Biochimica et Biophysica Acta, 470 (1977) 230—241

BBA 77816

THE STRUCTURAL TRANSITIONS OF ERYTHROCYTE MEMBRANES INDUCED BY CYCLIC AMP

S.V. KONEV [a], I.D. VOLOTOVSKII [a], V.S. FININ [a], A.V. KULIKOV [b], V.A. KIRILLOV [a] and E.I. ZAICHKIN [c]

[a] *Institute of Photobiology, Academy of Sciences of the BSSR, Minsk;* [b] *Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow and* [c] *Institute of Biochemistry and Physiology of Microorganisms, Academy of Sciences of the USSR, Puschchino-on-Oka (U.S.S.R.)*

(Received January 21st, 1977)
(Revised manuscript received May 5th, 1977)

Summary

The generalized structural transitions of erythrocyte membranes induced by cyclic AMP were registered by ESR, fluorescence, freeze-fracture and circular dichroism methods. Two transitions different in nature were revealed. One, which arises at 10^{-11}—10^{-10} M cyclic AMP, is cooperative and may be considered as a consequence of interaction of cyclic AMP with a receptor. It was calculated that a structural rearrangement in one erythrocyte ghost is induced by three cyclic AMP molecules. As a result of it the membranes are "loosened".

The other transition arises at 10^{-10}—10^{-8} M cyclic AMP and depends on the activity of the protein kinase system. This transition was shown to be non-cooperative and due to phosphorylation of membranous proteins. During this rearrangement the membranes are "stiffened".

Both transitions were demonstrated to relate to the membrane integrity.

Introduction

The problem of biological amplification and regulation of biochemical processes is one of the considerations of modern cell biology.

Cyclic adenosine 3′,5′-monophosphate is known to be a universal mediator for different hormones and other biologically active compounds [1—3]. The participation of cyclic AMP in the regulation of cell growth, membrane transport, byosynthetic reactions has been proved [4].

However, a concrete mechanism of coupling between cyclic AMP and the

Abbreviation: IPK, inhibitor of cyclic AMP-dependent protein kinase.

subsequent biochemical and physiological events in the membranes and cells remains to be elucidated. The hypothesis of induction of the cellular protein kinase system does not explain the pleiotropic and polyfunctional action of cyclic AMP [5].

Recently a great deal of data emphasizing an important role of generalized structural transitions of cellular membranes in the regulation of life processes have been accumulated [6,7]. The structural transitions of biological membranes are initiated by factors both exogenous (physiologically moderate temperatures, light etc.) and endogenous (metabolites, hormones including cyclic AMP) [6—10].

The present paper summarizes experimental results relating to the nature and character of the structural transitions induced by cyclic AMP in erythrocyte membranes. The data obtained show that cyclic AMP causes the structural transition of the membrane, involving mainly its protein phase. The existence of two transitions was demonstrated.

Materials and Methods

Erythrocyte membranes were prepared by the technique of Dodge et al. [11] from fresh donor blood. The membranes were resuspended in hypoosmolar buffers to prevent resealing of ghosts. As was shown by the control experiments, formation of resealed ghosts did not take place. This excluded involvement of the permeability effects.

For registration of structural transitions ESR, protein fluorescence, circular dichroism and freeze-fracture methods were used. ESR spectra were recorded in the X-band of frequencies on the spectrometer EPR-2. As spin probes the nitroxide derivatives of stearic acid (probes I and II) and benz-γ-carboline (probe III) were used. The average values from 5—7 experiments are given (S.E.M. $< 0.7\%$).

The incorporation of probes into the membranes was achieved by mixing them with ethanol probe solution (10^{-2} M). The unbound probe was separated from incorporated material by centrifugation at 15 000 $\times g$, 20 min. The protein fluorescence measurements were carried out on a double-beam absolute spectrofluorimeter FICA-55 and circular dichroism measurements on spectropolarimeter "Spectropol-1" equipped with a special attachment. In these experiments we registered tryptophan fluorescence intenstiy, which reflects a quantum yield of protein emission. Also the half-widths of differential spectra associated with shifts of the fluorescence spectra were measured.

In circular dichroism experiments the molar ellipticity at 222 nm, reflecting mainly the α-helix content of membranous proteins, was determined.

Freeze-fracturing or freeze-etching was done by standard techniques [12]. Membrane samples for freeze-fracturing were pelleted and frozen in their reaction medium without special cryoprotectors on bare copper grids which were put into propane at liquid nitrogen temperature. Freezing, fracturing, etching and shadowing were realized in JEE-4c apparatus (JEOL). Platinum-carbon replicas were investigated in a JEM-100B (JEOL) electron microscope.

The protein concentration in the samples was measured by the method of Lowry et al. [13].

Protein kinase inhibitor (IPK) was isolated from rabbit muscles according to the technique of Walsh et al. [14]. Experiments were carried out in 50 mM Tris · HCl buffer, pH 7,0. The pH value remained constant upon addition of effectors.

Results

ESR spectroscopy of spin probes

ESR spectra of probes I, II, III in erythrocyte membrane are presented in Fig. 1. Fig. 1 gives an idea of the basic parameters from which τ, the motion parameter (probe I) [15], S, the order parameter (probe II) [16] and parameter P (probe III) were calculated. The parameter P is a ratio of amplitudes of the restric-

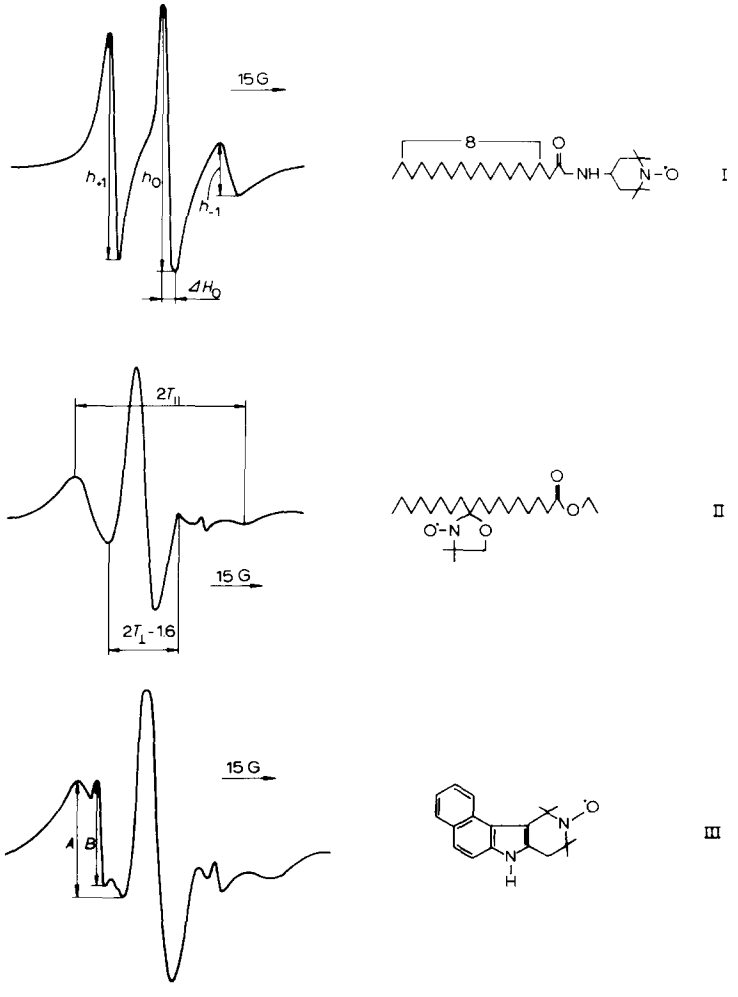
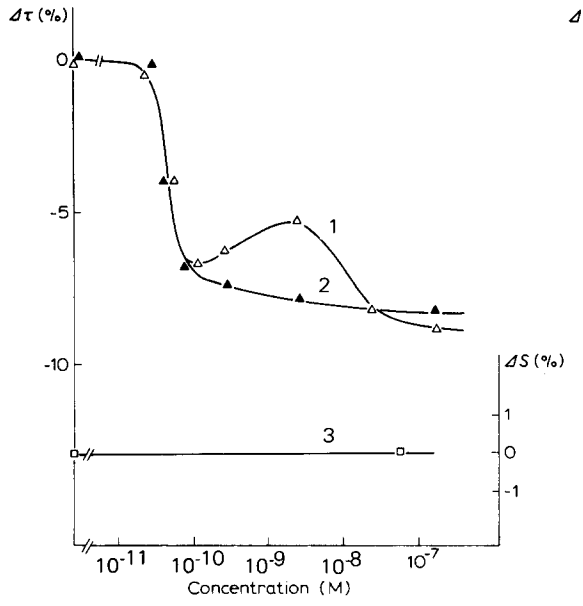


Fig. 1. Electron spin resonance spectra of spin probes incorporated into the erythrocyte membrane. Measurements were made in a medium of the following composition: 50 mM Tris · HCl buffer, pH 7.0; 50 mM NaCl. Protein concentration 10 mg/ml.



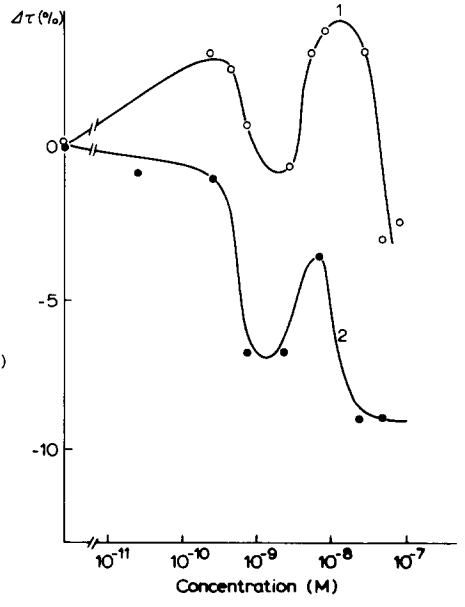


Fig. 2. Dependence of the motion parameter of probe I (τ, open and closed triangels) and the order parameter of probe II (S, open square) in the erythrocyte membrane on the cyclic AMP concentration. 1 and 3, erythrocyte ghosts only; 2, erythrocyte ghosts with 20 μg/ml of IPK.

Fig. 3. Dependence of the motion parameter (τ) of probe I in the erythrocyte membrane on cyclic AMP concentration. 1, erythrocyte ghosts with 1 mM $MgCl_2$ and 0.2 mM ATP; 2, the same as in 1 but with 20 μg/ml IPK.

ted and more restricted components of the first derivative of the low-field of the ESR spectrum that correlates with membrane fluidity.

The data obtained with membranes without Mg · ATP are presented in Fig. 2. At the cyclic AMP concentration of $2 \cdot 10^{-11}$—10^{-10} M the probe I mobility in the membrane is greatly enhanced. In the presence of IPK (curve 2), $\Delta\tau$, after reaching its maximal value, is not changed any more with subsequent increase in the cyclic AMP concentration. Without IPK (curve 1) a decrease (10^{-9} M) following some increase in the probe I mobility is observed.

Addition of ATP and Mg^{2+} required for phosphorylation transforms the dependence $\tau = f$ (cyclic AMP). In this case cyclic AMP decreases the probe I mobility. The whole process is described by a complex curve with two peaks at $2 \cdot 10^{-10}$ and $2 \cdot 10^{-8}$ M separated by the minimum at $2 \cdot 10^{-9}$ M (Fig. 3, curve 1).

In the presence of IPK (curve 2) the probe I mobility first drops (minimum at 10^{-9} M) and then rises (maximum at $8 \cdot 10^{-9}$ M).

Quite different data were obtained with probe II. The order parameter (S) remains practically unchanged under all cyclic AMP concentrations used (Fig. 2, curve 3).

The data with probe III associated with the hydrophobic zones of the membrane are shown in Table I. The parameter (P) of ESR spectra increases at 10^{-7} M cyclic AMP. The essential influence upon structural response of the mem-

TABLE I

EFFECT OF CYCLIC AMP ON PARAMETER P OF SPIN PROBE III INCORPORATED INTO THE ERYTHROCYTE MEMBRANE

Experimental material	Without cyclic AMP	With 10^{-7} M cyclic AMP
Intact membrane	0.41 ± 0.01	0.55 ± 0.02
After treatment of intact membrane with cyanuric chloride	0.33 ± 0.01	0.37 ± 0.02
After treatment of intact membrane with iodacetamide	0.31 ± 0.02	0.36 ± 0.01

brane was exerted by group-specific protein reagents. After treatment of the membrane with cyanuric chloride and iodacetamide reacting with sulphydryl, imino and amino groups the effect of cyclic AMP on the P value decreases.

Treatment with glutaraldehyde stabilizing the membrane structure exhibits the same effect. Cyclic AMP does not affect the mobility of probes after mild ultrasonic disintegration of membranes.

It should be mentioned that recording of ESR spectra of spin probes in the membranes was carried out in the presence of the phosphodiesterase inhibitor (10^{-3} M) theophylline. Owing to that, the concentration of cyclic AMP in the system was kept at a constant level. In the absence of theophylline the effects were observed at a much higher cyclic AMP concentration (by about three orders of magnitude) than in the presence of the inhibitor.

Fluorescence and circular dichroism data

Erythrocyte membranes reveal the typical protein spectra of fluorescence with emission maximum at 330—331 nm. This indicates the hydrophobic environment of tryptophan residues in the membranous proteins.

Fig. 4 presents the dependence of the intensity and relative half-width of the differential spectrum of tryptophan fluoresence of erythrocyte membrane proteins and molar ellipticity versus the cyclic AMP concentration.

As is shown in Fig. 4, addition of cyclic AMP results in appreciable changes of these parameters. Fluorescence quenching, an increase in the half-width of spectra and a decrease in molar ellipticity at 222 nm are observed. The control measurements indicated that changes in the fluorescence parameters observed do not arise from such trivial optical effects as screening of excitation light, reabsroption of fluorescence or from light scattering.

Similarly, the methodical artefacts which arise from superposition of dichroism of cyclic AMP and light scattering do not cause a decrease in molar ellipticity. This conclusion follows from the fact that cyclic AMP at the concentration used does not possess any optical activity, and light scattering of membranes with cyclic AMP remains practically unchanged.

It is characteristic that noncyclic analog of the nucleotide 5-AMP did not induce any noticeable changes in fluorescence and circular dichroism parameters. The membranes treated with mild doses of ultrasound do not show the effects described.

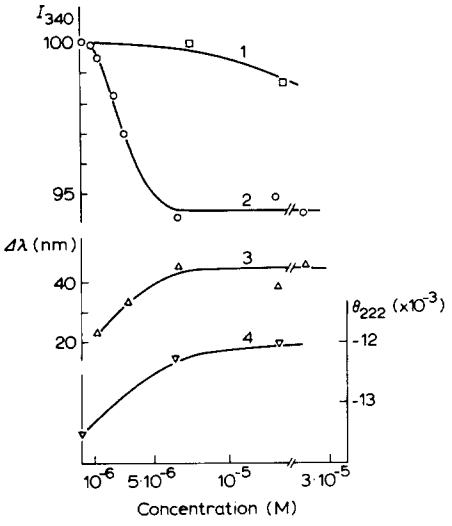


Fig. 4. Dependence of fluorescence intensity (I_{340}) (2), halfwidth of differential spectra ($\Delta\lambda$) (3) and molar ellipticity (θ_{222}) (4) of etythrocyte ghosts on the cyclic AMP concentration. Curve 1 shows the noncyclic 5'-AMP efficiency.

It should be emphasized that the effective cyclic AMP concentration necessary for the transitions differs for spectroscopic and spin probe experiments by a factor of ten. This paradoxal discrepancy is due to the following: (a) theophylline distorts the real effect because of its screening and optical activity; (b) totally hemoglobin-free ghosts are used in optical experiments in contrast to the ESR-spectroscopic ones. Hemoglobin depletion is known to be followed by the distortion of the ghosts' integrity and by partial removal of the corresponding receptor site [17].

Indeed, the control measurements using spin probes demonstrate that in the theophylline absence the structural response of maximally washed ghosts is shifted to the concentration range characteristic for optical probing.

Freeze-fracturing of erythrocyte ghosts

Freeze-fracturing electron micrographs of erythrocyte membranes are presented in Fig. 5.

The outer half of the fractured control membranes viewed from the outside of the cells (protoplasmic fracture face [18]) demonstrates a random distribution of many intramembrane particles measuring 80—100 Å in diameter. There are also some aggregates consisting of several particles. According to the calculations there are about 3000 particles in μm^2 of the fracture face of the control samples (Fig. 5a).

After the addition of Mg · ATP ($2 \cdot 10^{-4}$ M) to the medium there are no changes in the quantity of intramembrane particles in the fracture face (Fig. 5c). Only the tendency towards association of the particles with a change in the form and distribution of particle-free zones is manifested.

In contrast, addition of cyclic AMP (10^{-8} M) results in an essential change of freeze-fracturing data (Fig. 5b,d): the quantity of intramembrane particles per

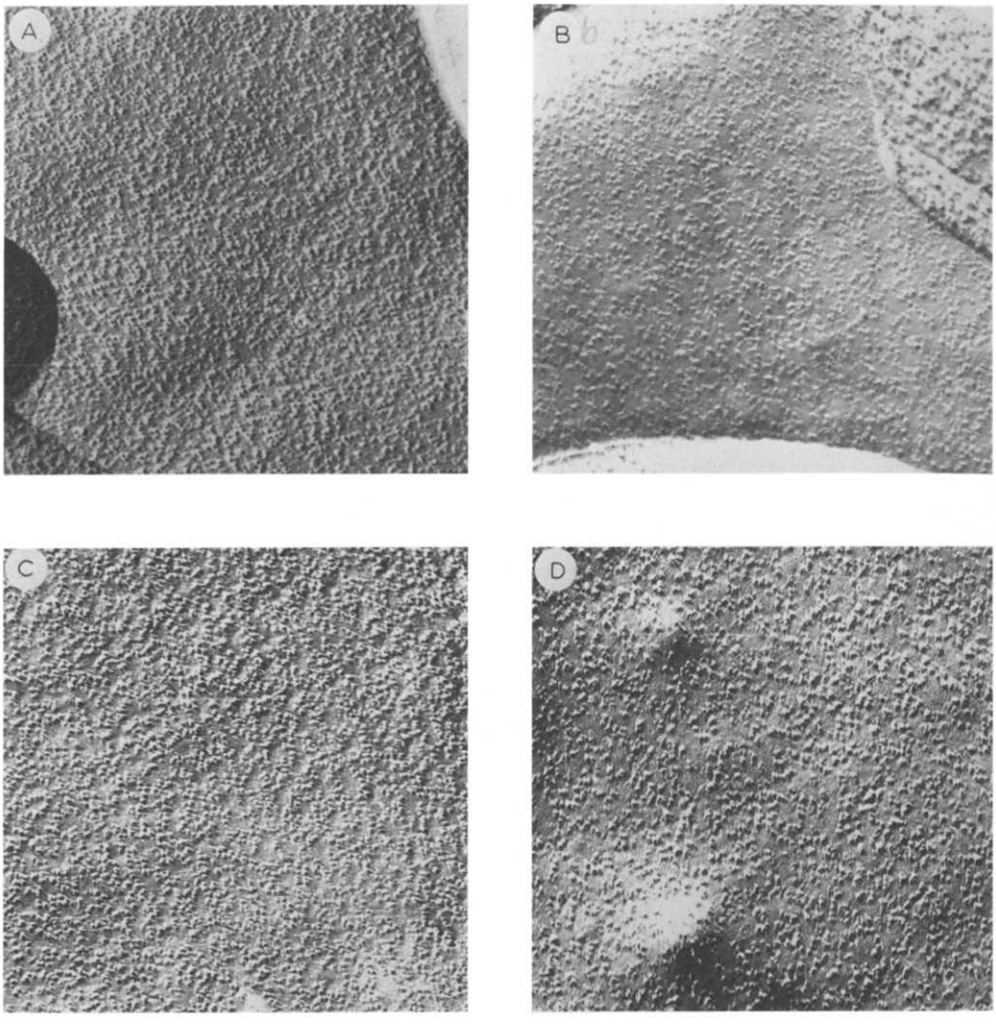


Fig. 5. Effect of cyclic AMP on the erythrocyte membrane revealed by freeze-fracture electron microscopy. The protoplasma fracture faces are shown. Magnification × 52 000. A, Erythrocyte ghosts only; B, erythrocyte ghosts with 10^{-8} cyclic AMP and 20 μg/ml IPK; C, erythrocyte ghosts with 1 mM $MgCl_2$ and 0.2 mM ATP; D, erythrocyte ghosts with 1 mM $MgCl_2$, 0.2 mM ATP and 10^{-8} M cyclic AMP.

μm^2 significantly decreases in the presence of Mg · ATP (1760 ± 70) or without it (1800 ± 70). Simultaneously the size of particles increases. However, their distribution does not become regular. As in control samples the intramembrane particles are scattered on the fracture face, forming aggregates comprising 3—5 particles. The areas on the fracture face free of particles increase correspondingly. Similar behaviour is revealed for two experimental variations (with and without Mg · ATP). At the same time large hump-shaped protrusions of about 1000 Å diameter appear on the fracture face after addition of cyclic AMP and Mg · ATP.

Discussion

The results of the experiments with spin probe I are alone enough to conclude that cyclic AMP induced the structural transitions of erythrocyte membrane. The character of structural changes registered by the probe I mobility is modified by the activity of the protein kinase system. When the protein kinase system is "switched off", τ of probe I is decreased under the cyclic AMP action. This corresponds to "loosening" of the membrane zones with which probe I is associated. On the contrary, when membranous protein kinases are active cyclic AMP addition results in τ increase, i.e. "stiffening" of the membrane. Such complex character of the dependence of structural membrane state on "switching off" of protein phosphorylation does not explain the observed effects on the basis of the idea of only one structural transition. At the same time the simultaneous consideration of curves in Figs. 2 and 3 permits us to describe them satisfactory by two structural transitions of different nature. One of these is not connected with protein phosphorylation and results in a decrease in the probe mobility (the S-shaped curve). Another transition does depend on phosphorylation and is accompanied by an increase of the probe mobility (the cupola shaped curve). Curve 2 (Fig. 2) corresponds to the first transition. The minimum and high-concentration maximum of curves 1—2 (Fig. 3) probably represent the superposition of both transitions with variable contribution of phosphorylation. The relatively small maximum of curve 1 (Fig. 2) is due to the weak protein phosphorylation by endogenous ATP.

What components of erythrocyte membrane are involved into these structural transitions? It is known that nitroxide derivatives of stearic acid might interact both with proteins and with phospholipids [19]. The observed modification of ESR spectra may be thought to be due to the change of the structural state of both protein and lipid phases of the erythrocyte membrane. However this is not likely to be the case. For probe I only the aliphatic ends of stearic acid penetrate into the lipid bilayer, and nitroxide radicals together with polar end groups remain near the membrane surface where the probability of limitation of their motion by proteins is naturally higher. On the contrary, the nitroxide group of probe II is buried into the lipid bilayer of the membrane together with the nonpolar hydrocarbon chain of stearic acid. The data of Bieri and Wallach [19] are in favour of such a suggestion. These authors showed that probe 5NS (stearic acid with nitroxide group in the 5th position) interacts with the proteins of erythrocyte membrane much stronger than probe 16NS (16th position). Taking that into account, it is reasonable to suggest that the appreciable change in the probe I mobility is due to the modification of the structure of the membrane protein system. At the same time it is possible that parameter τ of the label I also reflects a state of polar heads of phospholipids localized at the membrane surface.

It is worth noting that upon ESR cross scanning of membrane the amplitude of changes decays in direction from surface to the center. Thus at 10^{-7} M of cyclic AMP the change in mobility of probe I (iminoxyl radical in the stearate polar head) is about 9%, that of the parameter S (free radical at C-5 position) is about 1% [10] while the S parameter of probe II (radical at C-10) is not changed at all. This means that the main changes of fluidity are localized in hy-

drophilic surface of membrane rather than in the hydrophobic lipid bilayer.

There are other data indicative of the presumably protein nature of cyclic AMP induced structural transition. The structural response of the membrane is repressed by group-specific bifunctional reagents mainly to the protein NH_2 groups. Such inhibition could be due to the stabilization of the protein framework of the membrane by some kind of molecular cross-links caused by cyanuric chloride and glutaraldehyde. In similar way SH group blockage by iodacetamide prevents the membrane modification induced by cyclic AMP (Table I). It is worthwile to note that the major quantity of NH_2 and especially SH groups belongs to the proteins rather than lipids.

Also the fluorescence and circular dichroism data indicate the modification of the protein phase of the membrane. It is known that their parameters depend on the structural state of protein macromolecules in a membrane [20]. For example the short-wave shift of the fluorescence spectrum suggests a polarity decreased in the microenvironment of tryptophanyls which may be considered as statistically distributed natural probes sensitive to protein conformation. Broadening of spectra and fluorescence quenching probably reflect the growth of tryptophanyl microenvironment heterogeneity. A drop in molar ellipticity observed as a response to the cyclic AMP action reflects a decrease in α-helix content of membranous proteins and, as a consequence of that, a change in conformation. Finally, a decrease in the quantity and distribution of aggregates of intramembrane particles on a fracture face of erythrocyte membrane unequivocally indicates the structural changes in the protein framework of the membrane [21]. The nature of the intramembrane particles has been shown to be a protein one [22,23] and their aggregation is of physiological importance [24,25]. Furthermore, the large hump-formed protrusions on the fracture face of the erythrocyte membrane induced by cyclic AMP and Mg · ATP (i.e. under the optimal conditions for a second phosphorylation-dependent transition) may be an indication of a large-scale structural modification of the membrane. It is very likely that the contractile elements of erythrocyte membrane are involved in the formation of these protrusions. It is also known that mobility of intramembrane particles is restrained in the membranes by spectrin [26] viewed as one of the main substrates of protein kinase in erythrocyte membrane [27].

Thus the data obtained allow us to make the conclusion that cyclic AMP induced at least two structural transitions in the protein phase of the erythrocyte membrane. One of them is at low concentration (10^{-11}—10^{10} M) of cyclic AMP and is not connected with phosphorylation. It may be considered as a high-rate physical structural reaction. It is likely that a rapid change of the fluorescence parameters of erythrocyte membranes is due to such a reaction. The second transition is under the control of the protein kinase system. It is greatly influenced by phosphorylation of membranous proteins and may be viewed as chemical structural reaction, i.e. a structural rearrangement associated with mechanochemical displacements of the membrane molecular components after binding of inorganic phosphorus to contractile proteins.

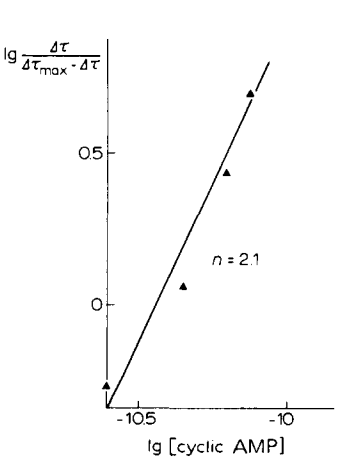
On the whole our interpretation of this structural transition is close to that of Kury and McConnell [10] with sufficient difference, however, for us to emphasize the predominant role of proteins rather than lipids in generalization of

structural effects. Also, some data from ref. 10 might be considered as indirect evidence of ability of cyclic AMP to induce the membrane modification which is not causally connected with phosphorylation. Indeed, an increase of spin probe parameter S under the influence of cyclic AMP in the presence of Mg^{2+} and ATP was observed along with a decrease of S upon substitution of GTP for ATP. In the latter case phosphorylation has been blocked both because exgenious ATP and Mg^{2+} were absent and due to the inhibitory effect of GTP on protein kinase activity [27]. In other words, the conditions were as with the presence of protein kinase inhibitor without ATP and Mg^{2+} (Fig. 2, curve 2).

It is quite evident that observed transitions are of a generalized nature because luminescence and circular dichroism parameters reflect the structural state of the whole pool of protein molecules rather than some of their fractions. Similar information is obtained from the ESR measurements.

The effect of physical generalization is particularly demonstrated in those transitions which are not connected with the protein kinase system when the mechanism of chemical amplification through enzyme phosphorylation is "switched off". According to the calculations, the structural state of the membrane is changed after binding of approx. 3 molecules to the cyclic AMP receptor sites. At the same time the lipid bilayer of the membrane may also be incorporated to some extent in the structural rearrangements as follows from the negligible changes in the parameters of lipid spin probes according to Kury and McConnell [10].

There are other data suggesting the generalized nature of the transitions: (a) physical structural transition is realized at a comparatively narrow range of low cyclic AMP concentrations, and (b) the plot of the fluorescence intensity versus the cyclic AMP concentration is distinctly S-shaped. This means that the structural rearrangement obeys the co-operative law. In support of that the data in the Hill coordinates may be presented. So, the Hill numbers calculated for cur-



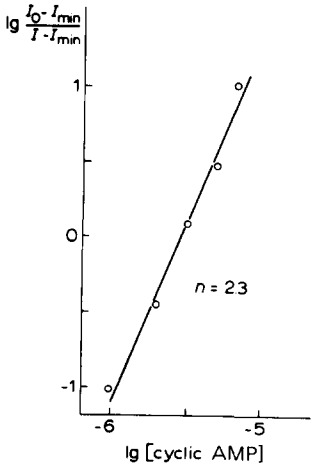


Fig. 6. The Hill plot for the motion parameter (τ) of spin probe I incorporated into the erythrocyte ghosts as a function of the cyclic AMP concentration. The data of Fig. 2 (curve 2) were used.

Fig. 7. The Hill plot for the fluorescence intensity (I_{340}) of erythrocyte ghosts as a function of the cyclic AMP concentration. The data of Fig. 4 (cruve 2) were used

ves 2 (Fig. 2) and 2 (Fig. 4) were found to be correspondingly equal to 2.1 and 2.3 (Figs. 6 and 7). The second transition (the chemical structural reaction) is nonco-operative, as judged by the smooth changes in probe I mobility.

The condition necessary for both structural reactions is integrity of membrane. The importance of the integrity, i.e. intactness of the native network of molecular interactions, is evident from the experiments with ultrasound-treated membranes. The mild ultrasonic fragmentation of erythrocyte ghosts totally removed the cyclic AMP induced rearrangements, as is seen from the results of ESR, fluorescence and circular dichroism measurements. For example, the rotational mobility of probe I remains unchanged in the sufficiently large membrane fragments clearly visible in the phase-contrast microscope. This emphasize the role of long-range forces in the realization of structural transitions.

Finally, the question of the nature of the receptors from which the observed structural transitions are initated is of great importance. It is very likely that these receptors are different in nature. Besides the regulatory subunits of protein kinase being the well-known cyclic AMP-binding site [3] other receptors of protein nature possessing high affinity to cyclic AMP without any protein kinase activity have been demonstrated [28,29].

Summing up, we consider a possible biological role of the cyclic AMP induced structural transitions. One may suggest that both structural transitions are related to pleiotropic regulatory action of cyclic AMP. The location and mobility of membrane components which are under the control of the phosphorylation level of contractile proteins are of some importance for final regulation processes. The changes in passive permeability, transport of substances, membrane potential and activity of a number of enzymes may be a consequence of such transition.

Acknowledgements

We are especially grateful to Dr. R.I. Zhdanov for kindly presented samples of nitroxide derivatives of stearic acid. We are also thankful to Dr. S.L. Aksentsev and Miss T.S. Kortneva for their valuable assistance in the preparation of the English version of the paper.

References

1 Robinson, G., Butcher, R. and Sutherland, E. (1971) Cyclic AMP, Academic Press, New York
2 Smith, H.M. (1968) Ann. N.Y. Acad. Sci. 150, 191—467
3 Bowman, W.C. and Nott, M.W. (1969) Pharmacol. Rev. 21, 27—72
4 Posternak, T. (1974) Ann. Rev. Pharmacol. 14, 23—34
5 Kuo, J. and Greengard, P. (1969) Proc. Natl. Acad. Sci. U.S. 64, 1349—1355
6 Konev, S.V., Aksentsev, S.L. and Chernitskii, E.A. (1970) The cooperative transitions of proteins in a cell (Russian) Nauka i Tekhnika, Minsk
7 Konev, S.V., Chernitskii, E.A., Aksentsev, S.L., Mazhul, V.M., Volotovskij, I.D. and Nisenbaum, G.D. (1975) Mol. Cell. Biochem. 7, 5—17
8 Meyers, M. and Swislocki, N. (1974) Arch. Biochem. Biophys. 164, 544—550
9 Konev, S.V., Volotovskij, I.D. and Finin, V.S. (1975) Doklady AN SSSR (Russian) 223, 1473—1476
10 Kury, P.C. and McConnell, H.M. (1975) Biochemistry 14, 2798—2803
11 Dodge, B.T., Mitchel, C. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119—130
12 Moor, H. (1971) Philos. Trans. R. Soc. Lond., Ser. Biol. Sci. 261, 121—131
13 Lowry, O.H., Rosenbrough, N.J., Farr, A.J. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
14 Walsh, D.A., Ashby, C.P., Gonzales, C., Calkins, D., Fischer, E. and Krebs, E.G. (1971) J. Biol. Chem. 246, 1977—1985

15 Lichtenstein, G.I. (1974) Method of Spin Labels in Molecular Biology (Russian) Nauka, Moscow
16 Hubbel, W.L. and McConnell, H.M. (1971) J. Am. Chem. Soc. 93, 314—326
17 Burger, S.P., Fujii, T. and Hanahan, D.J. (1968) Biochemistry 7, 3682—3700
18 Branton, D., Bullivant, S., Gilula, N.B., Karnovsky, M.J., Moor, H., Mühlethaler, K., Northcote, D.H., Packer, L., Satir, B., Speth, N., Staehelin, L.A., Steere, R.L. and Weistein, R.S. (1975) Science 190, 54—56
19 Bieri, V.G. and Wallach, D.F.G. (1975) Biochim. Biophys. Acta 406, 415—423
20 Konev, S.V. (1967) Fluorescence and Phosphorescence of Proteins and Nucleic Acids, Plenum Press, New York
21 Wallach, D.F.G., Bieri, V.G., Verma, S.P. and Schmidt-Ulrich, P. (1975) Ann. N.Y. Acad. Sci. 264, 142—160
22 Guidotti, G. (1972) Annu. Rev. Biochem. 41, 731—752
23 Singer, S.J. (1974) Adv. Immunol. 19, 1—66
24 Ojakian, G.K. and Satir, P. (1974) Proc. Natl. Acad. Sci. U.S. 71, 2052—2056
25 Chevalier, J., Bouget, J. and Hugon, J. (1975) Biophys. J. 15, 125a
26 Nickolson, G. and Painter, R. (1973) J. Cell Biol. 59, 395—405
27 Guthrow, C.E., Allen, J.E. and Rasmussen, H. (1972) J. Biol. Chem. 247, 8145—8153
28 Doskeland, S.O. and Ueland, P.M. (1975) Biochem. Biophys. Res. Commun. 66, 606—613
29 Khe-Ching Yuh and Tao, M. (1974) Biochemistry 13, 5220—5226