INTERACTION OF BASIC POLYAMINO ACIDS WITH THE RED BLOOD CELL

I. COMBINATION OF POLYLYSINE WITH SINGLE CELLS

by

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INTRODUCTION

Basic polypeptides which agglutinate bacteria and inhibit their growth and respiration have been isolated from various natural sources – fungi, bacteria, and higher plants and animals[1,2]. Synthetic basic polyelectrolytes have been shown to have similar effects: thus STAHMANN *et al.* and BURGER AND STAHMANN[3] found that polylysine decreases the virulence of tobacco mosaic virus; and BURGER AND STAHMANN[4] and E. KATCHALSKI *et al.*[5] found that polylysine agglutinates bacteria and inhibits their growth. These effects were reversible[3,4,5]. RUBINI *et al.*[6] observed that polylysine agglutinates chicken red blood cells, and DE VRIES *et al.*[7] found that it agglutinates as well as hemolyses human red blood cells.

Clearly, these effects are due to the binding of the polyelectrolyte to the cell. However, there has been little investigation of the mechanism involved. Experiments were therefore carried out to clarify the reaction by which positively charged polyelectrolytes combine with cell surfaces. For this purpose synthetic basic polyamino acids were chosen because their structure has been defined and their physicochemical properties are known; red blood cells were used because their structure is simpler than that of most cells.

The first part of this study is devoted to the interaction by the basic polypeptide, polylysine, with single red blood cells. The second part, to be published subsequently, deals with the mechanism of agglutination of red blood cells by polybases.

EXPERIMENTAL

(a) *Preparation of red blood cell suspensions*

Isotonic, buffered NaCl solutions of pH 7.2 and ionic strength 0.15 were prepared by mixing 100 volumes of a 0.9% NaCl solution (analytical grade) with 4 volumes of veronal-HCl buffer, prepared according to BRITTON[8].

9 volumes of human blood, obtained by venipuncture, were mixed with 1 volume of 0.1 molar sodium oxalate. The cells were separated from the plasma by centrifuging for 15 minutes at 1500 r.p.m., and were then washed 4 times with 6 volumes of the buffered NaCl solution and resuspended in this medium.

All experiments were performed with cell suspensions less than 8 hours old.

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(b) *Preparation of red cell "ghosts"*

5 volumes of distilled water were mixed gently with 1 volume of packed washed cells, and allowed to stand for 30 minutes. The suspension was then filtered through loose cotton and the filtrate was centrifuged. The ghosts were separated from the hemolysate and washed 4 times with the buffered NaCl solution. The ghosts were still found to contain 10–15% of the total hemoglobin of the blood cell.

(c) *Polyamino acids*

Poly-DL-lysine hydrobromide (PL), of an average degree of polymerization $n = 36$ (determined by amino end-group analysis), was prepared according to KATCHALSKI *et al.*[9]. As the magnitude of the effects of the polymer was found to vary somewhat with the batch, all experiments were carried out with PL of a single batch.

(d) *Microscopic electrophoresis*

The electrophoretic measurements were carried out according to ABRAMSON *et al.*[10], with a Northrop Kunitz-type micro-electrophoresis apparatus, equipped with reversible electrodes of copper in saturated copper sulfate. The strength of the electric field in the cell was calculated from the current strength, the conductivity of the solution, and the cross section area of the cell.

The electrophoretic mobilities of the red cells in any one suspension varied only within the limits of experimental error. To secure a reliable mean, measurements on at least 10 cells were taken for each mobility determination. Reproducibility of mobility measurements was within ± 2%. Constant and reproducible mobility values of red cells in polybase solutions were obtained five minutes after suspending the blood cells in the polyamino acid solution. The mobility of the cells in polyamino acid solution remained unchanged for several hours.

It was found that red cell suspensions suitable for electrophoretic measurements must be fairly dilute, preferably containing not more than 10^7 cells per ml. When mobility determinations were required on red cell suspensions too concentrated for measurement, the cells were thrown down at low speed centrifugation until a suspension sufficiently dilute for electrophoretic measurement was obtained. Where agglutination occurred, discrete cells were always found in addition to the aggregates. The mobilities of the discrete cells and of the cell aggregates in the same suspension were found to be equal in all cases. Therefore in determining the mobility of red cells under conditions of agglutination, the mobility of the discrete cells may be taken as representative of the suspensions as a whole.

It was found that basic polyelectrolytes are irreversibly adsorbed on glass; therefore a glass surface in contact with a polylysine solution is always covered with a concentrated layer of the polyelectrolyte. In the microelectrophoresis cell, where the ratio of surface area to volume is large, the adsorption of the polybase by the glass wall may decrease the PL concentration significantly in solutions of low concentration.

If red blood cells are allowed to settle on the PL-coated glass surface and then resuspended, their mobility may change markedly.

When glass coated with paraffin or with plastics (silicone, lucite) was used, mobility of the cells was not affected. However, because of the difficulty and inconvenience of cleaning the coated electrophoretic cell, measurements were made in the untreated glass apparatus. Constant and reproducible results were obtained if the following precautions were taken:

The electrophoresis apparatus was first rinsed with the solution of the polyamino acid; cells were not allowed to settle on the glass walls of the electrophoretic apparatus and of the vessels, and mobility measurements were made immediately after introducing the blood cell suspensions.

Between experiments, the adsorbed polybase was removed by rinsing the microelectrophoretic cell and the glassware with 1 *N* NaOH, and washing thoroughly with distilled water.

RESULTS

Preliminary experiments have shown that low concentrations of all the basic polyelectrolytes tested agglutinated the red blood cells and markedly reduced their electrophoretic mobility; neither of these effects was produced by neutral or acidic polyelectrolytes, or by any of the monomers, whether acid, basic or neutral.

Since basic polyelectrolytes of very different chemical groups produce similar effects, it is clear that the effects of the various polybases are due to their polyelectrolyte character and not to any specific chemical composition. The fact that only polybases, but not their monomers, affect the cells indicates that the factor chiefly responsible for

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their interaction with the oppositely charged cell surfaces is the strong electrostatic field of the polyion, which is due to its high charge density.

I. Electrophoretic behaviour of red blood cells in polylysine solutions

All experiments were performed in isotonic buffered NaCl solutions of pH 7.2 and ionic strength 0.15.

Polylysine tends to change the normally negative mobility of red blood cells to positive*; *i.e.* – with increasing PL concentration the negative mobility of the cells decreases to 0, thereafter it becomes positive until, at high PL concentrations, it approaches asymptotically a maximum value (Fig. 2).

Clearly these changes in mobility are due to adsorption of the positively charged PL on the negatively charged surface of the red blood cell. Their magnitude depends both on the PL concentration and on the number of cells per ml.

(a) *Reversibility of polylysine adsorption on the red blood cell*

It has been shown that the adsoption of PL on the red cell is a reversible reaction; it rapidly reaches a state of equilibrium which is dependent only on the final composition of the system. The *electrokinetic potential* of the red blood cell in PL solutions, *at a given ionic strength and pH*, is consequently *a single valued function of the equilibrium concentration of free PL in the solution.*

The reversibility of the reaction is proved by the following typical experiment:

A series of suspensions of $2 \cdot 10^7$ cells per ml was prepared in buffered NaCl solutions containing the following amounts of polylysine hydrobromide (PL) (a) 0 micrograms per ml, (b) 2 micrograms per ml, (c) 10 micrograms per ml; (d) same final composition as (b), prepared by mixing 4 parts of suspension (a) with 1 part of suspension (c). The mobilities of the cells in the suspensions are given in Table I.

TABLE I

MOBILITY OF RED BLOOD CELLS SUSPENDED IN SOLUTIONS OF PL IN BUFFERED NaCl, OF IONIC STRENGTH 0.15 AND pH-7.2

All the suspensions contained $2 \cdot 10^7$ cells/ml. The preparation of the suspensions is described in the experimental part.

Suspension	*Polylysine-HBr micrograms/ml*	*Mobility μ/sec/volt/cm*
a	0	−1.08
b	2	0.00
c	10	+1.18
d	2	0.00

It will be observed that cells of suspensions (b) and suspension (d) have the same mobility. Moreover, suspension (d) was electrophoretically homogenous – all cells had the same mobility – proving a homogenous redistribution of PL over all the cells.

* "Negative" and "positive" are used to denote the mobilities of negatively charged and of positively charged particles, respectively.

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(b) *Variation of electrophoretic mobility of red blood cells with number of cells at constant PL concentration*

The mobility of red blood cells was determined in a series of suspensions at equal PL concentrations but at different cell-concentrations. The results are given in Fig. 1.

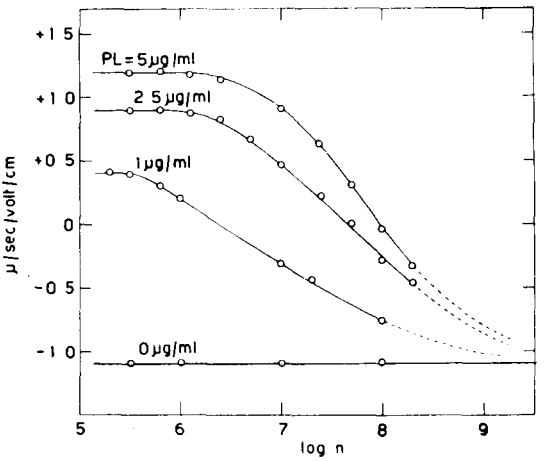

Fig. 1. Dependence of red blood cell mobility on number of cells per ml (n) at constant PL concentration.

The mobility increases with increasing dilution of the cell suspension, reaching an upper limiting value at very high dilution. On the other hand, when the number of cells per ml is very great, mobility approaches that of cells in a solution free of polylysine.

The curves may be explained as follows: as the red cells adsorb PL, the concentration of free PL in solution at equilibrium is less than the total PL concentration. Increasing the number of cells per ml decreases the equilibrium concentration of free PL and hence decreases the potential of the red cells. At sufficiently high cell-concentrations, practically all the polyamino acid is adsorbed by the cells, and the concentration of free PL approaches zero. Correspondingly, the amount adsorbed *per cell* is so small that the potential of the cell does not differ significantly from that of the cell in a solution free of polylysine.

On the other hand, at sufficiently low cell-concentrations, the total amount of polybase adsorbed is negligible, and the free PL concentration does not differ significantly from the total PL concentration. Hence the mobility of the red cell reaches a maximum which is characteristic of the total PL concentration; this will henceforth be termed the "*limiting mobility*" for that concentration.

(c) *Variation of electrophoretic mobility of red blood cells with PL concentration, at constant number of cells*

A curve representing red cell mobility as a function of equilibrium concentration of free PL, is obtained by plotting the limiting mobility versus PL concentration (Fig. 2 curve a). Curves b and c (Fig. 2) represent the mobilities of red cells in suspensions, containing 10^7 and 10^8 cells respectively, versus total PL concentration.

All mobility curves approach, asymptotically, the same maximum mobility value (+ 1.90 μ/sec/volt/cm). However, the PL concentration at which maximum mobility is approached is dependent on cell concentration. Thus the limiting mobility curve ($5 \cdot 10^5$ cells/ml) approaches maximum mobility at approximately 60 μg/ml, while at $2 \cdot 10^7$ cells/ml it is approached at approximately 100 μg/ml.

II. Determination of the amount of polybase adsorbed on red blood cells from mobility data

At the low PL concentrations with which most of this work is concerned, the well known methods of polyamino acid assay, ninhydrin colorimetric method and microbio-

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logical assay, are not sufficiently accurate for our purposes, as well as time consuming.

Attempts to develop a general method for the determination of polyelectrolytes, based on titration with polyelectrolytes of opposite sign and using the turbidity of the

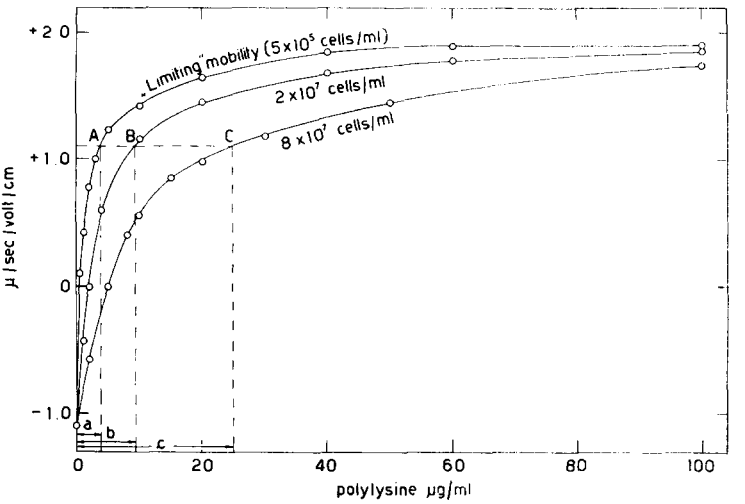

Fig. 2. Red blood cell mobility versus total PL concentration for constant number of cells per ml.

product of their interaction as indicator, were promising for pure polyelectrolyte solutions; but in the presence of traces of hemoglobin, usually encountered in red blood cell suspensions, no reliably reproducible results could be obtained.

Electrophoretic mobility data, however, can be used as sensitive indicators from which both the concentration of polybase in solution and the amount adsorbed on the cells can be readily evaluated.

As was shown above, equal red cell mobilities correspond to equal free PL concentrations, regardless of the total PL and cell concentrations. In the case of the limiting mobility the concentration of free PL is known, being equal to the total given PL concentration. Therefore, the limiting mobility curve may be used for the evaluation of free PL concentration. Thus in Fig. 2, points A, B and C, not only represent the same mobility, but express the same free PL concentration, which is equal to that at point A on the limiting mobility curve. Hence, the amount of PL adsorbed on the cells at points B and C is obtained by subtracting the free PL concentration – a – from the total PL concentration – b or c.

Verification of our assumptions is obtained from further analysis of Fig. 2. The amount of PL adsorbed per red blood cell should be the same in all suspensions in which the cells have the same mobility, hence the amount adsorbed per cell at points A, B and C should be the same and equal to

$$\frac{b-a}{10^7} = \frac{c-a}{10^8}$$

It will be seen from the data in Table II that the correspondence is satisfactory.

Additional verification of the validity of the method is derived from the following typical experiment:

A red blood cell suspension, represented *e.g.*, by point B Fig. 2, was centrifuged, and

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TABLE II

THE AMOUNT OF PL ADSORBED PER RED CELL IN SUSPENSIONS OF VARYING RED CELL CONCENTRATIONS AT EQUAL RED CELL MOBILITIES

Points on curves of Fig. 3	*Total PL μg/ml*	*No. of cells per ml*	*Red cell mobility μ/sec/volt/cm*	*Free PL μg/ml*	*PL* adsorbed per cell, μg*
A	3	10^5	+ 1.0	3	
B	7.5	$2 \cdot 10^7$	+ 1.0	3	$2.25 \cdot 10^{-7}$
C	20.5	$8 \cdot 10^7$	+ 1.0	3	$2.20 \cdot 10^{-7}$
A′	1.4		+ 0.6	1.4	
B′	4.0	$2 \cdot 10^7$	+ 0.6	1.4	$1.3 \cdot 10^{-7}$
C′	11.0	$8 \cdot 10^7$	+ 0.6	1.4	$1.2 \cdot 10^{-7}$

* The figures in this column represent the amount of polylysine hydrobromide (PL) equivalent to the amount of polyion adsorbed.

the clear supernatant was separated from the cells. According to previous reasoning, the PL concentration in the supernatant should equal a and therefore the limiting mobility in the supernatant should equal the mobility of the red cells at B. The limiting mobilities in the supernatants of several suspensions were determined by introducing a small number of cells ($5 \cdot 10^5$/ml) and measuring their mobilities. The mobilities of the cells in the suspensions and the limiting mobilities in the supernatants were found to be equal in all cases.

From the mobility data given above for different PL concentrations and numbers of red cells a single adsorption isotherm has been constructed (Fig. 3).

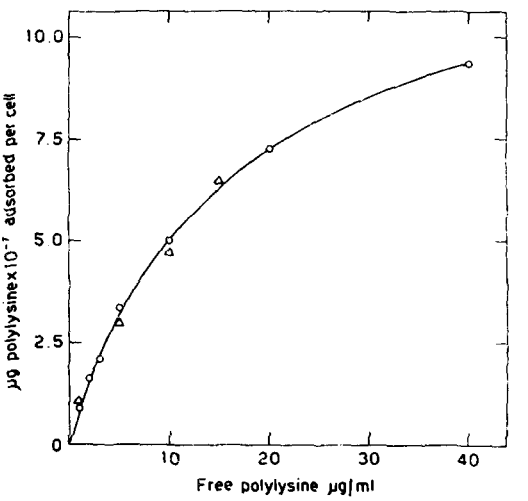

Fig. 3. Amount of polylysine adsorbed per red blood cell versus free polylysine concentration. ○ red blood cells; △ "ghosts".

Fig. 3 represents the amount of PL adsorbed per cell as a function of the equilibrium concentration of free PL. The isotherm is a parabola of the Freundlich type. It reaches a saturation value of approximately 10^{-6} μg per red blood cell. This adsorption isotherm is characteristic of the interaction of PL with the red blood cell in physiological saline solution of ionic strength 0.15 and pH 7.2.

Preliminary experiments have shown that the run of the absorption isotherms is dependent on both ionic strength and pH.

III. Location of the site of PL adsorption in the red blood cell

In order to ascertain whether the membrane alone or both membrane and interior of the cell participate in the binding of PL, experiments on red cell ghosts were carried out. It has been shown by TEORELL[11] and others that the membrane's electrophoretic behavior and permeability properties are unaltered in the ghost, which may be considered therefore as a practically intact red cell membrane.

It was reasoned that if the PL does not penetrate into the interior of the cell, whole

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cells and ghosts should adsorb equal amounts of PL. On the other hand, as additional experiments have shown that hemoglobin binds considerable quantities of PL, with penetration of the PL the whole cells should adsorb markedly more than the ghosts.

Using the same technique as described for red blood cells, it was found that both the limiting mobility curves and adsorption isotherms for ghosts were identical with those obtained for whole cells, within the limits of experimental error (see Fig. 3). It may be concluded therefore that the PL does not penetrate into the cell.

Supporting evidence for this conclusion may be obtained as follows. If the adsorption is in the membrane only, then hemolysing a suspension of red cells in PL solution should cause additional adsorption of PL from the solution by the liberated hemoglobin. Consequently, the mobility of the ghosts and the limiting mobility of the supernatant hemolysate should be lower than that of the unhemolysed cells. On the other hand, if PL penetrates the membrane and combines with the hemoglobin, the amount of adsorbed PL should be approximately the same for the unhemolysed cells and for the hemolysed cells plus the hemolysate. The results of several experiments with different PL concentrations are given in Table III.

TABLE III

CHANGE IN MOBILITY OF RED CELLS IN PL SOLUTION DUE TO HEMOLYSIS

All suspensions contained $2.5 \cdot 10^7$ cells/ml. The hemolysate was prepared by hemolysing 1 volume of a 20% red cell suspension with 8 volumes of water, after which it was brought to the final composition of $2.5 \cdot 10^7$ cells/ml, 0.15 molar NaCl, and the given PL concentration.

Total PL conc. μg/ml	*Mobility of red cells before hemolysis μ/sec/volt/cm*	*Limiting mobility in supernatant after hemolysis μ/sec/volt/cm*
10	+ 1.02	+ 0.20
15	+ 1.38	+ 0.71
20	+ 1.54	+ 0.90

It will be seen from Table III that the hemoglobin liberated from the cells into the bulk of the solution pronouncedly lowers the mobility of the red cells, *i.e.* it binds considerable amounts of PL. This indicates, as did the previous experiments, that the polylysine does not penetrate into the cell.

IV. Adsorption of PL on glass

It was of interest to compare the adsorption of PL on the dynamic structure of the erythrocyte membrane with that on static surfaces of solids such as glass or quartz.

The specific surface area of powdered glass was determined by comparing the amount of methylene blue required to cover the glass powder with a monomolecular layer with the amount required to provide a monomolecular layer on 1 cm^2 surface of glass beads. Glass beads of known diameter were immersed in a series of methylene blue solutions of various concentrations, and the concentration in the supernatant was determined colorimetrically at $\lambda = 6600$A. The amount adsorbed was plotted against the total concentration (Fig. 4).

The horizontal part of the curve represents a complete monomolecular layer. The adsorption of methylene blue per mg of powdered glass was determined and plotted in a similar manner. The specific area of the glass powder was calculated from the two sets of figures obtained.

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Determination of the specific area of powdered glass by this method should be more accurate than the alternative method of calculating the surface area from the number of molecules in a monomolecular layer and from the area of the molecule. Comparison of both methods in a few control experiments showed good agreement.

The amount of PL adsorbed per unit area of glass surface was determined as follows:

Suspensions of glass particles, containing 1 mg of glass powder per ml, were prepared in PL solutions of various concentrations. After 10 minutes the suspensions were centrifuged and the concentration of the PL in the supernatant was determined by the limiting mobility technique with a small number of red cells. The mobilities of the glass particles in the suspensions were measured as well. The results are given in Table IV.

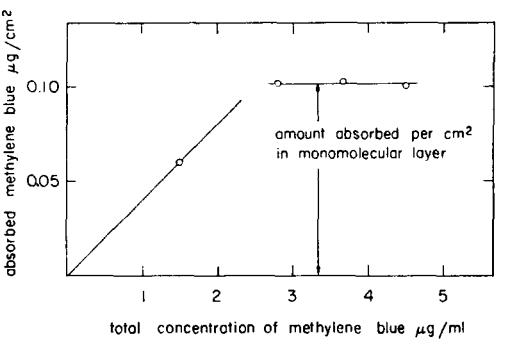

Fig. 4. Amount of methylene blue adsorbed per cm^2 surface of glass beads versus total methylene blue concentration.

TABLE IV

AMOUNT OF PL ADSORBED PER cm^2 OF GLASS SURFACE

Total PL conc. μg/ml	*PL conc. in supernatant μg/ml*	*Adsorbed PL* μg per cm^2 glass surface*	*Mobility of glass particles μ/sec/V/cm*
0			−1.90
2	0.93	0.107	+1.75
4	3.05	0.095	+1.78
6	4.90	0.110	+1.76

* The figures in this column represent the amount of polylysine hydrobromide (PL) equivalent to the amount of polyion adsorbed.

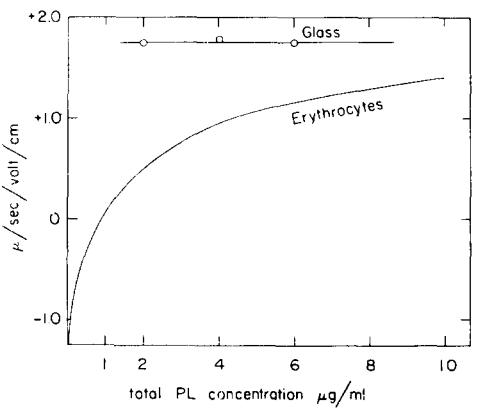

Fig. 5. Electrophoretic mobilities of glass particles and of erythrocytes in PL solutions. Surface area per ml of suspension in both cases equals 10 cm^2.

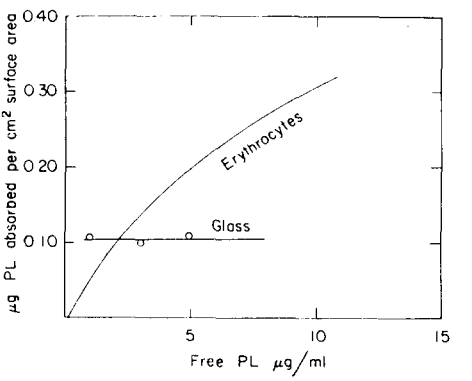

Fig. 6. Amount of PL adsorbed per cm^2 surface area on erythrocytes and on glass particles, versus free PL concentration.

When glass particles and red cells were compared with respect to mobility and amount of PL adsorbed (Figs. 5 and 6), the following points were brought out:

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(a) While the mobility of red cells increases gradually, approaching asymptotically the maximum (+ 1.90 μ/sec/V/cm) at PL concentrations of 70–80 μg/ml, with glass particles, a constant maximum mobility is reached at a PL-concentration of 1 μg/ml or less.

The maximum mobility of glass particles in PL solutions is somewhat less than that of erythrocytes, *i.e.* + 1.76 μ/sec/V/cm for glass compared to + 1.90 μ/sec/V/cm for red cells.

(b) With glass, maximum adsorption amounts to 0.1 μg/cm^2, while with red cells it is approximately 0.6 μg/cm^2.

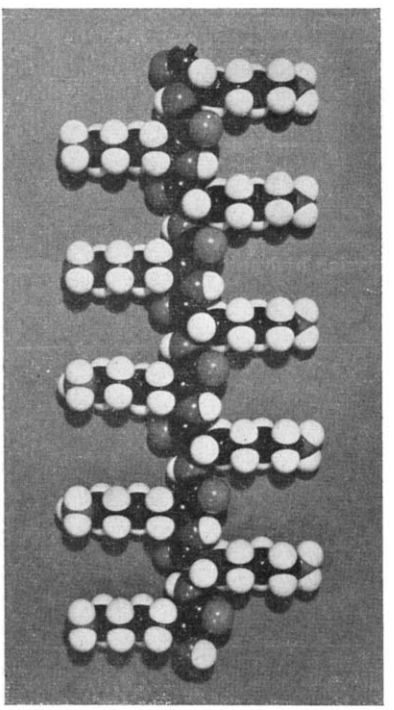
Adsorption of PL on glass is practically irreversible, as shown by the following observations:

1. Dilution or washing does not remove the PL from the glass.

2. At concentrations below saturation value, the mobilities of the glass particles in the suspension are not homogeneous.

DISCUSSION

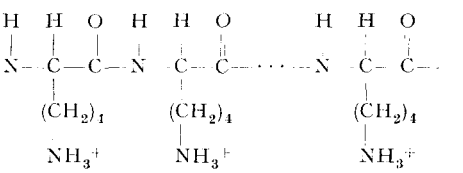
It was concluded that the PL bound by the red blood cell is concentrated in the membrane only. That at least part, if not all, of the bound PL is adsorbed on the exterior surface of the membrane, is proved by the changes in the potential of the erythrocytes in PL solutions.



Under the experimental conditions, at pH 7.2 and ionic strength 0.15, the PL molecule is fully ionized, *i.e.* every lysine residue carries a positive charge, and the polypeptide chain is almost fully extended (Fig. 7).

The fully extended chain is coplanar, with both the C=O oxygen atoms and the N–H hydrogen atoms in the plane of the chain. The side chains, attached to the carbon atoms, point alternately to the left and upward from the plane of the chain, and to the right and downward. The distance along the chain corresponding to one amino acid residue is 3.67 A.

Fig. 7. Model of fully extended polylysine molecule (10 monomeric units).



If the PL molecule lies flat, with the side chains parallel to the adsorbing surface, two arrangements of the molecules in a monomolecular layer are possible:

1. The molecule may be considered to occupy the area of a rectangle whose long axis is formed by the polypeptide backbone and whose width is equal to twice the length

of the side chain, plus the projected width of the central peptide links, plus the Van der Waals distance between two neighboring molecules.

2. Since the thickness of the aliphatic side chain (3.4 A) is less than half the distance between successive residues on one side of the molecule (7.3 A), an interlaced arrangement is possible, with each side chain of one molecule lying between two side chains of its neighbor (Fig. 8). The width of the rectangular area thus effectively covered by one molecule is equal to the length of the side chain, plus the Van der Waals distance between the spine and the amino group of the side chain, plus the projected width of the spine.

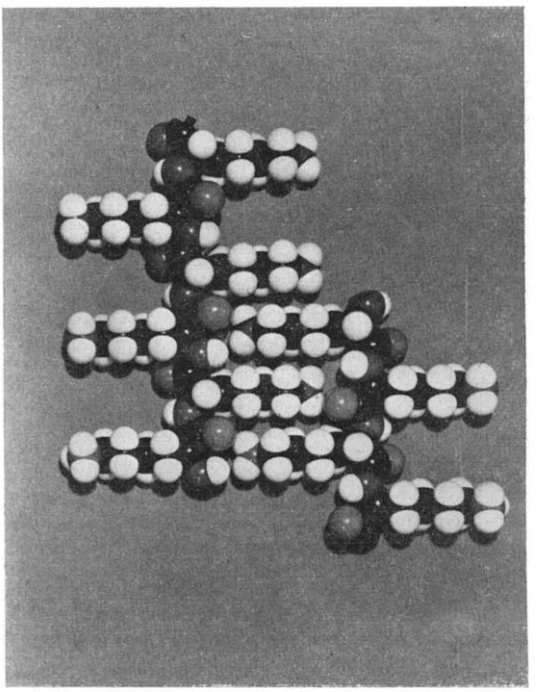


Fig. 8. Model of section of two polylysine molecules in "interlaced" arrangement.

The effective area covered per monomeric unit would be 59 A^2 in case (1) and 36 A^2 in case (2), and the amount of PL required to cover 1 cm^2 of surface area would be 0.059 μg in the first case and 0.096 μg in the second.

The actual amount adsorbed on glass has been found to be 0.104 ± 0.05 $\mu g/cm^2$ which is in good agreement with the calculated amount for the interlaced arrangement in (2).

Since it was found that glass binds PL strongly and almost irreversibly we may well imagine that this strong attraction forces the molecule to acquire the entropically less probable flattened out arrangement.

It was found that the amount of PL adsorbed per cm^2 surface area of the erythrocyte was 0.6 μg, which is equivalent to about six monomolecular layers of the type described. This discrepancy may be due to any one—or all—of several causes:

(a) A different orientation of the PL molecules on the surface may permit closer packing, as shown for example, in Fig. 9.

This arrangement would be accompanied by a smaller decrease in configurational entropy.

(b) Adsorption may take place not only on the exterior of the cell membrane but inside the structure as well, particularly on the fibrillar protein constituents. It should be noted, in this connection, that fibrillar proteins have been found to bind about a hundred times more PL than do globular proteins[12], a phenomenon analogous to the fact that dyes are more strongly bound by stretched or partially coagulated proteins than by the native globular form[13].

The likelihood of intramembrane adsorption is supported by the observation that PL causes hemolysis, when present in comparatively large concentrations. These permeability changes are reversible,—when the cells are washed free of PL further hemolysis ceases. Recent electron microscopy of ghosts[14] has demonstrated that the red cell

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membrane is composed of a supporting web of fibrillar protein (stromatin) covered by a mosaic of plaques, which are probably lipoprotein in nature, with lipids in between. The water channels ("pores") are located between the placques. HILLIER suggests that hemolysis is due to opening up of the pores by a change in the relative position of the

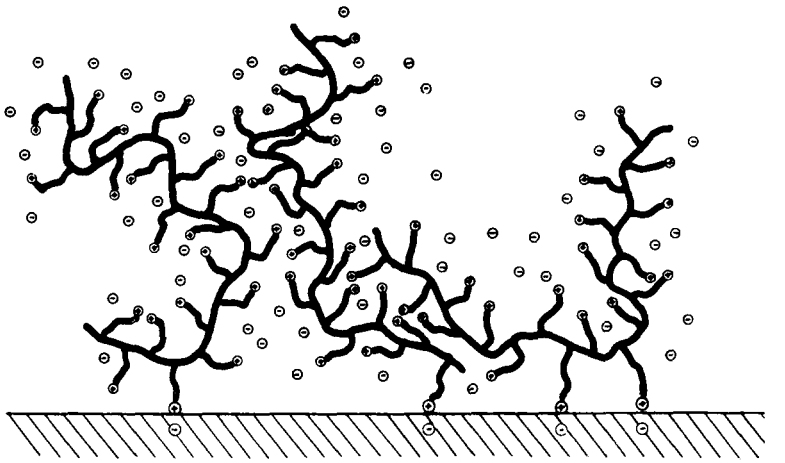

Fig. 9. Schematic representation of adsorption of polylysine-HBr on the surface of the erythrocyte. The PL molecules are depicted by the branched lines. The circles at the ends of the branches are $-NH_3^+$ groups; the free circles represent bromide ions.

placques. It can be thought that the PL may well produce such an effect by bridging the fibrils and/or the placques and distorting their regular arrangement.

If the amount of adsorbed PL is calculated from electrophoretic data, on the assumption that all the positive charges of the adsorbed PL contribute to the electrokinetic potential, a large discrepancy is found between the observed and calculated amounts. The actual amount adsorbed on erythrocytes is found to be greater than the calculated amount by a factor of approx. 75, and on glass, by a factor of 10. With bacteria, E. KATCHALSKI *et al.*[5] have found a similar but even greater discrepancy. These discrepancies may be due to various factors, (further ionization of the surface, adsorption of negative ions, expulsion of positive ions) and are now being investigated.

SUMMARY

1. The interaction between negatively charged cells and positively charged (basic) polyelectrolytes is mainly due to non-specific electrostatic attractive forces.

2. Adsorption of polylysine (PL) on the red blood cell is a reversible reaction which rapidly attains a state of equilibrium; consequently the surface potential of the erythrocyte in PL solutions is a single-valued function of the equilibrium concentration of free PL in solution.

3. A method is described whereby mobility data can be used to evaluate both the concentration of PL in solution and the amount adsorbed on the cells.

4. An adsorption isotherm of the Freundlich type was obtained which is characteristic of the interaction of PL with the red blood cell at pH 7.2 and ionic strength 0.15.

5. With red blood cells, PL is bound by the membrane only.

6. PL adsorption on the surface of glass particles and their electrophoretic mobilities were measured.

7. The orientation, and the hemolytic effect, of PL molecules adsorbed on the red cell membrane are discussed.

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RÉSUMÉ

1. L'interaction entre des cellules négativement chargées et des polyélectrolytes positivement chargés (basiques) est due principalement à des forces attractives électrostatiques non spécifiques.

2. L'adsorption d'une polylysine (PL) sur les globules rouges est une réaction réversible qui atteint rapidement un état d'équilibre; par conséquent le potentiel de surface des érythrocytes dans des solutions de PL est une fonction à une seule variable de la concentration à l'équilibre de la PL libre dans la solution.

3. Une méthode permettant, à partir de déterminations de mobilité, dévaluer à la fois la concentration en PL de la solution et la quantité adsorbée sur les cellules est décrite.

4. Un isotherme d'adsorption de Freundlich, caractéristique de l'interaction de PL avec les globules rouges à pH 7.2 pour une force ionique de 0.15, a été obtenu.

5. Dans le cas des globules rouges, PL est lié seulement à la membrane.

6. L'adsorption de PL à la surface de particules de verre et leur mobilité électrophorétique ont été mesurées.

7. L'orientation, et l'action hémolytique, des molécules de PL adsorbées sur la membrane des globules rouges sont discutées.

ZUSAMMENFASSUNG

1. Die Wechselwirkung zwischen negativ geladenen Zellen und positiv geladenen (basischen) Polyelektrolyten ist in der Hauptsache spezifischen elektrostatischen Anziehungskräften zuzuschreiben.

2. Die Adsorption von Polylysin (PL) durch rote Blutkörperchen ist eine reversible Reaktion, welche schnell ein Gleichgewichtsstadium erreicht. Demzufolge ist das Oberflächenpotential des Erythrocyten in PL-Lösungen eine von einer Unbekannten, nämlich der Gleichgewichtskonzentration von freiem PL in Lösung, abhängige Funktion.

3. Es wird eine Methode beschrieben, welche es ermöglicht, an Hand der Beweglichkeitsangaben, sowohl die Konzentration des PL in Lösung, als auch die auf den Zellen adsorbierten Mengen abzuschätzen.

4. Es wurde eine Adsorptionsisotherme Typ Freundlich erhalten, welche bei pH 7.2 und Ionenstärke 0.15, charakteristisch für die Wechselwirkung zwischen PL und den roten Blutkörperchen ist.

5. In roten Blutkörperchen wird PL nur von der Membran gebunden.

6. Es wurden Messungen betreffend die Adsorption von PL auf der Oberfläche von Glaspartikeln und deren elektrophoretische Beweglichkeit vorgenommen.

7. Die Orientation und die hemolytische Wirkung von auf der Membran der roten Blutkörperchen adsorbierten PL-Molekülen wird erörtert.

REFERENCES

1 R. L. M. SYNGE, *Quart. Reviews* (London), 3 (1949) 245.
2 E. BRICAS AND CL. FROMAGEOT, *Advances in Protein Chem.*, 8 (1953).
3 M. A. STAHMANN, L. H. GRAF, J. C. WALKER AND D. W. WATSON, *J. Biol. Chem.*, 189 (1951) 45. W. C. BURGER AND M. A. STAHMANN, *J. Biol. Chem.*, 193 (1951) 13.
4 W. C. BURGER AND M. A. STAHMANN, *Arch. Biochem. Biophys.*, 39 (1952) 27.
5 E. KATCHALSKI, L. BICHOVSKY AND E. B. VOLCANI, *Biochem. J.*, 55 (1953) 671.
6 J. R. RUBINI, M. A. STAHMANN AND A. F. RASMUSSEN Jr., *Proc. Soc. Exp. Biol. Med.*, 76 (1951) 659.
7 A. DE VRIES, Y. STEIN, O. STEIN, J. FELDMAN, J. GUREVITCH AND E. KATCHALSKI, *Proc. Fourth Internat. Congr. Internat. Soc. Hematol.*, VII-8 (1954) 385.
8 H. T. S. BRITTON, *Hydrogen Ions*, Chapman and Hall, London, 1932.
9 E. KATCHALSKI, I. GROSSFELD AND M. FRANKEL, *J. Am. Chem. Soc.*, 70 (1948) 2094.
10 H. A. ABRAMSON, L. S. MOYER AND M. H. GORIN, *Electrophoresis of Proteins*, New York, Reinhold Publishing Corporation, 1942.
11 T. TEORELL, *J. Gen. Physiol.*, 35 (1952) 669.
12 Unpublished results.
13 R. J. GOLDACRE, in *International Review of Cytology*, Vol. I, p. 135.
14 J. HILLIER AND J. F. HOFFMAN, *J. Comp. Physiology*, 42 (1953) 203.

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