Polycation-Cell Surface Interactions and Plasma Membrane Compartments in Mammals. Interference of Oligocation with Polycationic Condensation

Stefan Antohi and Vlad Brumfeld

Institut de Virusologie "St. S. Nicolau", 285 Mihai Bravu, R 79650 Bucarest 77, Romania

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At lower concentrations, polyarginine, polylysine, protamine, histones H1, H2A, H2B and H3 cause lysis of human erythrocytes, whereas at higher concentrations inner histones are not hemolytic but induce only surface condensation and alterations in the cell-shape. Antibody coated erythrocytes treated with polyarginine result in ghost-like spheres having globular bodies 1 μm in diameter on the surface. Human fibroblasts and lymphocytes, and Ehrlich ascites cells treated with polyarginine also form surface globular bodies similar in size. Nucleate cell-polyarginine mixtures with lower polycation doses result in cytolysis, while higher polycation doses produce pyknosis of the cell surface accompanied by reorganization of a membrane-like structure. Changes in spectrofluorometric values result from 1,6-diphenyl-1,3,5-hexatrien binding to cell lipids, match the plasma membrane alterations. Reciprocal shake incubation amplifies and/or conditions these polycation-induced alterations. The homogeneity of pyknotic surface bodies and the apparent polycationic membrane reorganization requiring oscillatory friction forces suggest the preexistence of a multizonal glycocalyx distribution corresponding to plasma membrane compartments. The possible role of this compartmentalization in receptor and membrane recycling, as well as the involvement of reversible catalytic-like polycation condensation in macromolecular changes are discussed.

Introduction

The reiterative distribution of carboxyl and sulfate groups of the glycocalyx [1–3] confers upon the cell surface the configuration of a giant polyanion which will be able to interact pyknotically with repetitive cationic groups of natural or synthetic basic polypeptides. Such condensation interactions might be similar to those occurring in the multizonal polyaction pyknosis of the bacterial wall [4–6]. These previously reported findings and the polyanionic similarities between the eukaryotic glycocalyx and the bacterial wall, prompted us to investigate whether cells of higher eukaryotes, suspended and incubated with basic polypeptides under conditions similar to those described in bacteria [6], might display analogous condensations. The present paper describes the variable capacity of poly-L-arginine (p(Arg)), poly-L-lysine (p(Lys)), protamine, and of four histone classes to produce globular condensation on the surface of human erythrocytes, fibroblasts and lymphocytes, and of mouse Ehrlich ascites cells. These surface globularizations, resembling those described in bacteria [6], finally result either in hemolysis or in the fragmentation of nucleate cells. As in the case of *Escherichia coli* cells [6], higher p(Arg) concentrations cause apparent cell surface reorganization, representing a kind of "rescue" from cytolysis of the cell. Under shake incubation conditions, the diversity of p(Arg) inducing surface alterations at different polycation concentrations is in good agreement with the cell binding of hydrophobic fluorescent probe 1,6-diphenyl-1,3,5-hexatrien (DPH). These polycation induced structural changes shall be taken into account as a basis for modeling possible plasma membrane compartmentalization which apparently fits the restricted lateral mobility of the receptor [7–9], receptor reutilization [10] and membrane recycling [11]. Furthermore it is suggested that the apparent catalytic-like activities of polycationic proteins, having oligocations (*e.g.* spermidine) as cofactors, induce reversible and repetitive conformational changes of anionic proteins and nucleic acids.

Reprint requests to Dr. St. Antohi.

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Materials and Methods

Initial cell suspensions

The diploid line of human embryo fibroblasts IVN3CDU (kindly supplied by Dr. I. Aderca, "St. S. Nicolau" Institute of Virology Bucharest) was

grown in Lactalbumin Hydrolysate Medium [12]. Cultures of 48–72 h in cofluent layer on rectangular glass flasks were harvested by versene-trypsin treatment (versene 0.2% in PBS – 30 ml, trypsin NBC 1/300 0.25% in PBS – 1 ml) for 5 min at 37 °C, suspended by pipetting, pelleted at $500 \times g$ and resuspended in acetate-saline [6], at a concentration of 3×10^6 cells/ml. Fresh human blood group A (Center of Hematology, Bucharest) was used as a source of erythrocytes and peripheral lymphocytes: 40 ml of blood were mixed with 20 ml balanced salt solution [13] having 90 U/ml heparin (Sigma); lymphocytes were isolated by the ficoll-paque technique [13] and finally resuspended in acetate-saline, 3×10^6 cells/ml; for the initial erythrocyte suspension, 0.6 ml blood-heparin suspended in 20 ml acetate-saline was pelleted at $500 \times g$ for 5 min and finally resuspended also in acetate-saline, 45×10^6 cells/ml. Ehrlich fluid ascites cells from albino mice, obtained after 10 days tumoral growth (furnished by Dr. Elisabeta Nastac – "St. S. Nicolau" Institute of Virology, Bucharest), were diluted 10^6 cells/ml, pelleted at $200 \times g$ for 5 min and resuspended in acetate-saline, 3×10^6 cells/ml. All the initial cell suspensions were used immediately.

Polycations and oligocations

p(Arg) hydrochloride MW 60000, p(Lys) hydrobromide MW 13000, protamine sulfate, histones III S (lysine rich, H1), VI S (H2A), VII S (H2B) and VIII S (H3) received from Sigma, were separately dissolved in tridistilled water 2 mg/ml, distributed in 1 ml aliquots, kept at −10 °C and used within 30 days. Diamino heptane (Merck) used as cationic analogue of spermidine and diamino butane (putrescine dihydrochloride-Mann) separately dissolved in tridistilled water 4 mg/ml were kept at 4 °C.

Estimation of polycation-induced cytolysis

Separate series of samples (final volumes – 2 ml) in tubes of 18/180 mm, corresponding to different cell types and different polycations were prepared by dispensing: 1.7 ml acetate-saline, 0.2 ml polycation stock solution, prediluted when necessary to obtain the final concentrations mentioned in Fig. 1, 0.1 ml initial cell suspension. For the nucleate cell series, the final polycation concentrations were those mentioned in Fig. 3. Each cell series included polycation-free control samples completed with 0.2 ml tridistilled water. The different series were then incubated at 35 °C for 30 min with reciprocal shaking at 120 strokes/min, and a shaking pathlength of 30 mm; the sample tubes were fixed at a 20° angle with respect to the horizontal. Parallel identical series of samples were statically incubated at 35 °C for 30 min. Hemolysis was evaluated by OD_{340} as described [4, 5]. Cytolysis of nucleate cells was estimated by cell scoring using a counting chamber; the final figures were average values calculated from different mean values of 18 assay sample series. Viable cells were counted when necessary using the trypan blue (Sigma) procedure [14].

Morphological changes of the cells in prelytic stages as well as in the "rescue" state brought about by polycation overdosage

Series of cell-polycation mixtures prepared and incubated as described, but only for 5 min, were pelleted at $500 \times g$ for 5 min. The supernatant was discarded, each pellet resuspended in 0.1 ml acetate-saline, slides prepared for phase contrast microscopy and immediately examined. For the study of polycation overdosage "rescue", slides were likewise prepared from samples incubated for 30 min as above. All the other morphological examinations were done as described here.

Nucleate cell recondensation at high p(Arg) concentrations after cell-disruption initiated by low polycation concentrations

Samples with 5 μg/ml p(Arg) and 1.5×10^5 cells/ml were shake incubated at 35 °C as above, and 200 μg/ml p(Arg) were added at 5, 10 and 20 min intervals, incubation being continued until 30 min. Morphological examination and cell scoring were then carried out.

Treatment of specific antibody coated erythrocytes with p(Arg)

Initial cell suspensions – 4 ml, were mixed with antithetic human serum (complement inactivated hemotest serum group B, Center of Hematology – Bucharest) prediluted 1/2 in acetate-saline – 4 ml. The mixture, incubated at 37 °C for 10 min, was pelleted at $500 \times g$ for 5 min, washed once and resuspended in 10 ml and 4 ml acetate-saline, respectively; the latter sample was mixed with

antihuman horse serum (Cantacuzino Institute – Bucharest) prediluted 1/5 with acetate-saline – 4 ml, and incubated for 10 min at 37 °C. After sedimentation at 500 × *g* for 5 min, the cells were washed and resuspended as above, 0.1 ml aliquots were added to separate samples of p(Arg) 20 µg/ml, prepared as above. Samples were shake incubated as described for hemolysis but for 5 min, pelleted at 500 × *g* for 5 min and examined morphologically.

The action of diamino heptane and putrescine on the "rescue" from hemolysis and cell disruption by polycation overdosage

Separate erythrocyte samples prepared as described for hemolysis and having histones H2A, H2B and H3 200 µg/ml, respectively, were shake incubated as above for 20 min, diamino heptane 100 µg/ml and putrescine 200 µg/ml were separately distributed in different histone series and shake incubation continued for 10 min. In other erythrocyte-histone sample series the oligocations were preadded to the overdosed polycation distribution, the shake incubation being correspondingly inverted (10 then 20 min). Similar oligocationic pretreatments were carried out with fibroblast – p(Arg) 100 µg/ml samples. OD_{340} estimation, cell scoring and morphological examinations were then performed.

Estimation of the fluorescence intensity of p(Arg) treated fibroblasts in the presence of DPH added before and after shake incubation

To a series of p(Arg)-fibroblast mixtures with the p(Arg) concentrations mentioned in Fig. 3, was added DPH (Sigma) 2×10^{-3} M in tetrahydrofuran (Merck)-5 µl/sample; the samples were kept for 15 min at 23 °C, then shake incubated as above for 30 min. In another identical series, DPH was added after shake incubation of the samples. Both series had p(Arg)-free control samples. Fluorescence intensity was measured at $\lambda = 392$ nm using an Aminco SPF 500 corrected spectrofluorometer.

Results

Diversity of the hemolytic pattern of polycationic polypeptides in relation to static and shake incubation

Under shake incubation, both synthetic polycations, p(Arg) and p(Lys), and the natural ones

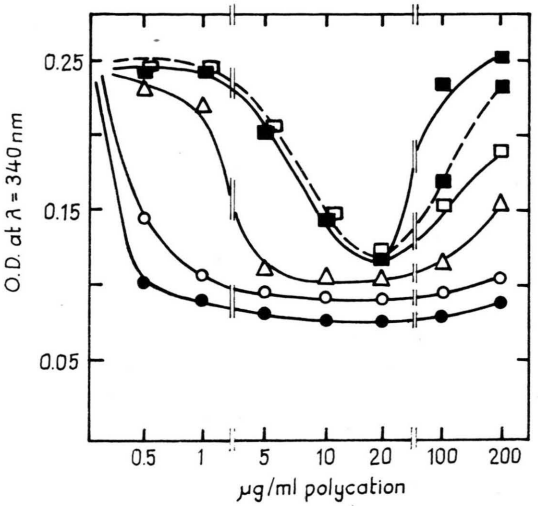

Fig. 1. Hemolysis caused by different basic polypeptides plotted as OD_{340} values against polycation concentrations: p(Arg) (●); p(Lys) (○); protamine and histone H1 (△); histone H2A (□—□); histone H2B (- - -■); histone H3 (——■).

(protamine and histones H1, H2A, H2B and H3) had hemolytic effects. The minimal hemolytic concentration of the synthetic polycations was taken as "threshold lytic dose" (Fig. 1) since the higher concentrations displayed similar lytic values. The hemolytic profile of protamine and histone H1 was similar, their threshold doses were however about ten times greater than those of the synthetic polycations, and displayed a slight increase of OD values at higher concentrations. This increase of OD values, corresponding to the decrease of hemolysis at higher polycation concentrations, was more clearly illustrated in the H2A, H2B and H3 series; the 200 µg/ml-dose of H3 (Fig. 1) was not at all lytic. Morphologic examination of histone sample pellets with higher polycation concentrations (Fig. 2) showed various erythrocyte deformities, such as marginal distorted condensations and "bell-like cell"forms.

Under static incubation, only p(Arg) and p(Lys) caused hemolysis which became evident at minimal polycation concentrations 5–10 times higher than in the case of reciprocal shaking. Although protamine and histones, under static incubation did not induce a significant decrease of OD values, evident morphological alterations of almost all red cells were observed: spherocytes and elongate forms at lower polycation concentrations; peripheral de-

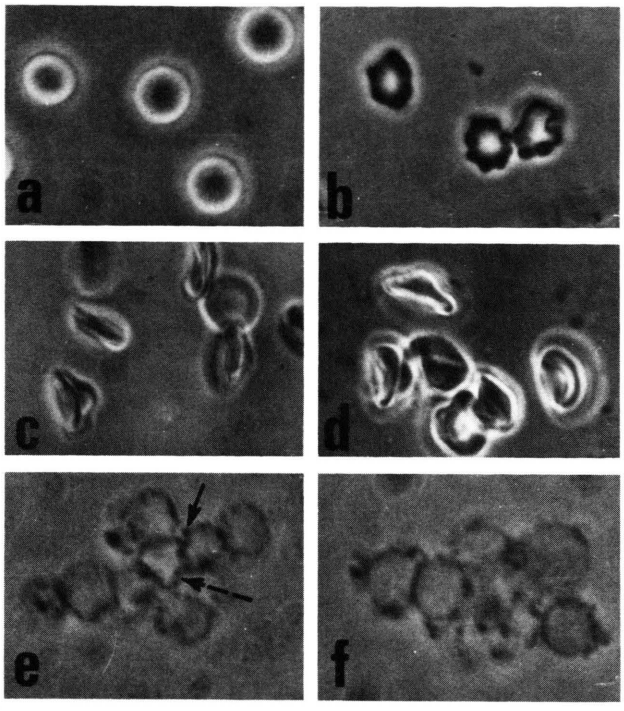

Fig. 2. Polycation induced morphological alterations of erythrocytes. Control sample cells (a). Cell deformities such as condensed distorted margins (b), and "bell"-like forms (c), (d) caused by histone H2B and H3 overdosage; ghost-like spheres (e, f) with globular bodies on their surface (—►) separated by pits (- - ►), induced by the action of p(Arg) on antibody coated cells (1000 ×).

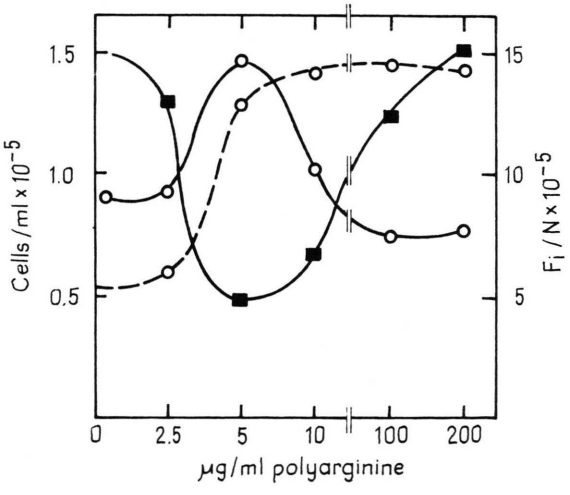

Fig. 3. Cell concentrations (■) and fluorescence given by DPH – lipid binding in the fibroblast – p(Arg) sample series plotted against different polycation concentrations. F_i/N = fluorescence intensity/cell number ratio. F_i/N values in the sample series with DPH added before (○- - -○) and after (○—○) shake incubation.

formities with marginal globular condensation at higher concentrations of the inner-nucleosomal histones.

Ghost-like erythrocyte forms with globular surface condensations resulting from the action of p(Arg) on antibody coated erythrocytes

The p(Arg) treatment of antibody coated erythrocytes resulted in the formation of ghost-like, frequently agglutinated, hemoglobin-free spheres (Fig. 2). The gost surface had discrete globular condensations of about one μm, separated by interglobular pits. p(Arg)-free control samples of antibody coated erythrocytes had neither ghost spheres nor surface globular condensations.

Disruption displayed by nucleate cells treated with low p(Arg) concentrations

Suspended fibroblasts, lymphocytes and Ehrlich cells treated with 5 μg/ml p(Arg) under shake incubation for 30 min, exhibited an approximately 65% decrease in cell concentration (Fig. 3). In addition to the remaining whole cells there were clustered filamentous cell fragments with many heterogeneous globular bodies. About 3.5% of the remaining cells were viable, *i.e.*, did not take up trypan blue stain. In the predisruption stages when the cell number decreased to about 70%, many cells, at least double in size, had dense homogeneous bodies protruding on the cell surface and ranging in diameter from 1 to 1.5 μm (Fig. 4). The distribution of these dense globular bodies on the cell surface was inferred from the fact that they are the first cell components to appear in the microscopic field at different levels of the cell thickness, and from their location at the extreme periphery of the cell when occurring along its contour. These cells appeared to be in an advanced predisruption stage preceding complete cellular break down, and are considered as "bursting" cells. The other polycations were less able to cause the appearance of bursting cells and cell-disruption, and hence did not lead to any significant decrease in cell numbers. The very low trypsin concentration used for the detachment of cultured fibroblasts did not apparently modify the polycation interactions observed here; there was no significant difference between the results obtained with different suspensions of fibroblasts, lymphocytes and ascites cells identically treated with versene-trypsin.

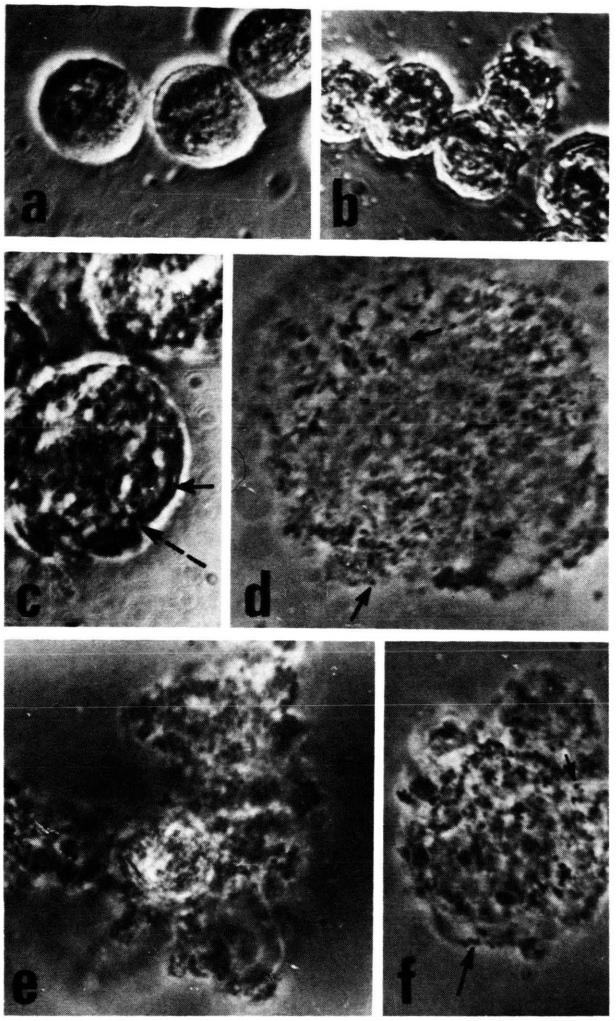

Fig. 4. Modification of nucleate cells treated with p(Arg). Control p(Arg)-free sample fibroblasts (a) and fibroblasts treated with p(Arg) 200 μg/ml (b) (400 ×) and (c) (1000 ×); thickened contour (⟶) and pits (- - ⟶), "Bursting" fibroblast (d) with surface globular bodies termed polionosomes (⟶) (1000 ×). Cell fragments resulting from fibroblasts treated with diamine heptane 100 μg/ml followed by p(Arg) 100 μg/ml treatment (e) (1000 ×). "Bursting" ascites cell with marginal polionosomes (f) (1000 ×).

Surface hyperpyknosis at higher polycation concentration; apparent "rescue" of the cells from cell-disruption

Decrease in the number of nucleate cells upon treatment with 5 μg/ml p(Arg) diminished with increase in polycation concentration; at p(Arg) doses greater than 100 μg/ml the cell numbers were the same as in polycation-free control samples, apparently representing "rescue" of the cells from disruption (Fig. 3). All the rescued cells were not however viable and displayed agglutinability. Their margins presented irregular thickenings and the surface seemed to have an embossed profile (Fig. 4). The rise in concentration to 200 μg/ml of p(Arg) in samples preincubated for 5 min and 10 min with cell-disruption-inducing p(Arg) doses resulted in the formation of condensed cells with the same irregular surface. In these samples the cell number fell to 70%, although incubation was continued. In the samples preincubated for 20 min, the increase to 200 μg/ml of the p(Arg) dose resulted in hyperpyknosis of the cell surface; however the cell number decreased to 40%, close to the value observed in control samples where the initial 5 μg/ml p(Arg) dose was not elevated. Cell surface hyperpyknosis by polycation overdosage occurred only under reciprocal shake incubation, and was more significant with p(Arg), as the other polycations included less condensation. The apparent surface hypercondensation was similar in all the three types of nucleate cells used.

Diversity in fibroblasts cell binding of the fluorescent probe DPH added before and after shake incubation

Addition of DPH before shake incubation to the p(Arg)-fibroblast sample series resulted in progressive increase in the values of fluorescence intensity/cell number ratios, as compared to the values recorded with polycation-free control samples. This increase was not diminished by higher p(Arg) concentrations. As shown in Fig. 3, the addition of DPH after shake incubation resulted in a similar increase in the intensity of fluorescence by 5 μg/ml p(Arg) but a decrease in fluorescence at higher p(Arg) doses; at doses > 100 μg/ml p(Arg) the values were lower than in the control polycation-free samples. The intensity of fluorescence was also slightly increased in the control p(Arg)-free samples to which the DPH was added after the shake incubation interval.

"Rescue" from hemolysis and cell-disruption due to polycation overdosage reduced by diamino heptane

When diamino heptane was added to the erythrocyte and fibroblast series, before and after the

addition of polycations, higher polycation concentrations were unable to ensure overdosage cell "rescue": 200 µg/ml histones H2B and H3 induced hemolysis estimated at an OD decrease of about 60%; 100 µg/ml p(Arg) induced cell-disruption corresponding to a decrease in cell number of about 70%. The pelleted cell fragments exhibited less condensed and delimited globular bodies (Fig. 4). In contrast, putrescine had no significant influence on the rescue from either hemolysis or cell disruption caused by polycationic overdosage. However it induced slight attenuation of the polycation surface condensation.

Discussion

In addition to polycation induced endocellular alterations of nucleate cells, the direct action of polycations on the plasma membrane has been discussed and considered to be doubtful [15]. In contrast, polycations have more recently been included among the membrane aggregation factors bearing an influence on mammalian membrane permeability [16]. The diversity of polycation induced cytolysis and deformity of the cell surface described in the present paper indicate once again the existence of extensive polycation-plasma membrane interactions. This diversity may also be observed with hemolysis, previously mentioned for p(Lys) (*cf.* [17]) since the cationic content and the chain length of protamine, histones [18, 19], p(Arg) and p(Lys) are heterogeneous. This correspondence strongly suggests the electrostatic condensation nature of the polycation-erythrocyte surface interactions. The erythrocyte deformities induced by inner histone overdosage suggest involvement in the interaction also of the spectrin cytoskeleton [20] which likewise has affinities for oligocationic polyamines [21]. In nucleate cells, the polycation-plasma membrane interactions are strongly amplified when p(Arg) is used, the cell samples with lower p(Arg) concentrations, under shake incubation, resulting in cell-disruption. These drastic cell alterations suggest as initial molecular mechanism multiglobular salt binding aggregations between the reiterative cationic groups of polycation molecules and the repetitive anionic groups of glycocalyx constituents, probably similar to that described in bacteria and operationally termed polionosomes [6]. The nascent polionosomes (Figs. 2 and 4), continuously exposed to oscillatory movements generating cell surface friction forces, would exercise traction on the glycocalyx protein roots causing dislocations in the lipid bilayer and osmotic imbalance, possibly analogous to those induced by complement [22].

Involvement of polionosomes in the defining of a model "glycocalyx compartmentalization" extended by protein roots within the entire plasma membrane

The multizonal distribution of surface polycation pkynosis in nucleate cells, the condensing morphopoieses of polionosomes to an almost similar extent, suggest that the anionic groups of the glycocalyx might have been previously distributed in distinct zones. These zones, richer in glycocalyx with high anionic reactivity, would condense separately with exocellular p(Arg) chains, yielding the corresponding polionosomes (Fig. 4), and would constitute compartments extending in the lipid bilayer by the rooted moieties of glycocalyx forming glycoproteins. The compartments, apparently extending on the surface over-distances greater than the diameter of a polionosome, would be similar in size and delimited from one another by intercompartmental areas poor in anionic glycocalyx. These areas might correspond to a network of coated pits since, as already emphasized (*cf.* [2]), the outside surface of the coated pits has a "modest" glycocalyx. In turn the compartmental distribution of anionic groups may account for zonal polycation pkynosis. In the polycation condensation process, the initial partially bound p(Arg) chains of a compartment would be unable to overreach the bordering coated pit areas, in spite of the oscillating movements caused by reciprocal shaking. Not managing to interact with the anion rich glycocalyx of neighbouring compartments, the polycationic chains interact electrostatically only with the glycocalyx constituents bordered by coated pits, and condense a poly-ionic body, *i.e.*, a polionosome. As it is formed by condensation, its diameter is less than the linear dimensions of the corresponding compartment. Thus the outside surface of the plasma membrane would be formed by plateau-like compartments delimited by pits which correspond on the cytoplasmic surface of triskelion clathrin complexes [23]. This compartmentalization might be structurally maintained by extensions of the cytoskeleton into the plasma membrane [7, 24, 25].

The uniformly distributed pyknotic zones on the ghost like spheres resulting from p(Arg) treatment of antibody coated erythrocytes (Fig. 2), also appear to lend support to the model of plasma membrane compartmentalization. As polycations interact with serum proteins (*cf.* [26]), these pyknotic globular bodies would also incorporate the coating antibodies, the phenomenon being an antibody mediated polycation condensation. Nevertheless, the uniform alignment of the condensed zones with clear-cut interspaces strongly suggests that antibody coating complexes and, implicitly, their corresponding surface antigens are distributed in discrete zonal compartments probably preexisting the p(Arg) action. A glycocalyx compartment with its bordering coated pits would make-up a functional unit of receptors [8, 10, 27] and membrane [11, 18] recycling. Within the context of plasma membrane compartmentalization, the restricted lateral mobility of the receptors [7, 9] would be confined to the limits of a glycocalyx compartment during the sequential steps of the receptor cycle in the membrane [8]. Hence, the receptors inserted in the glycocalyx compartments would move to the periphery, and internalize when reaching the coated pits. Since specific ligand binding on the receptor occurs in the lateral motion interval [9], the insertion of a receptor would take place close to the compartment centre, and would by lateral diffusion reach the internalizing border, thus having time to accomplish the specific ligand binding function. The reutilization of the receptors (*cf.* [10]) and their long range movements [24, 29] suggest that the receptor compartmental cycle would have an intercalated cytoplasmic step, allowing the reinsertion of a receptor in compartments adjacent or remote from that in which its internalization had occurred. This aparent recycling might distribute the receptors from one compartment to another and thereby the compartmentalization suggested here seems to be in agreement with the findings supporting the fluid mosaic model [1, 2, 30]. The continuous membrane flow from one membrane system to another [28] would be favoured by zonalization of the plasma membrane system extending over long cellular distances. The same compartmentalization would also be consistent with the catalytic sequences in which the reaction rate is restricted by lateral motion, such as transglutaminase activities engaged in receptor mediated endocytosis [31, 32] and the hormone-receptor-adenylate cyclase system [9]. The plasma membrane compartments suggested here are conceived as zonal units transiently organized during receptor and membrane recycling. The polycation globular condensation would fix one state of this continuously moving zonalization. From the viewpoint of evolution the glycocalyx compartments would have as remote ancestor the subunit organization of the bacterial wall [6] engaged in a more rigid and less specific form of macromolecule endocytosis.

Cell surface condensation by polycations interferred with by oligocations point out the common property of cationic proteins to organize dynamically various eukaryotic structures

The apparent rescue from cell-disruption at polycation overdosage would be due to membrane reorganization by aggregation of the nascent polionosomes. The hyperconcentrated polycationic strate adjacent to the glycocalyx would initiate nascent polionosomes. The "to and fro" shake movements make the polionosomes oscillate into new polycationic strata, thus establishing supra-added salt bonds and forming a plasma membrane-like structure. Both the fact that p(Arg) overdosage blocks the cell-disruption initiated by low p(Arg) concentration and the spectrofluorometric data lend support to such a reorganization sequence: (i) the p(Arg) overdosage in fibroblast-low dose p(Arg) samples, arrested the triggered cell-disruption since the cell number remained at precytolysis value; this suggests a membrane-like structure reorganization even in the case of "bursting" cells (Fig. 4); (ii) addition of DPH before shake incubation facilitated DPH-lipid binding as the initial multizonal condensing alterations, which increase in proportion to the polycation concentration, expose the lipids to a greater extent; the subsequent shaking and polycation overdosage cause surface reorganization which also retain the DPH-lipid complexes already formed. This accounts for the progressive increasing intensity of fluorescence per cell; (iii) the DPH added after shake incubation finds the cell surface with a structure reorganized by p(Arg) overdosage, hiding the cell lipids, consequently fluorescence per cell drops.

The structural changes of the giant cell-surface polyanion brought about by polycations appear

to underline the common capacity of cationic proteins to assemble structures with reiterative distribution of anionic groups, also displaying self-organizing abilities [33]. These organizing assembles are widespread in eukaryotes: in addition to chromatin [34, 35] and ribosome [36] assemblies, there also are cytoskeletal microtubule assemblies aggregated by microtubule associated protein acting as polycation [37]. It may be assumed that the different polycation-organized assemblies are modified dynamically by oligocationic polyamines, as several reports pointed out the role of spermine, spermidine and putrescine in the regulation of DNA synthesis, cell division and differentiation (*cf.* [38–40]) and in modulation of the spectrin cytoskeleton [21]. According to our results, the polycationic cell-surface condensations, as apparent membrane reorganized structures, are influenced by oligocations since diamino heptane treatment abolished the histone overdosage "rescue" of erythrocytes from hemolysis and the p(Arg) overdosage "rescue" of fibroblasts from cell-disruption. Preadded diamino heptane impedes the condensation ability of polycations and postadded oligocation molecules cause dissociation of what had been previously organized by the polycations. There seems to be an interference between the less condensing electrostatic action of oligocations and the extensive salt binding condensations caused by long chain cationic proteins. Thus polycations and oligocations having the same substrates would participate in a "more condensing–less condensing" interplay which could account for the role of polyamines in the different regulation pathways. The polyamines would be a kind of cofactor inducing reversible and repetitive changes of many structural assemblies organized by polycations such as the cytoskeleton, chromatin and ribosomes. The catalytic-like participation of polycations in the conformational modification of their polyanionic substrates, not implying rearrangement of their covalently stabilized intramolecular topography, has suggested classification of these cationic proteins in a separate class of enzyme-like factors [6] – the stereases. This viewpoint seems to be consistent with recent concepts which consider enzymes as reactants rather than catalysts [41] and may be useful as it outlines the dynamic aspects of many polycation-organized structures. Moreover, the considerable diversity of polycationic factors in higher eukaryotes [19] as compared with those of lower eukaryotes and prokaryotes [42], suggests that the increase in polycationic polymorphism might have had an essential role in the second great evolutionary leap [43] from prokaryotes to eukaryotes. For instance polycationic sterease-type activities were required for an accurate intracellular "handling" of the increased number of genes in replication and gene expression, as well as in the structural organization of the cytoskeleton and of other tubulin based structures.

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