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Modification of fluid lipid and mobile protein fractions of reticulocyte plasma membranes affects agonist-stimulated adenylate cyclase. Application of the percolation theory

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Abstract

The technique of fluorescence recovery after photobleaching was used to measure the lateral mobility of membrane integral proteins in reticulocyte plasma membranes which were treated to modify the 'fluid' lipid or immobilized protein fractions, hence increasing the relative prevalence of obstacles to protein lateral motion. This was achieved by either: (1) treating the plasma membranes with phospholipase A_2 followed by extraction of the hydrolysis products using fatty-acid-free bovine serum albumin, resulting in a decrease in the membrane 'fluid' lipid portion; or (2) preincubating the plasma membranes with polylysines, resulting in plasma membrane protein aggregation and immobilization. As the prevalence of obstacles to lateral motion increased in plasma membranes through the treatments described above, the mobility of the membrane integral proteins diminished. Experimental results for the dependence of protein mobility on the prevalence of obstacles to lateral motion were compared to theoretical data in order to verify the applicability of the percolation theory to reticulocyte plasma membranes. The influence of a decrease in the 'fluid' lipid and an increase in the immobilized membrane protein fractions upon the hormone-stimulated adenylate cyclase activity has been studied as well. As the 'solid' lipid and immobilized membrane protein fractions decreased, both the hormone-stimulated adenylate cyclase activity and the fraction of β-adrenergic receptors with high affinity to hormone diminished. It was shown that this correlation can be caused by a decrease in membrane fraction accessible to the movement of the interacting proteins of the adenylate cyclase complex. Hormonal stimulation of adenylate cyclase is discussed in terms of the percolation theory.

Keywords: Percolation theory; Adenylate cyclase; Fluorescence recovery; FRAP; Reticulocyte; Plasma membrane

1. Introduction

In recent years, ample evidence has been acquired supporting the view that the plasma membrane has a heterogeneous domain structure [1]. Lateral motion of membrane proteins takes place in domains of 'fluid' lipids (lipids in the liquid-crystalline phase), whereas domains of 'solid' lipids (lipids in the gel phase) and immobilized membrane proteins can prevent such a motion [2–5] (i.e., they are obstacles to lateral motion). This conception of the plasma membrane has led to the description of the plasma membrane as a percolation medium [6–10]. The extended branched pathway of motion for mobile particles in such a medium forms the so-called percolation cluster; that part of the cluster which has no branches and dead ends is called the percolation cluster backbone [11]. At a certain obstacle concentration (the percolation threshold), the percolation cluster disappears and diffusion over long-range distances becomes impossible. Extensive theoretical analysis of membrane models based on percolation theory had been undertaken by Saxton (see [6,12]).

It is known [13] that the lateral mobility of membrane components determines various cellular processes. Changes in the plasma membrane, a medium for the lateral mem-

Abbreviations: BSA, bovine serum albumin; FITC, fluorescein-5-isothiocyanate; DTAF, 5-(4,6-dichlorotriazinyl)aminofluorescein; [^{3}H]DHA, 1-(4,6-propyl[^{3}H])dihydroalprenolol; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; FRAP, fluorescence recovery after photobleaching; R_H, fraction of β-adrenergic receptors with a high affinity to hormone.

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brane component motion, can affect the rate of lateral diffusion and, as a consequence, the rate of biological processes dependent on this movement [7–9,14–19]. Examining theoretically the plasma membrane as a percolation medium, Saxton [6], we [7–9,17,20] and Thompson et al. [10] predicted how differences in membrane structure could influence biochemical reactions within the membrane. The adenylate cyclase system can serve as an example of a membrane localized enzyme complex, whose activity may be determined by membrane protein lateral motion [7–9,13,18,20–24]. Our earlier studies of adenylate cyclase activity [7–9,17,20] yielded experimental evidence supportive of description of the plasma membrane in terms of percolation theory. It was shown, in particular, that the formation of the hormone-β-adrenergic receptor-G_s-protein ternary complex was in conformity with this theory.

New experimental evidence for description of the plasma membrane as a percolation medium is given in the present paper. We changed the portion of 'fluid' lipids and immobilized membrane proteins in reticulocyte plasma membrane and investigated an influence of these changes upon mobility characteristics of the integral membrane proteins. It was examined also how hormone-stimulated adenylate cyclase activity depends on these changes in plasma membrane.

2. Materials and methods

2.1. Reagents and fluorescent probes

Fatty-acid-free bovine serum albumin (BSA), fluorescein-5-isothiocyanate (FITC)-labeled poly(L-lysine) (molecular mass = 18 kDa), poly(L-lysines) (molecular masses = 24.4 kDa; 90 kDa; 140 kDa; 354 kDa), EDTA, 5-(4,6-dichlorotriazinyl)aminofluorescein (DTAF), L-isoproterenol, caffeine, DL-propranolol, cAMP, ATP, creatine phosphokinase and creatine phosphate were from Sigma; β-mercaptoethanol, EGTA were from Serva; 1-(4,6-propyl[^{3}H])dihydroalprenolol ([^{3}H]DHA) and [α-^{32}P]ATP were from Amersham; bee venom phospholipase A_2 was from Calbiochem.

2.2. Preparation of reticulocyte plasma membrane

Reticulocytosis in Wistar male rats weighing 200–250 g was induced by hypodermic injection of phenylhydrazine-HCl [25]. Percentage of reticulocytosis was not less than 95%. Isolation of reticulocyte plasma membranes was as described previously [17].

2.3. Treatment of plasma membranes with phospholipase A_2

Hydrolysis of plasma membrane phospholipids was carried out at 24° C for 15 min in a buffer containing 50 mM Tris-HCl, pH 7.6, 0.25 mM $CaCl_2$, and 0.04–2.00 μg/ml phospholipase A_2 [17]. Hydrolysis was stopped by adding EGTA (up to 5 mM). The membranes were incubated with fatty-acid-free BSA for 10 min at 24° C in order to extract the hydrolysis products. The membranes were then washed by centrifugation (three times) in 50 mM Tris-HCl buffer (pH 7.6) containing 1 mM EGTA and 1 mM β-mercaptoethanol at 4° C.

2.4. Determination of 'fluid' lipid fraction

The 'fluid' lipid fraction was determined using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) according to McConnell's method with modifications [26,27]. From the ESR spectra of a spin probe, TEMPO, added to the membranes in the presence of chromooxalate the fraction of TEMPO dissolved in 'fluid' lipids was determined. Changes in the 'fluid' lipid fraction were calculated after normalization of the data according to the formula: (treatment/control − 1) · 100% [8].

2.5. Preparations for FRAP experiments

Reticulocyte plasma membranes were labeled with DTAF according to Sheetz et al., whereby greater than 90% of the label were linked to the major integral protein of erythrocyte plasma membranes Band 3 [28]. Plasma membranes were then sedimented at $1000 \times g$ for 5 min upon a fatty-acid-free slide covered with collagen film in buffer I (50 mM Tris-HCl, 0.5 mM EDTA, pH 7.6). In the case of polylysine treatment, plasma membranes were incubated at 25° C for 15 min in the polylysine solution in buffer I. Sedimented membranes were covered by a cover slip. The distance between the cover slip and the slide was fixed with hydrophobic films to avoid membrane deformations. The preparation was then sealed with paraffin.

2.6. Measurements of the integral protein lateral mobility by FRAP technique

The technique of fluorescence recovery after photobleaching [29] was used to measure the lateral diffusion coefficient of integral membrane proteins. The computer-controlled setup used consisted of a microscope (Reichert) and 3W argon ion laser (LGN-402, Ukraine) tuned to 488 nm. A focusing lens and 100 × objective (N.A. 1.32) were used to achieve a laser beam radius of 1.7 μm with a photobleaching beam intensity of 200 W/cm^2. After photobleaching, attenuation of the laser beam intensity to the reduced measuring level was achieved using an electromagnet operated switch system [30]. The measurement parameters were: bleach duration and half-time for fluorescence recovery, 500 ms and 3–20 min; time interval between measurement points, 20 s; increment of time interval, 700 ms; depth of bleaching, 30–70%. Fluorescence from the object was registered by a photomultiplier

(FEU-79, Russian Federation). Curve-fitting to the fluorescence recovery data was performed as described [31] to calculate mobility parameters. The temperature at the object was maintained at 25.0 ± 0.1° C using heating stage and objective.

2.7. Determination of adenylate cyclase activity

The incubation mixture for the assay of adenylate cyclase activity contained 50 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 0.5 mM [^{32}P]ATP (20–40 cpm/pmol), 0.75 mM cAMP, 5 mM caffeine, 10 mM creatine phosphate, 1.0 mg/ml creatine phosphokinase, 0.2 mM EGTA, 1 mM β-mercaptoethanol, and 20–30 μg of reticulocyte plasma membranes. The incubation was carried out for 15 min at 25° C. The resulting cAMP was purified on columns with alumina as described [32].

2.8. [^{3}H]DHA binding to plasma membranes

[^{3}H]DHA ($0.1 \cdot 10^{-9}$–$8 \cdot 10^{-9}$ M) binding to plasma membranes (40–60 μg of protein) was performed in 200 μl volume of 40 mM Tris-HCl buffer, pH 7.6, with 10 mM $MgCl_2$, 1 mM β-mercaptoethanol, 1 mM EDTA, 1 mM ascorbic acid for 50 min at 25° C. To stop the reaction, ice-cold incubation buffer was quickly added to the sample and the mixture was filtered through GF/C glass-fiber filter (Whatman). Non-specific binding was determined in the presence of 10 μM DL-propranolol.

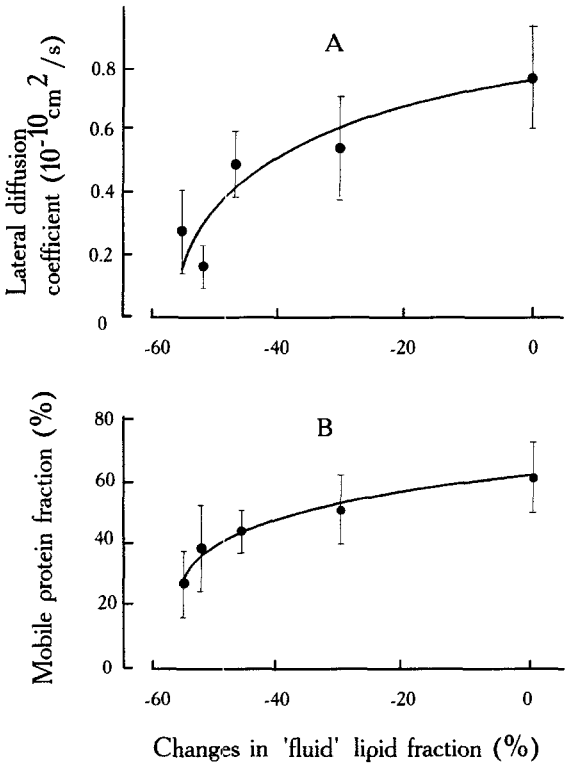


Fig. 1. Dependence of the lateral diffusion coefficient (A) and the mobile fraction (B) of the integral membrane proteins measured by FRAP on the variation of the 'fluid' lipid fraction in rat reticulocyte plasma membranes. The 'fluid' lipid fraction in plasma membranes was changed by treating the plasma membranes with phospholipase A_2 followed by extraction of the hydrolysis products using fatty-acid-free BSA (see Materials and methods). The data are presented as means ± S.E. of five experiments.

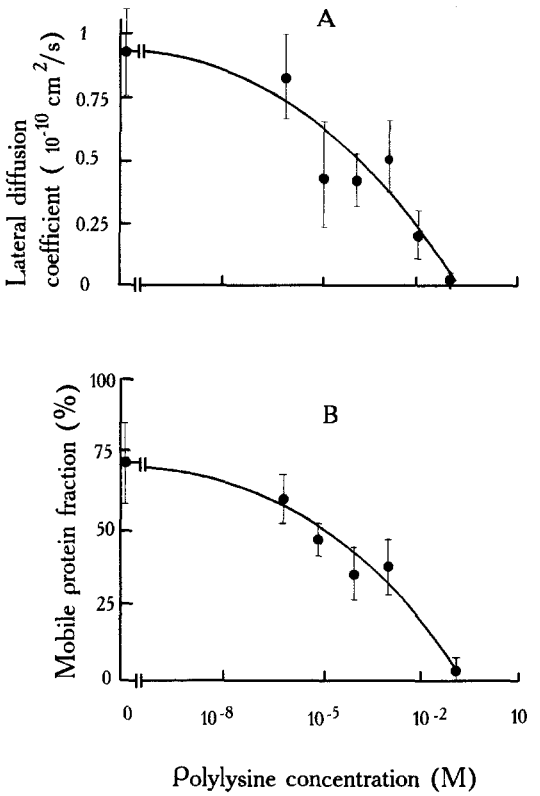


Fig. 2. Dependence of the lateral diffusion coefficient (A) and the mobile fraction (B) of the integral membrane proteins in rat reticulocyte plasma membranes measured by FRAP on the variation of the polylysine concentration (molecular mass = 24.4 kDa). The data are presented as means ± S.E. of three experiments.

2.9. Determination of the fraction of high affinity β-adrenergic receptors (R_H)

The R_H percentage was determined from curves of displacement of [^{3}H]DHA ($2 \cdot 10^{-9}$ M) by a hormone according to the model of Feldman et al. [33] with allowance for non-specific binding [34]. The parameters were estimated by the deforming polyhedron method [35].

3. Results

3.1. Lateral mobility of integral proteins in reticulocyte plasma membranes

Analysis of a spin probe TEMPO spectra from ESR measurements on reticulocyte plasma membranes showed that the treatment of plasma membranes by phospholipase

A_2 with subsequent extraction of hydrolysis products by fatty-acid-free BSA led to a decrease in the 'fluid' lipid fraction and, as a consequence, to an increase in the relative fraction of 'solid' lipids in plasma membranes [17]. We have previously shown [7–9,17,20] that such a treatment does not lead to a damage of membrane proteins such as adenylate cyclase, G_s-protein, or β-adrenergic receptor. This may indicate that the native structure of the membrane proteins and, as a consequence, the ability for the lateral motion does not change. FRAP was used to measure the lateral diffusion characteristics of integral membrane proteins in rat reticulocyte plasma membranes labeled with DTAF. The results of the experiments showed that decreasing the 'fluid' lipid fraction by 56.3% from control membranes induced by treatment of plasma membranes with phospholipase A_2 resulted in a decrease in the lateral diffusion coefficient and the mobile fraction of the integral membrane proteins from $0.74 \cdot 10^{-10}$ to $0.27 \cdot 10^{-10}$ cm^2/s and 62.8 to 25.5%, respectively (Fig. 1).

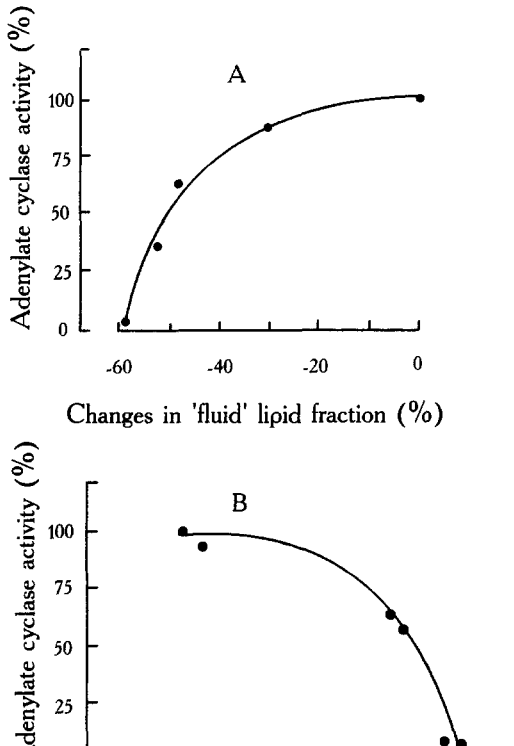


Fig. 3. Dependence of adenylate cyclase activity stimulated by L-isoproterenol on the variation of the 'fluid' lipid fraction (A) and on the variation of the immobile protein fraction (B) in rat reticulocyte plasma membranes. The 'fluid' lipid fraction in plasma membranes was changed as in Fig. 1. The immobile protein fraction was changed by preincubating of the plasma membranes with polylysine (molecular mass = 24.4 kDa, 10^{-6}–10^{-1} M) calculated from the results of FRAP experiments as (100% − % mobile fraction). 100% adenylate cyclase activity correspond to the activity of control membranes with 10^{-4} M isoproterenol + 10^{-4} M GTP. The adenylate cyclase activity was measured in the presence of 10^{-4} M of isoproterenol + 10^{-4} M GTP. The data are presented as means of four experiments.

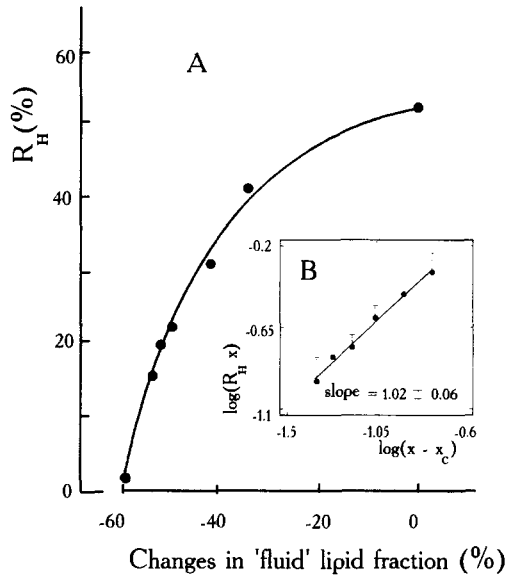


Fig. 4. (A) Dependence of R_H on the varied 'fluid' lipid fraction in rat reticulocyte plasma membranes. The 'fluid' lipid fraction in plasma membranes was changed as in Fig. 1. R_H corresponds to the activity of control membranes with 10^{-4} M isoproterenol + 10^{-4} M GTP. (B) The data of Fig. 4A replotted according to the percolation model (see the text) as $\log(R_H \cdot x)$ vs. $\log(x - x_c)$ (correlation coefficient $r = 0.98$).

Using optical and electron microscopy, it was shown [36,37] that incubation of red blood cell plasma membranes with polylysines led to protein aggregation in the plasma membrane resulting from the interaction of polylysine with negatively charged domains of the proteins [38]. Results of FRAP measurements of the mobility of DTAF-labeled proteins in plasma membranes showed that as the polylysine concentration increased, so did the fraction of immobilized proteins, resulting in the reduction in the lateral diffusion coefficient of mobile proteins (Fig. 2). FRAP measurements of the mobility of the FITC-labeled polylysine (molecular mass = 18 kDa, 10^{-3} M) on the plasma membrane surface indicated that the polylysine was immobile (lateral diffusion coefficient less than 10^{-13} cm^2/s). This demonstrates the lack of mobility of membrane proteins aggregated by polylysines. Clearly, a decrease in the lateral diffusion coefficient cannot be explained by an increase in the size of mobile protein particles through aggregation by polylysines.

3.2. Adenylate cyclase activity in reticulocyte plasma membrane

Our studies of hormone-stimulated adenylate cyclase activity and R_H in plasma membranes with altered 'fluid' lipid fraction indicated that treatment of plasma membranes with phospholipase A_2 with subsequent reducing the 'fluid' lipid fraction by 62% from control membranes

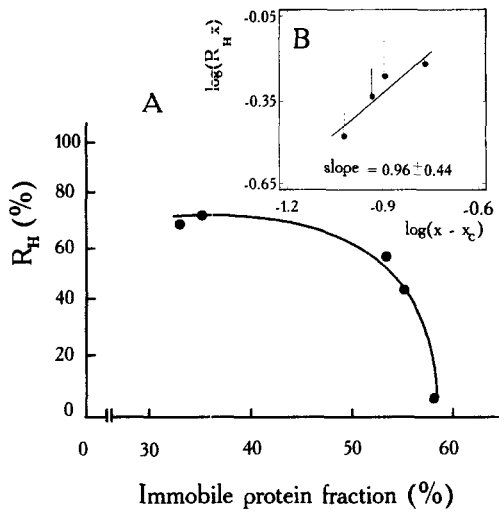


Fig. 5. (A) Dependence of R_H on the varied immobile protein fraction in rat reticulocyte plasma membrane. The immobile protein fraction was changed as in Fig. 3. R_H corresponds to the activity of control membranes with 10^{-4} M isoproterenol + 10^{-4} M GTP. (B) The data of Fig. 5A replotted according to the percolation model (see the text) as $\log(R_H \cdot x)$ vs. $\log(x - x_c)$ (correlation coefficient $r = 0.84$).

lead to a decrease in hormone-stimulated adenylate cyclase activity and R_H to zero value (Figs. 3A and 4A). It was shown also that the dissociation constant and the total binding site number for the β-adrenergic receptor antagonist [^{3}H]DHA were unaltered in plasma membranes with reduced 'fluid' lipid fraction, and both β-adrenergic receptor and G_s-protein and G_s-protein and catalytic subunit capabilities for interaction were unchanged [7,8,17,20].

Studies of the adenylate cyclase activity in reticulocyte plasma membranes preincubated with polylysine showed that as the fraction of the DTAF-labeled immobilized proteins measured by FRAP increased, both hormone-stimulated adenylate cyclase activity and R_H decreased (Figs. 3B and 5A). [^{3}H]DHA binding to β-adrenergic receptors in plasma membranes preincubated with polylysine did not differ from that in control plasma membranes (Table 1). Polylysines with larger molecular weight at constant residue concentration inhibit the hormone-stimulated adenylate cyclase activity to a greater extent (Table 2). The inhibition of adenylate cyclase activity by direct interactions of polylysine with the proteins of the adenylate cyclase complex can be discounted, since the number of reactive groups of polylysines with different molecular weight does not change at constant residue concentration.

Table 1
Effect of changes in the polylysine concentration on the total number of [^{3}H]DHA binding sites and the dissociation constant for [^{3}H]DHA binding to β-adrenoreceptors in rat reticulocyte plasma membranes

Polylysine concentration (M)	K_d (nM)	B_{max} (fmol/mg protein)
0	0.91±0.29	732±214
$5 \cdot 10^{-5}$	1.03±0.32	800± 44
$5 \cdot 10^{-3}$	0.84±0.40	651± 66

The plasma membranes were preincubated for 15 min at 25° C with polylysine (molecular mass = 24.4 kDa) at the different concentration and then the dissociation constants (K_d) and the total number of [^{3}H]DHA binding sites (B_{max}) were determined (see Materials and methods). Results are means ± S.E of three similar experiments.

Table 2
Dependence of adenylate cyclase activity in rat reticulocyte plasma membranes on the variation of the molecular weight of polylysines

Molecular mass (kDa)	Adenylate cyclase activity	
	pmol/mg protein per min	P
0 (control)	1955±62	
24.4	1673±59 [a]	< 0.025
90	1661± 5 [b]	< 0.025
140	1623±11 [c]	< 0.025
354	1382±41 [d]	< 0.001

Adenylate cyclase activity was measured with 10^{-4} M isoproterenol + 10^{-4} M GTP at constant residue polylysine concentration (10^{-6} M). The results are means ± S.E. of three similar experiments. The significance of the differences between the adenylate cyclase activity in the control plasma membranes and the plasma membranes, preincubated with polylysines, was calculated with Student's t-test and is indicated by P.
[a,c] For this pair $P < 0.025$.
[b,d] For this pair $P < 0.01$.

4. Discussion

The functions $f(x)$, used in percolation theory to describe diffusion in a medium possess the form [11]:

$$f(x) \propto (x - x_c)^{\omega} \quad (1)$$

where x is the space fraction accessible for protein movement; x_c the critical x value (at $x \leq x_c$ there is no percolation cluster); and ω a critical exponent.

In particular, for two-dimensional space (from [11,39–43]):

$$D(x) \propto (x - x_c)^{\mu}, \ \mu = 1.1604 \quad (2a)$$

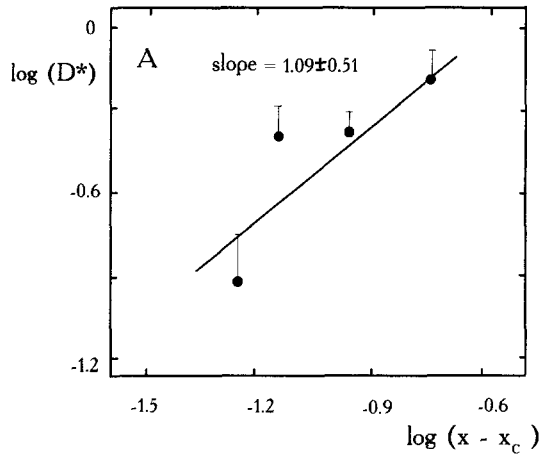
$$P_B(x) \propto (x - x_c)^{\beta_B}, \ \beta_B = 0.5 \quad (2b)$$

where $D(x)$, the diffusion coefficient along the whole backbone of the percolation cluster; $P_B(x)$, the probability that a particular point belongs to the backbone of the percolation cluster; $\mu = 1.1604$ and $\beta_B = 0.5$, the theoretical values of the critical exponents.

For our investigation we used the model of continuous two-dimensional percolation, which is suitable to describe the plasma membrane [6], and appropriate for this model x_c value of 0.675 [43,44] was chosen. In accordance with percolation theory, the critical exponent is independent of the chosen model and, therefore independent of x_c, and is determined only by space dimensionality [11,42].

x can be calculated from the experimental values of 'fluid' lipid and immobilized fractions using simple transformations (see Appendix). To obtain dimensionless value

D^* the experimental value of protein lateral diffusion coefficient D was normalized by D_{max} (the lateral diffusion coefficient in the medium without obstacles, recalculated by extrapolation of the plot of experimental dependence of D on x to line $x = 1$). Plotting the dependence of D^* on $(x - x_c)$ in logarithmic coordinates made it possible to find a slope of the experimental line (Fig. 6). A slope of the plot obtained from the results of the experiments on membranes with the altered 'fluid' lipid fraction is equal to 1.09 ± 0.51 (Fig. 6A) and in case of membranes with the altered immobilized proteins fraction slope is equal to 1.04 ± 0.08 (Fig. 6B). In both cases the values of the critical exponent obtained experimentally were in accordance with the theoretical one (see Eq. (2a)) regardless of the type of obstacles. This indicates the validity of describing the mobility of integral membrane proteins without considering the nature of the obstacles.



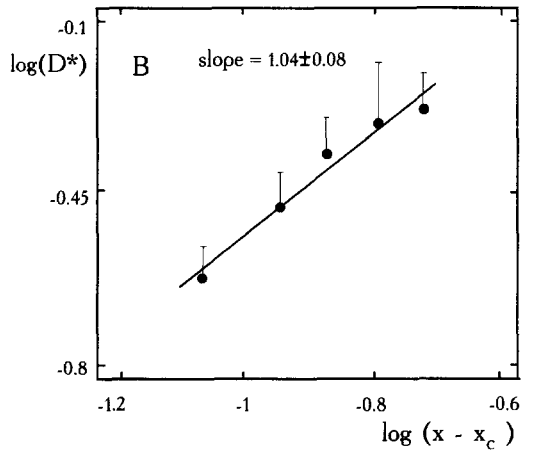


Fig. 6. Log-log plot of the normalized measured values of the lateral diffusion coefficients of the integral membrane proteins, D^*, and $(x - x_c)$ according to the percolation model (see the text) in rat reticulocyte plasma membranes treated with phospholipase A_2 (0.04–2.00 μg/ml) (correlation coefficient, $r = 0.83$) (A) and in rat reticulocyte plasma membranes preincubated with polylysine (molecular mass = 24.4 kDa, 10^{-6}–10^{-1} M) (correlation coefficient $r = 0.99$) (B).

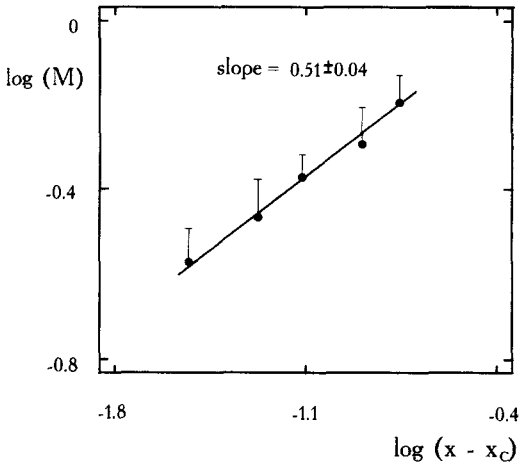


Fig. 7. The data of Fig. 2B replotted according to the percolation model (see the text) as $\log(M)$ vs. $\log(x - x_c)$ ($r = 0.99$); M, the mobile fraction of the integral membrane proteins.

It is possible to verify whether the expression (2b) is satisfied for the plasma membrane. Let us imagine that there is a percolation cluster on the membrane surface spot examined under the laser beam. The fluorescence recovery on the bleached spot will be a result of diffusion of labeled proteins mainly in the percolation cluster backbone. Our experiments with repeat bleaching of the same spot (curve not shown) demonstrated that the mobile fraction decreased not more than by 5.3%; this result means that the contribution from other parts of the percolation cluster and/or finite clusters at the edge of the bleached spot is small enough (not more than 5.3%). Therefore, the value of the mobile protein fraction determined from FRAP measurements in plasma membranes with the altered 'fluid' lipid fraction will correspond to $P_B(x)$. The slope from the plot of the mobile protein fraction dependence on $(x - x_c)$, plotted in logarithmic coordinates, 0.51 ± 0.04, (Fig. 7) coincided with the theoretical value with good accuracy (see Eq. (2b)). (In the case of the experiments using polylysines, a part of the mobile labeled proteins interacts with the polylysine molecules and becomes immobilized; therefore, the value of mobile fraction in this case will not correspond to $P_B(x)$). Thus, expressions (2a) and (2b) are satisfied for proteins diffusing in plasma membranes. This may indicate that protein mobility in the plasma membrane and the plasma membrane itself may be described in terms of the percolation theory.

Our results here show that the hormone-stimulated adenylate cyclase activity decreased with both a decreased 'fluid' lipid fraction and an increased immobile protein fraction (Fig. 3). Reducing the 'fluid' lipid fraction and increasing the immobile protein fraction also led to a decrease in the percentage of β-adrenergic receptors with high affinity to hormone (Figs. 4A and 5A). It is known [46] that transition of β-adrenergic receptor from a low to a high affinity state induced by formation of the hormone-receptor-G_s-protein ternary complex is a prerequisite stage

in the stimulation of adenylate cyclase by hormone. Since both the value of the R_H fraction and the hormone-stimulated adenylate cyclase activity approached zero at the same values of the immobilized protein fraction and reduced the 'fluid' lipid fraction, it can be assumed that R_H formation is a limiting step in adenylate cyclase inhibition in plasma membranes pretreated with phospholipase A_2 or preincubated with polylysines. [^{3}H]DHA binding and other experiments ([7–9,17] and Table 1) showed that decreased hormone-stimulated adenylate cyclase activity was not due to changes in the functional activity of the proteins of the adenylate cyclase complex in the case of phospholipase A_2 treated plasma membranes; and could not be exclusively explained by loss of the protein function in case of plasma membranes preincubated with polylysines (see Results). It was shown above that the lateral diffusion of the integral proteins diminished in plasma membranes treated with phospholipase A_2 or preincubated with polylysines. It was shown also [20,24,47] that at least a part of the components of the adenylate cyclase complex are laterally mobile in plasma membranes and that the hormone-stimulated adenylate cyclase activity may be determined by lateral mobility of these components [13,17,18,21–23]. It can be supposed that an increase in the immobile protein fraction and the relative increase in the portion of 'solid' lipids may result in a slowing-down of the lateral mobility of these proteins. As a consequence of this, the probability of formation of the ternary complex resulting from the interaction of the laterally mobile components may be reduced followed by a decrease in adenylate cyclase activity (see also Ref. [19] for results for reduced vasopressin-stimulated adenylate cyclase activity through reducing vasopressin receptor lateral motion).

It was shown above that the plasma membrane could be considered as a percolation medium and that protein diffusion in this medium could be described in terms of the percolation theory. We have tried to apply the percolation theory to describe the adenylate cyclase complex. Expressing the immobile protein fraction and the portion of 'fluid' lipids through x (the membrane surface fraction accessible for protein movement) (see Appendix), one can note that as the membrane surface accessible for motion was reduced, the hormone-stimulated adenylate cyclase activity approached zero (Fig. 8). Such a correlation is in agreement with the one theoretically predicted for the chemical reaction whose rate is determined by lateral diffusion of its components in a percolation medium [6].

In the preceding paper [17], it was evaluated:

$$R_H \propto D(x)/x \tag{3a}$$

and if the percolation processes take place in plasma membranes:

$$R_H \propto (x - x_c)^{\mu}/x \tag{3b}$$

We replotted the data of Figs. 4A and 5A using coordinates $\log(R_H \cdot x)$ vs. $\log(x - x_c)$. The straight lines can be drawn through the experimental points for the plasma membranes both treated with phospholipase and preincubated with polylysine (Figs. 4B and 5B, accordingly). In both cases the slopes of the experimental lines are close to the theoretical value of $\mu = 1.1604$ (see Eq. (2a)). This accordance may evidence that the proteins of the adenylate cyclase complex (the β-receptors and/or G_s-proteins) behave as the particles diffusing in the percolation cluster.

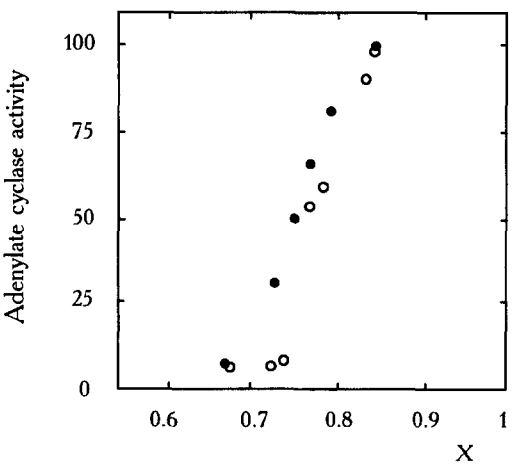


Fig. 8. Dependence of adenylate cyclase activity stimulated by L-isoproterenol on the plasma membrane fraction accessible for protein movement, x, in rat reticulocyte plasma membranes with varied, ○, immobile protein fraction, ●, 'fluid' lipid fraction. The raw data come from Fig. 3.

As shown recently [1], plasma membranes may be heterogeneous, what may greatly influence the percolation properties [6–10,17,20], through, for example, the presence of proteins immobilized by cytoskeleton etc. It is known [48,49] that the level of the hormone-stimulated adenylate cyclase activity is higher in rat reticulocytes than in erythrocytes, and that extensive cytoskeletal changes accompany the differentiation of reticulocyte to erythrocyte. It can be assumed that an increase in the number of membrane proteins immobilized by the cytoskeleton and, as a consequence, changes in the percolation properties of plasma membranes during erythrocyte maturation may be responsible for the sharp decrease in hormone-stimulated adenylate cyclase activity in mature erythrocytes.

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Appendix 1

Let us assume that plasma membranes can be divided into two classes according to accessibility to protein movement: (1) those which are accessible to protein movement

and may be called 'fluid', their total area is F; and (2) those which can be considered as 'obstacles', their total area is S. S consists of immobilized proteins and so-called 'solid' lipids, i.e., lipids in the gel phase. Experimentally estimated changes in the 'fluid' lipid fraction, ΔF, and in the 'obstacles', ΔS, are related with F and S as follows:

$$\Delta F = (F - F_0)/F_0 \quad (4a)$$

$$\Delta S = (S - S_0)/S_0 \quad (4b)$$

where F_0 - a total area of 'fluid' parcels and S_0 - a total area occupied by 'obstacles' before any membrane treatment.

Let F_c and S_c be the threshold values of F and S, accordingly (at $x = x_c$), hence

$$\Delta F_c = (F_c - F_0)/F_0 \quad (5a)$$

$$\Delta S_c = (S_c - S_0)/S_0 \quad (5b)$$

x can be determined from F and S as follows:

$$x = F/(S + F) \quad (6a)$$

$$x_c = F_c/(S_c + F_c) \quad (6b)$$

Let $x_c = 0.675$ (theoretical value [43,44]), $\Delta F_c = -0.62$ (fitted earlier from our data by minimization of the sum of squares of residuals in the double logarithmic plot [17]) and the total area occupied by membrane protein is 0.253 [45]; then taking into consideration these numeral values and from Eqs. (4a–6b) one can obtain for x in case of plasma membranes with the altered 'fluid' lipid fraction:

$$x = (1 + \Delta F)/(1.183 + \Delta F) \quad (7a)$$

and in the case of plasma membranes with the altered immobile protein fraction:

$$x = 0.845 - 0.155 \cdot \Delta S \quad (7b)$$

Detailed deducing of the expressions (7a) and (7b) was given elsewhere [17,45].

References

[1] Edidin, M. (1990) Curr. Top. Membr. Transport 36, 81–96.
[2] Devaux, P.F. and Seigneuret, M. (1985) Biochim. Biophys. Acta 822, 63–125.
[3] Peters, R. (1981) Cell Biol. Int. Rep. 5, 733–760.
[4] Pink, D.A., Lookman, T., Macdonald, A.L., Zuckerman, M.J. and Jan, N. (1982) Biochim. Biophys. Acta 687, 42–56.
[5] Kapitza, H.G., Puppel, D.A., Galla, H.-J. and Sackmann, E. (1984) Biophys. J. 45, 577–584.
[6] Saxton, M.J. (1982) Biophys. J. 39, 165–173.
[7] Sobolev, A.S., Rosenkranz, A.A. and Kazarov, A.R. (1984) Biosci. Rep. 4, 897–902.
[8] Sobolev, A.S., Kazarov, A.R., Rosenkranz, A.A. and Ganchev, T. (1984) Dokl. Akad. Nauk. SSSR (Proc. Acad. Sci. USSR) 277, 501–503 (in Russian).
[9] Kazarov, A.R., Rosenkranz, A.A. and Sobolev, A.S. (1989) Stud. Biophys. 134, 67–71.
[10] Thompson, T.E., Sankaram, M.B. and Biltonen, R.L. (1992) Comm. Mol. Cell Biophys. 8, 1–15.
[11] Stauffer, D. (1979) Phys. Rep. 54, 1–74.
[12] Saxton, M.J. (1989) Biophys. J. 56, 615–622.
[13] Axelrod, D. (1983) J. Membr. Biol. 75, 1–10.
[14] Orly, J. and Schramm, M. (1987) Proc. Natl. Acad. Sci. USA 84, 1721–1725.
[15] De Foresta, B., Rogard, M., Le Maire, M. and Gallay, J. (1987) Biochim. Biophys. Acta 905, 231–251.
[16] Jans, D.A., Peters, R., Jans, P. and Fahrenholz, F. (1990) Exp. Cell Res. 191, 121–128.
[17] Sobolev, A.S., Kazarov, A.R. and Rosenkranz, A.A. (1988) Mol. Cell. Biochem. 81, 19–28.
[18] Jans, D.A. (1992) Biochim. Biophys. Acta 1113, 271–276.
[19] Jans, D.A., Peters, R., Jans, P. and Fahrenholz, F. (1991) J. Cell Biol. 114, 53–60.
[20] Kazarov, A.R., Rosenkranz, A.A. and Sobolev, A.S. (1986) Biokhimiya (Biochemistry USSR) 51, 287–295 (in Russian).
[21] Tolkovsky, A., Braun, S. and Levitzki, A. (1982) Proc. Natl. Acad. Sci. USA 79, 213–217.
[22] Bergman, R.N. and Hechter, H. (1978) J. Biol. Chem. 253, 3238–3250.
[23] Clapham, D.E. and Neer, E.J. (1993) Nature 365, 403–406.
[24] Tolkovsky, A.M. and Levitzki, A. (1978) Biochemistry 17, 3811–3817.
[25] Larner, A.C. and Ross, E.M. (1981) J. Biol. Chem. 256, 9551–9557.
[26] McConnell, H.M., Wright, K.M. and McFarland, B.G. (1972) Biochem. Biophys. Res. Commun. 47, 273–278.
[27] Vistnes, A.I. and Puskin, J.S. (1981) Biochim. Biophys. Acta 644, 244–250.
[28] Sheetz, M., Schindler, M. and Koppel, D. (1980) Nature 285, 510–512.
[29] Axelrod, D., Koppel, D.E., Schlessinger, J., Elson, E. and Webb, W.W. (1976) Biophys. J. 16, 1055–1069.
[30] Eremenko, S.I., Skalchinskii, O.B., Weiner, L.M., Gromova, O.A. and Lyakhovich, V.V. (1986) Biolog. Membr. (Biological membranes, Russia) 3, 253–260 (in Russian).
[31] Yguerabide, J., Schmidt, J.A. and Yguerabide, E.E. (1982) Biophys. J. 39, 69–75.
[32] Counis, R. and Mongongu, S. (1978) Anal. Biochem. 84, 179–185.
[33] Feldman, H., Rodbard, P. and Levin, D. (1972) Anal. Biochem. 45, 530–556.
[34] Hancock, A.A., De Lean, A. and Lefkowitz, R.J. (1979) Mol. Pharmacol. 16, 1–9.
[35] Himmelblau, D.M. (1972) Applied Nonlinear Programming, McGraw-Hill, New York.
[36] Elgsaeter, A., Shotton, D.M. and Branton, D. (1976) Biochim. Biophys. Acta 426, 1–156.
[37] Clague, M.J. and Cherry, R.J. (1989) Biochim. Biophys. Acta 980, 93–99.
[38] Duffon, M.J., Cherry, R.J., Coleman, J.W. and Stanworth, D.R. (1984) Biochem. J. 223, 67–71.
[39] Rintoul, M.D. and Nakanishi, H.J. (1992) J. Phys. A25, L945–L948.
[40] Normand, J.M., Herrmann, H.J. and Hajjar, M. (1988) J. Stat. Phys. 52, 441–446.
[41] Havlin, S. and Ben-Avraham, D. (1987) Adv. Phys. 36, 695–798.
[42] Stauffer, D. (1985) Introduction to percolation theory, p. 70, Tailor and Francis, Philadelphia.
[43] Gawlinski, E.T. and Stanley, H.E. (1981) J. Phys. A14, L291–L299.
[44] Rosso, M. (1989) J. Phys. A22, L131–L136.
[45] Zakharova, O.M., Rosenkranz, A.A. and Sobolev, A.S. (1994) Biolog. Membr. (Biological membranes, Russia) 11, 412–419 (in Russian).
[46] Kent, R.S., De Lean, A. and Lefkowitz, R.J. (1980) Mol. Pharmacol. 17, 14–33.
[47] Kwon, G., Neubig, R.R. and Axelrod, D. (1992) Biophys. J. 61, A96.
[48] Bilezikian, J.P. (1978) Biochim. Biophys. Acta 542, 263–273.
[49] Beckman, B.S. and Hollenberg, M.P. (1979) Biochem. Pharmacol. 28, 239–248.