Also, we found that some of the polyoxyethylene coumpounds display marked lytic activity toward the mouse leucose L-1210 ascite cells while manifesting absolutely no hemolytic action (unpublished results). It is common knowledge that commercial saponin allows the elimination of erythrocytes from any natural cell mixture due to this compound's selective lytic action. Thus, it seems obvious that the lytic action of a given amphiphile on a given cell membrane depends on the agents's properties as well as on those of the membrane.

We believe that the most interesting amphiphiles in the above respect must be those including naturally occuring groups and residues and, in the first stage of research along this line, the most available compounds appear to be *N*-acyl derivatives of various amino acids, carbohydrates, etc. Synthesis and the study of the membrane-perturbing and lytic action of these compounds are in progress in our laboratory at present.

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