[Chem. Pharm. Bull. 18(11)2158—2163(1970)]

UDC 615.273.2.015.44

Effect of Ionic Head of Cationic Surface Active Agents on Their Hemolytic Activity

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(Received February 13, 1970)

An investigation was made of the effect of ionic head of cationic surface active agents on their hemolytic activity. When the number of carbon atoms in the alkyl radical is less than 8, there appeared to exist a close relation between the adsorbability and the hemolytic activity (amount adsorbed per cell necessary to cause hemolysis) of the cationic agents. However, no direct correlation could be found if the number of carbon atoms exceeds 8. This was interpreted as implying that too high the adsorbability of surface active cations with a long hydrophobic tail and a large ionic head may adversely affect the hemolytic activity of these ions.

Our previous works[2–4] have revealed the importance of the interaction of surface active cations with phospholipid anions in red cell membrane in hemolysis. In our latest paper,[5] a mechanism of hemolysis by cationic surface active agents has been proposed, based on the experimental findings accumulated in our laboratory. Namely, surface active cations were suggested to be adsorbed on the lipoprotein layer of red cell membrane by hydrophobic bond and interact electrostatically with phospholipid anions under the conditions of physiological pH and ionic strength to liberate them into the surrounding medium, thereby causing the alteration in protein confomation of the cell membrane, which gives rise to hemolysis. The adsorption of surface active cations was also suggested to take place on the outer mucoprotein layer of the membrane where they are captured by carboxyl groups to form ionic bonds. The role of this adsorption in hemolysis was concluded, however, to be less important than that of the adsorption on the lipoprotein layer. According to this mechanism, low hemolytic concentrations of higher members of a given homologous series[6–9] can be plausibly explained by their increased adsorbability to red cell membrane.

On the other hand, it was not established yet how the hemolytic concentrations of cationic agents with the same hydrophobic tail vary with their ionic head. Literature survey also indicates that there have been very few reports on this problem.[10,11] The difference in ionic head of cationic agents would not cause any essential alteration of the mechanism by which they hemolyze red cells but would affect their hemolytic concentration through the disparity in their adsorbability.

The present paper was planned, therefore, to find the dependence of hemolytic activity of cationic surface active agents on their ionic head.

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Experimental

Materials——The series of alkylamine hydrochlorides ($C_nH_{2n+1}NH_3Cl$, $n=4,6,8,10$, and 12) were obtained as precipitates by passing dried hydrogen chloride through the benzene solution of the amines. The precipitates were collected and recrystallized from ethanol. The series of alkylpyridinium iodides ($C_nH_{2n+1}PyI$, $n=4,8,10$, and 12) were prepared and purified by the method already described.[12] The synthesis and purification of the series of alkylquinolinium bromides ($C_nH_{2n+1}QuBr$, $n=4,8$, and 12) were made as follows. Quinoline and alkylbromides were purified by distillation just before use. To a known amount of quinoline in a round bottom flask an approximately equivalent amount of alkylbromide was added in dropwise with stirring, and the mixture was heated with further stirring for 4 hr at 100°. At the end of this period, the reaction mixture was cooled and washed with carbon tetrachloride, followed by recrystallization from acetone. The purity of the salts was checked by thin-layer chromatography.

The red cell suspension used was prepared from dog blood in the following way. Citrated blood was centrifuged and the settled cells were washed three times with the phosphate-buffered isotonic saline (pH 7.4) or nonbuffered isotonic saline (aqueous 0.9% NaCl solution). The former was used for the hemolysis experiments by alkylpyridinium and alkylquinolinium salts, whereas the latter for those by alkylamine hydrochlorides.

The washed and packed cells were then suspended in the same medium as that used in washing to give a suspension of a desired concentration. A concentration of 1% v/v of the suspension was found in a hemocytometer to correspond to a cell count of 1.75×10^8/ml.

Hemolysis Techniques——The determination of the degree of hemolysis was made in the following manner. Two milliliters of the cell suspension were pipeted into test tubes, into which an equal volume of various concentrations of cationic surface active agents was added quickly by a syringe to prevent any locallysis. The mixtures were then allowed to react for an hour in a water bath controlled at 30 or 37 ± 0.1°.

At the end of this period, the mixtures were immediately centrifuged to remove the unhemolyzed cells. The degree of hemolysis was estimated by determining spectrophotometrically the amount of hemoglobin released in the supernatant liquid.

Adsorption of Surface Active Cations on Red Cells——The amount of surface active actions adsorbed on dog red cells was estimated by the method adopted in previous works.[5,13]

Thus, the total amount of surface active cations, c_x, needed to produce x% hemolysis after 1 hr reaction time was determined as a function of cell count, N, in unit volume of the system.

$$c_x=a_xN+b_x$$

The values of a_x and b_x give the amounts adsorbed and unadsorbed of surface active cations per cell, respectively, and were determined by the least-squares method at various degrees of hemolysis. The adsorption isotherms were obtained by plotting a_x against b_x.

Surface Tension Measurements of Hemolytic Agent Solutions——The surface tension measurements were carried out using a du Nouy tensiometer at room temperature.

Result and Discussion

Hemolytic Concentrations

In Table I are presented the minimum concentrations of cationic surface active agents required to cause complete hemolysis for 4% v/v red cell suspension. The minimum concentration necessary for a given surface active agent to produce hemolysis is called its hemolytic concentration and is regarded as a measure of its hemolytic activity. Thus, a surface active agent which gives lower hemolytic concentration than another does is said to be more hemolytically active than the latter.

The critical micelle concentrations are given in Table II for some of the cationic agents listed in Table I since these values may serve as a measure of their adsorbability[14] and the adsorption of surface active ions should play an important role in hemolysis. In view of this, a parallelism is expected between the hemolytic concentrations and the critical micelle concentrations of the cationic agents with the same hydrophobic tail but different ionic head.

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Table I. Minimum Concentrations of Cationic Surface Active Agents Required to Produce 100% Hemolysis for 4% v/v Red Cell Suspecsion (M)

Compound	30°	37°
$C_{12}H_{25}NH_3Cl$	3.70×10^{-4}	2.95×10^{-4}
$C_{10}H_{21}NH_3Cl$	1.95×10^{-3}	1.35×10^{-3}
$C_8H_{17}NH_3Cl$	4.05×10^{-2}	2.40×10^{-2}
$C_6H_{13}NH_3Cl$	4.58×10^{-1}	3.15×10^{-1}
$C_4H_9NH_3Cl$	1.71	1.53
$C_{12}H_{25}Pyl$[a]	4.45×10^{-4}	3.00×10^{-4}
$C_{10}H_{21}Pyl$[a]	4.55×10^{-3}	3.00×10^{-3}
$C_8H_{17}Pyl$[a]	3.03×10^{-2}	2.33×10^{-2}
C_4H_9Pyl[a]	5.55×10^{-1}	4.80×10^{-1}
$C_{12}H_{25}QuBr$[b]	1.95×10^{-4}	1.53×10^{-4}
$C_8H_{17}QuBr$[b]	1.21×10^{-2}	1.03×10^{-2}
C_4H_9QuBr[b]	1.95×10^{-1}	9.60×10^{-2}

a) abbreviation for alkylpyridinium iodides
b) abbreviation for alkylquinolinium bromides

Table II. Critical Micelle Concentrations of Cationic Surface Active Agents at Room Temperature

Compound	CMC (M)	Compound	CMC (M)
$C_{12}H_{25}NH_3Cl$	1.4×10^{-2}	$C_{10}H_{21}Pyl$	1.6×10^{-2}
$C_{10}H_{21}NH_3Cl$	4.8×10^{-2}	$C_8H_{17}Pyl$	6.4×10^{-2}
$C_8H_{17}NH_3Cl$	1.75×10^{-1}	$C_{12}H_{25}QuBr$	5.0×10^{-4}
$C_{12}H_{25}Pyl$	4.0×10^{-3}	$C_8H_{17}QuBr$	5.2×10^{-2}

A comparison of Table I with Table II indicates, however, that there is not necessarily such a parallelism. For instance, the critical micelle concentrations of dodecylamine hydrochloride, dodecylpyridinium iodide, and dodecylquinolinium bromide are 14.0, 4.0, and 0.5 mM, respectively, while these agents are in the order, dodecylpyridinium iodide>dodecylamine hydrochloride>dodecylquinolinium bromide, in the hemolytic concentration. This may arise in part from the fact that the hemolytic concentration of surface active agent is not independent of the concentration of red cell suspension but a function of it.[5,13,15] Actually, in some cases, a reversal of hemolytic concentrations of surface active agents is observed when the red cell concentration is changed.[15] In order to compare the hemolytic activity of various surface active agents in a proper way, therefore, the use of hemolytic concentration determined for red cell suspension of a fixed concentration is inadequate and a measure independent of the red cell concentration should be sought. In this sense, the amount adsorbed per cell of surface active ions would be appropriate.

Adsorption of Surface Active Cations

Fig. 1 and 2 show the hemolysis curves and the hemolytic concentration versus cell concentration curves for butylpyridinium iodide at 37°. Similar results were obtained with all other cationic agents employed. These figures clearly demonstrate that the hemolytic concentration is dependent on the red cell concentration.

Adsorption isotherms for the cationic agents with butyl and dodecyl radical are shown in Fig. 3 and 4, respectively. The amount adsorbed of the agents necessary to produce lysis appears to be strongly affected by their ionic head.

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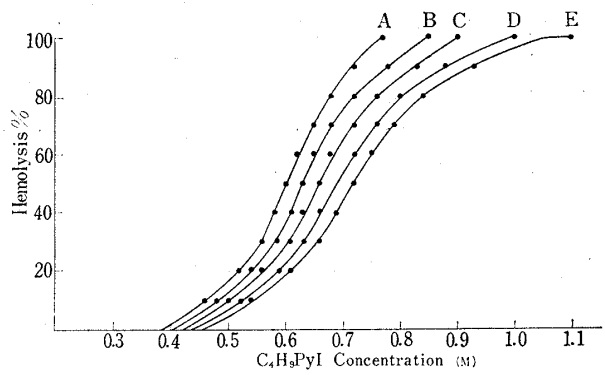

Fig. 1. Percent Hemolysis versus C_4H_9Pyl Concentration Curves for Dog Red Cell Suspension at 37°

cell concentration % v/v; A,1; B,2; C,3; D,4; E,5

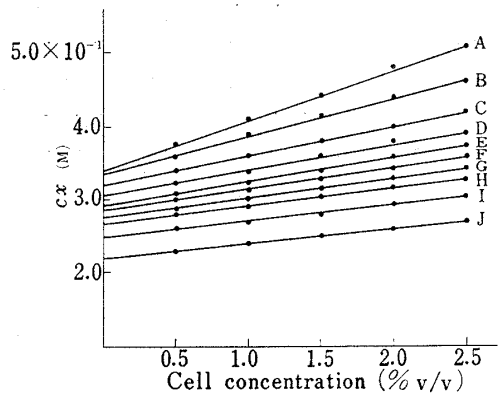

Fig. 2. C_4H_9Pyl Concentration Needed for Various Degrees of Hemolysis, c_x, versus Cell Concentration at 37°

hemolysis percent: A,100; B,90; C,80; D,70; E,60; F,50; G,40; H,30; I,20; J,10

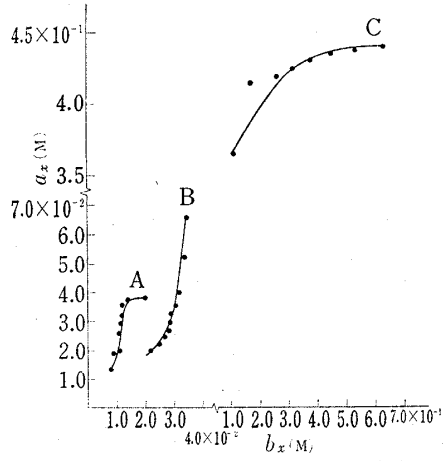

Fig. 3. Adsorpition Isotherms for Cationic Surface Active Agents with Butyl Radical at 37°

A: C_4H_9QuBr B: C_4H_9PyI C: $C_4H_9NH_3Cl$

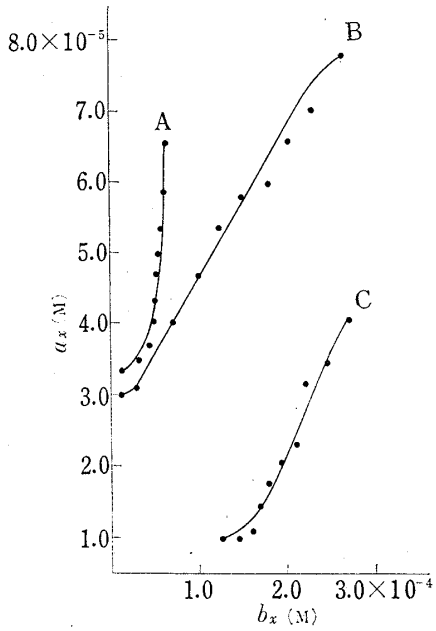

Fig. 4. Adsorption Isotherms for Cationic Surface Active Agents with Dodecyl Radical at 30°

A: $C_{12}H_{25}QuBr$ B: $C_{12}H_{25}PyI$
C: $C_{12}H_{25}NH_3Cl$

In Table III are summarized the value of a_{100} and b_{100} at 30° and 37° for alkylamine hydrochlorides, alkylpyridinium iodides, and alkylquinolinium bromides. An inspection of the table indicates the following facts. First, both a_{100} and b_{100} decrease with increasing alkyl chain length, showing the higher adsorbability for higher members of any homologous series. Secondly, an increase in temperature resluts in a decrease in a_{100} and b_{100}, due presumably to the increased sensitivity of red cells to surface active agents at higher temperatures.[5,6,13] Thirdly, there seems to be a close relation between the adsorbability and a_{100} of the cationic agents when the number of carbon atoms in their hydrophobic tail is less than 8. That is, the values of a_{100} increase in the order, alkylamine hydrochlorides<alkylpyridinium iodides< alkylquinolinium bromides. This would reflect the higher adsorbability for surface active

cations with larger ionic head since the increased size brought about by the substitution of a pyridine or quinoline ring for the amine group attached to alkyl chain should cause an increase in attractive forces between the surface active cations and components of red cell membrane. On the other hand, in the case of dodecyl derivatives, the values of a_{100} are in the increasing order, dodecylpyridinium iodide>dodecylquinolinium bromide>dodecylamine hydrochloride, being indicative of the apparent contradiction to the above argument.

Table III. Values of a_{100} and b_{100} at 30° and 37° for Three Homologous Series of Cationic Surface Active Agents (ions/cell)

Compound	30°		37°	
	a_{100}	b_{100}	a_{100}	b_{100}
$C_{12}H_{25}NH_2HCl$	1.40×10^{8}	1.00×10^{9}	1.25×10^{8}	7.70×10^{8}
$C_{10}H_{21}NH_2HCl$	5.71×10^{8}	5.62×10^{9}	4.43×10^{8}	3.74×10^{9}
$C_{8}H_{17}NH_2HCl$	3.11×10^{10}	7.78×10^{10}	6.92×10^{9}	6.92×10^{10}
$C_{6}H_{13}NH_2HCl$	3.51×10^{11}	8.78×10^{11}	1.25×10^{11}	8.39×10^{11}
$C_{4}H_{9}NH_2HCl$	1.58×10^{12}	2.75×10^{12}	1.51×10^{12}	2.28×10^{12}
$C_{12}H_{25}Pyl$	2.39×10^{8}	1.08×10^{9}	2.32×10^{8}	5.78×10^{8}
$C_{10}H_{21}Pyl$	1.73×10^{9}	1.23×10^{10}	1.68×10^{9}	7.06×10^{9}
$C_{8}H_{17}Pyl$	2.47×10^{10}	5.29×10^{10}	1.99×10^{10}	4.05×10^{10}
$C_{4}H_{9}Pyl$	3.18×10^{11}	1.28×10^{12}	2.28×10^{11}	1.20×10^{12}
$C_{12}H_{25}QuBr$	2.29×10^{8}	2.11×10^{8}	1.83×10^{8}	1.57×10^{8}
$C_{8}H_{17}QuBr$	5.67×10^{9}	3.07×10^{10}	2.87×10^{9}	3.00×10^{10}
$C_{4}H_{9}QuBr$	2.62×10^{11}	1.47×10^{11}	1.32×10^{11}	6.88×10^{10}

In order to make this situation clearer, two graphs are drawn demonstrating the relationships between log carboxy methyl cellose (CMC) and alkyl chain length, and between log a_{100} and alkyl chain length in Fig. 5 and 6, respectively. These two relationships are linear for all homologous series withih the experimental error of $\pm5\%$ in this work. It is evident from the two graphs that the values of a_{100} of the corresponding members of the three homologous series are not always in the same order with their CMC's when the number of carbon atoms in their alkyl chain is more than 8. This would be interpreted as implying that the surface active cations with very high adsorbability resulting from the combination of a long hydrophobic tail and a large ionic head are easily trapped in the outer part of red cell membrane and consequently a large amount of these ions is needed further to reach the inner part of the membrane, where they are adsorbed and interact electrostatically with phospholipid anions to release the latter out of the membrane. In other words, too strong the adsorption may adversely affect the hemolytic action of cationic surface active agents. For example, dodecylpyridinium ions will be adsorbed more strongly on red cell membrane than dodecylammonium ions since the former has larger ionic head than the latter. As a concequence, larger amount of dodecylpyridinium ions will be required than are dodecylammonium ions to be adsorbed sufficiently on the inner lipoprotein layer of the membrane to interact electrostatically with phospholipid anions. This would result in a higher a_{100} value for the pyridinium iodide than the amine hydrochloride. Similar trend will be expected for hexadecylquinolinium and hexadecylpyridinium salts because the straight lines of these salts in Fig. 6 tend to cross each other at a point corresponding to the carbon atom number of about 14 if they are extended on the graph.

It may be concluded, therefore, that the increase in the size of ionic head increases the hemolytic activity of cationic agents due to their increased adsorbability to red cell membrane when their hydrophobic tail is short but not necessarily so and even affects adversely if the carbon atom number in the chain exceeds 8.

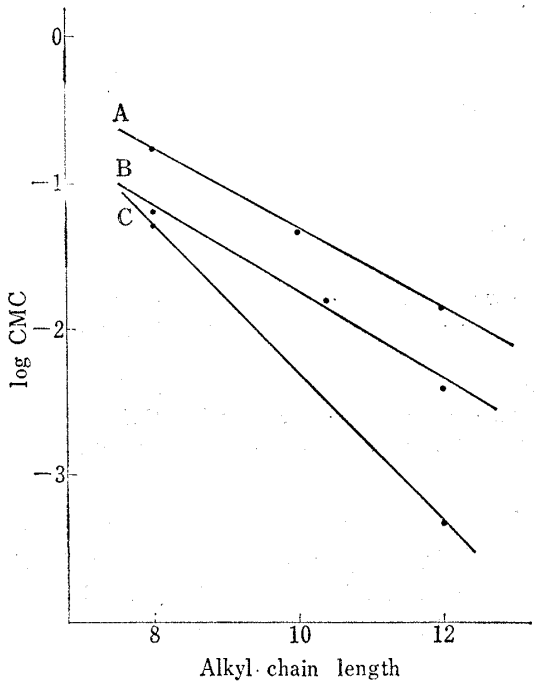

Fig. 5. Relation between log CMC and Alkyl Chain Length

A: RNH_3Cl B: RPyI C: RQuBr

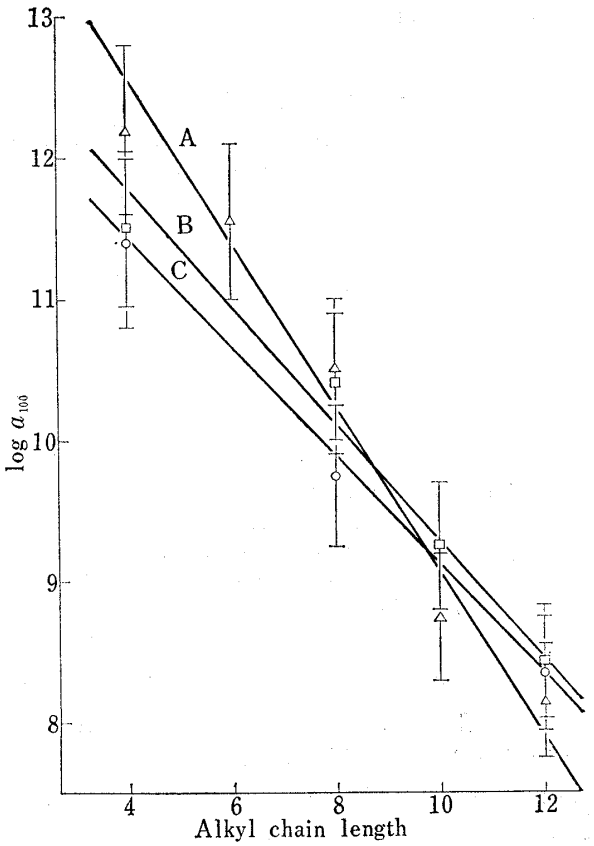

Fig. 6. Relation between log a_{100} and Alkyl Chain Length at 30°

A: RNH_3Cl B: RPyI C: RQuBr

Acknowledgement The authors wish to express their hearty thanks to the experimental assistance of Misses S. Ito, M. Saito, A. Usami, and M. Shibuya.