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Polyelectrolytes in Immunology: Fundametals and Perspectives

Mehmet I. MUSTAFAEV
Bakü State University, Department of Chemistry,
Bakü-AZERBAIJAN
İstanbul Technical University, Department of Chemistry,
80626 Maslak-İstanbul-TURKEY
TUBİTAK-MRC, Research Institute for Genetic Engineering
and Biotechnology, Kocaeli-TURKEY

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Physico-chemical criteria for the construction of artifical immunomodulators and immunogens(and vaccines) on the basis of synthetic polyelectrolyte(PE) and polyelectrolyte complexes(PEC) with low molecular weight ligands and protein (and polysaccharide) antigens are considered. The role of electric charge, chain length, conformation, hydrophobicity, polydispersity and supramolecular structure in the immunomodulating activity of PE(PEC) and the conditions facilitating the transformation of their suppressor capacity into immunostimulating one in the same polymerhomologues series are discussed. A structure-function relationship of immunogenicity of PE(PEC), a structural model of an artificial polymer- subunit immunogen and some practical applications of them as valuable tools in theoretical immunological studies are described.

Introduction

The great Louis Pasteur a hundred years ago laid down the scientific bases of vaccine production. His fundamental principle of immunization is the use of weakened or attenuated strains of microorganisms. At the same time inactivated microbes and proteins extracted from them, contain a great number of antigens. That is way after vaccination the immune system produces very many antibodies against redundant antigens, which lead to complications (allergy, etc.) upon vaccination.

An interesting and original approach to the solution of the problems being after artificial synthesizing of unique specific determinants of relevant antigens, each of which would be typical for this or that infections desease agent. Examples of such artficial antigens are synthetic polypeptides: linear compolymers of amino acids and artificial polyamino acids with braced chains. The structure of these macromolecules imitates that of the neutral ones. Besides, the effective immune response to synthetic antigens constructed in this way is developed in the presence of adjuvants. Adjuvants are widely used to increase antbody formation in experimental animals, for example, Freund's incomplete adjuvant, a water-in- oil emulsion containing the antigen, and Freund's complete adjuvant, which is the same but with killed tuberculosis bacilli. These

adjuvants can not be used in man because the mineral oil base is not degraded and persists at the injection site. Particularly with the complete adjuvant, unacceptable granulomas can be formed. In other hand as antigens they do not immunize so effectively as live attenuated microbes. This connected with the fact that the genotype, so the genetic structure of each given body is active in producing antibodies. The formation of antibodies is controlled by special genes. There is a real need for safe and effective adjuvant for use in human immunisation programmes.

During last thirty years or so there has been a steady increase in the number of publications dealing with biologically active synthetic polymers (or "polymeric drugs"). Many of these reports contain pharmocological test data (on antibacterial, antihelminitic, antifungal, antiviral, antineoplastic, herbicidal, enzymatic, etc., activity), and sufficient data relative to polymer properties are available to allow some very tentative structure-activity relationships to be made. But the most tempting results were obtained when weak antigen molecules(haptens, proteins, polysaccharides and whole cells) in combination with their synthetic (non-natural)polyelectrolytes(PE) were administered to animals. These combined administration brings about an enhancement (by one or two orders) of the immune response to respective antigens, e.g. synthetic PE have immunoadjuvant activity. Besides, the use of PE as carriers for microbial and viral antigens whose components are firmly linked together to form a stable complex (or conjugate) made it possible not only to increase by several orders of magnitude the immune responsiveness of the organism but also afforded effective immune protection which, in turn, opened the way of the construction of artifical vaccines against the unconquered infections. The present short review is aimed at a comparative analysis of the most recent data concering the immunomodulating and immunogenic properties of PE and polyelectrolyte complexes(PEC) in relation to their physical and chemical parameters in aqueous system with special reference to the structure-function relationships. In Section I of the review the properties of the polyelectrolytes-immunomodulators, the role of electric charge, chain length, conformation, hydrophobicity, polydispersity, and supramolecular structure in the immunomodulating activity of PE will be examined. In Section II, we describe the physical and chemical structures of various polyelectrolyte complexes (PE-surfactants, PE-metals, PE-haptens, PE-proteins, PE-polysaccharides) and their immunological activity and structural-activity relationships. A clue to the practical solution of problem of artificial immunogens (or vaccines) with predetermined specificity and of the physico-chemical mechanism of action of polymer- antigen systems is the use of polyelectrolyte complexes in which polymeric carriers are attached to high and low molecular weight ligands by "mobile" electrostatic and hydrophobic bonds. Preparation of PEC whose composition and chemical structure can be regulated and analysis of the role of structural and chemical transformations of their constituent components not only demonstrated their practicability as a reliable basis for the creation of new highly immunogenic drugs but also showed them to be valuable tools in theoretical immunological studes. Rapid progress in this area has been attained through the modelling of complex multicomponent biological systems from simple polymer-antigen mixtures during the analysis of PE interactions with biopolymers *in vitro* with further extrapolation of these physico-chemical regularities to living organisms. For clarity, it may become necessary to discuss the biological interactions and finally, future research emphasis and challenges are summarized also in Section II.

Polyelectrolytes

This article attempts to interpret the growing literature dealing with immunologically active synthetic polymers from the point of the view of the important physico-chemical characteristics of macromolecules. Synthetic polymers are known to exhibit broad and varied activities with biological systems which have

been discussed in detail in previous reviews [1–4]. During the last years there was a steady increase in the number of publications with immunologically active synthetic high molecular weight compounds of polyelectrolyte(PE) origin, e. g., macromolecules bearing positive and negative charges or capable of being charged at physiological conditions have been shown to have immuno-adjuvant activity in several model systems. Synthetic polyanions, such as polyacrylic acid, pyran copolymer and dextran sulphate have all been demonstrated to enhance the primary and secondary antibody responses to sheep erythocytes(sRBC) in *vivo*[5–9]. Cationic polymers, such as polyvinyl-pyridines, polyvinylimidazoles, polyconidines and its derivatives-several quaternary ammonium polymers being injected to animal simultaneously with the sRBC increased their immune response to the immunizing antigen and thus produced an adjuvant effect [10–13]. Several synthetic polycations, particularly polyvinylimidazoline, have shown strong adjuvant activity with diptheria and tetanus toxoids [14]. In addition, immunoadjuvant properties for this and related polycations have been observed with experimental tumors in mice [15] Polylycine, for example, when added to synthetic cationic polymers such as diethylaminoethyl-dextran, enhances the interferon- inducing activity of the polyamines[16]. *In vivo* administration of anionic and cationic polymers despite the differences in their electric charge or chemical structure (or even in the chemical nature of their constituent components) displayed approximately a similar immunostimulating activity. Synthetic PE activate the reaction of hypersensitivity of the delayed type and also the graft versus-host reaction. It is possible to stimulate the T-independent immune reactions to various antigens-haptenes, proteins, polysaccharides, foreign cells-and in different species of laboratory animals-mice, rats, rabbits, guinea pigs and dogs. At the same time, neutral and uncharged polymers as well as polymers incapable of acquiring an electric charge in aqueous media (poly-N-vinylpyrolidone, polyethylene glycol, dextran, etc.) had no effect on the immune response to sRBC and thus exhibited no adjuvant activity. Monomeric derivatives of polymeric adjuvant components were also devoid of immunogenic activity. Quite an opposite effect was observed when copolymers were used as adjuvants. For instance, in contrast with homopolymers, copolymers of acrylic acid with methylvinylpyridine whose polymeric chains contain both acidic and basic groups produced immunosuppressor effects and inhibited the immune response by inducing selective elimination of T-B interactions[17]. The adjuvanticity of dextran sulfate was feld to be a function of the polyanionic nature of the agent, as neutral dextran had no effect on the antibody response while a positively charged derivative, diethylaminoethyl- dextran, had an inhibitory effect[9]. These findings led the authors to suppose that the action mechanism of PE adjuvants is not directly linked with the fine peculiarities of their chemical structure (nonspecificity towards the monomeric linke of PE) and that their immunomodulating activity is due to some common properties that are conferred on them by their polymeric origin[3,12,19,20].

Effect of Molecular Mass

Within the framework of above mentioned hypothesis it seemed reasonable to investigate the influence of the average molecular mass or the molecular mass distribution (MMD) (polydispersity) of PE on their adjuvanticity, since early studies in this area were conducted with the use of unfractionated polymers. In the case of polyampholytes it is their structural heterogeneity that should be taken into consideration alongside with their MMD.

A large number of polymers have been tested wherein molecular mass has been stated to be, a structural change that affects biological activity [3,21,22]. But at the same time not much detailed work has been reported concerning molecular-weight with narrow polydispersity and immunologically activity.

The critical point which determined, to a large extent, the successful outcome of these studies was the use of PE fractions with a predetermined molecular mass. This very circumstance made it possible

to establish differences in the immunomodulating activity of PE not only at the level of their individual components but also at the level of the macromolecule as an entity.

Analysis, of polacrylic acid, polyvinylpyridine, polyvinylimidazole, polyconidine and their quaternary salts revealed that the dependence of the immunostimulating activity of these compounds expressed as the ratio of the number of antibody-forming cells (AFC) in experimental and control samples on the polymerization degree (chain length, n) is a critical factor which appears as an exponentially increasing function, "n". In all cases studied the adjuvant activity which was independent of the chemical structure of PE, was manifested only at certain "critical" values of the macromolecular length n_{crit}, showing, at first a drastic rise and then reaching a maximum [12,20].

Contrariwise, n_{crit} was found to depend on the chemical structure of PE, and it decreased during the transition from flexible-chain PE to rigid- chain ones. Macromolecules of polyconidine and its derivatives were less labile due to the presence in the main chain of their macromolecules of cyclic fragments which start to "operate" at much lower molecular weight of the macromolecules.

The critical type of dependence of the AFC number on the length of the PE chain was also observed during the analysis of PE effects on the reaction of endogenous colony formation as well as in the model of cooperative interactions of T- and B-lymphocytes. Interestingly that the dependence of the ability of PE to adhere to negatively charged polymeric latex beads measured by the increment in the optical density after addition of aqueous solutions of PE on "n" is qualitatively very similar[12].

Polyelectrolyte Complexes

The results of immunological and physico-chemical studies led to examine the hypothesis postulating the crucial role of cooperative adsorption of PE on the surface of immunocompetent cells in stimulation of hemopoiesis and to elaborate an entirely new principle of construction of artificial T- independent polymer-subunit immunogens.

Polyelectrolytes can distribute themselves in a living system by blood or lymphatic circulation, by cellular transport through the involvenment of mobile phagocytic cells, or by absorption on cell surface. The interaction of PE with blood proteins and cell surfaces is essential, not only for distribution, but also for specific reactivity since they readily interact with other macromolecules to form supermolecular structures. This association phenomenon occurs with neutral macromolecules because of weak van der Walls interactions, and more importantly, hydrogen bonding. Polycations and polyanions form much stronger electrostatic interactions through ionic bonds. Complex formation of blood proteins and serum or whole blood with PE are sensitive to molecular weight, charge density, concentration, ionic strength, stereochemistry and ratio of both the PE and the proteins[23–32]. Since the complexes formed by these polymeric interactions differ in stability, it should be possible for polymers to have an inherent selectivity and specificity [25–32]. Detailed evidence of these facts and relation to immunological systems is given in [33–58,20].

An important step to the elucidation of action mechanisms of synthetic adjuvant and their chemical synthesis was the clarification of the role of PE molecules in their immunomodulating activity[20]. A comparative analysis of the adjuvant effects of PE relative to their physical and chemical properties in aqueous solutions (structure-function relationships) under conditions close to those observed in living organisms shed additional light on their immunological activity. Of those polyelectrolyte polyionic complexes with low molecular weight ligands appeared to be the most informative ones.

Mixing of PE with soluble bifunctional low molecular weight complex-forming ligands allows one to obtain soluble polyelectrolyte complex (PEC) and to control the hydrophobic-hydrophilic balance of

their macromolecules, their conformation and, correspondingly, their supramolecular structure by using very simple experimental designs. Thus, the use of such model systems made it possible to establish a correlation between the structure of PEC and their immunomodulating activity.

Polyelectrolyte-SAS complexes. One of the approaches to the compactization of PE macromolecules in aqueous systems is the loading of polyionic chains with lateral nonpolar radicals or surfactants (surface active substance-SAS). This can be achieved either through covalent binding of SAS to functionally important groups of PE or by their binding, i.e., the formation of mobile electrostatic bonds between SAS and PE[23,30,41,60–62]. At a definite level of equilibrium between the free energy of PE and its hydrophobicity, hydrophobization of the macromolecule leads to marked conformational changes, such as compactization of PE chains. This effect is generally manifested at relatively small molar concentrations of lateral hydrophobic radicals (benzyl;dodecyl;cetyl-) (ca. 3-7 mol %) which allows one to analyse the role of the supramolecular structure in their immunomodulating activity at a low level of changes in the chemical composition of PE. Owing to their biphilic nature SAS confer on PE a certain "adhesivess", as a result of which PE acquire the ability to form complexes with proteins in which as play the role of crosslinking agents.

Within this context the use of SAS as regulators of the PE structure in aqueous solutions in the analysis of mechanisms underlying immunomodulating effects of PE seems to be a rather perspective approach.

The dependence of the immunostimulating activity of polyconidine covalently loaded with lateral radicals of varying hydrophobicity on the length of the polymeric chain, n, show, that the presence in the PE molecule of more hydrophobic cetyl or benzyl radicals able to "operate" at much lower values of "n" makes it possible to diminish the length of the polyionic chain [12]. Most probably, the hydrophobic cetyl and benzyl radicals present in PE stimulate the ability of their constituent components to be adsorbed on the surface of immunocompetent cells. Therefore cooperative adhesion of PE on the membrane can be achieved at a smaller chain length. Stipulating that membranes of some immune cells (T- and B-lymphocytes, macrophages, monocytes, etc.) differ markedly both by their structure and composition, it becomes obvious that the observed dependence of the cooperative binding on the hydrophobic-hydrophilic balance of PE allows a direct delivery of polymeric adjuvants to cells of a definite type. This, in turn markedly increases the selectivity of PE with minimum side effects. Such a selectivity has been shown for PAA which displays a mitogenic and stimulating activity towards B-lymphocytes with no effect on T-cells.

These studies led to the chemical synthesis of poly-4-vinylpridine loaded with N-alkyl radicals of varying lengths as well as to the analysis of their immunomodulating activity in respect to PE[30]. In the range of alkyl radical R_{10} (decyl)-R_{16} (cetyl) at the degree of quaternization of 3-4 mol % the macromolecules transition from the coil to the compact structure. The results of immunological experiments show that the adjuvant properties of the PE correlated with the conformational transition from the coil to the compact structure in the polymer molucule. In the region of the unfolding of the polymeric coils, PE exhibit a high immunostimulating activity, whereas in the case of more compact structures, they do not practically "operate".

Another way of hydrophobization of macromolecules is the formation of "mobile" (salt) bands between the polymeric molecule and the low molecular weight ligand (SAS).

Electrostatic complexes with SAS were obtained by simple mixing of polycations and polybases (poly-4-vinyl-N-ethylpyridinium bromides), with the anionogenic detergents, sodium dodecyl sulfate and sodium deoxycholate as well as by mixing of polyacids (PAA, AA copolymers with maleic anhydride, N-vinylimidazole and N-vinylpyrrolidone) with the cationic detergent, cetyltrimethylammonium bromide[20].

An addition of increasing concentrations of SAS to PE under physiological conditions resulted in the tight binding of the whole bulk of SAS to the polyion with subsequent formation of stable water-soluble PEC.

Depending on the value of the SAS/PE ratio and independently of the nature of SAS and PE two types of highly ordered structures were formed. In all the systems under study these complexes differed by the degree of hydrophobization of their microenvironment. At very low values of the SAS/PE ratio ([SAS]/[PE]$\ll$1) the random binding of SAS to PE resulted in the formation of unfolding structure with a rise in the SAS concentration in the reaction medium the hydrophobic (reciprocal) interactions of alkyl radicals of SAS caused the formation of intramolecular aggregates of SAS (intramolecular micellar aggregates). In the latter case the degree of compoctization of the PEC particles was much higher than in the first case which had a more compact spatial package of the SAS molecules within the micelles.

Immunological studies were performed on mice immunized with SAS or PE-SAS- complexes at varying concentrations of SAS (PE concentration was 0.8-1.0 mg per animal; the SRBC dose was 10^7). The formation of complexes at low values of the [SAS]/[PE] ratio was accompanied by the augmentation of the immunostimulating activity of PE. The PE-SAS complexes having a more folded structure of their constituent components carrying "adhesive" hydrophobic groups of SAS in the polyionic molecule potentiated the adjuvant effect of PE. Quite a different situation was observed after immunization of mice with PE-SAS complexes having a higher content of the detergent and a more compact organization of their constituent macromolecules.

Polyelectrolyte-metal complexes (PMC). The increasing interest of investigators in PMC is due, primarily, to the crucial role of metal ions in biological processes [62–68] as well as to the unique capabilities of the PMC proper whose physica-chemical characteristics differ drastically from those of the original components, polymer and metal (Me).

The use of such models allows one to get a deeper insight into the mechanisms of action of many naturally occurring polymers and to mimick their behaviour in the presence of transient metal ions. PMC were used as a basis for the construction of a vast variety of biomedical preparations and drugs. Transient metal ions as well as other biphilic low molecular weight compounds (e.g., SAS) possess the ability to bind to neutral or weakly charged water-soluble polymers, and they confer on them adhesive properties and the capacity to form complexes with complementary surfaces (proteins, etc.). Polyanions form ion-coordinate complexes with proteins by crosslinks via Me ions. Recent studies demonstrated the important role of some Me (Cu, Zn, Fe) in the functional activity of immunocompetent cells [64–68]. Thus, iron deficiency leads to the inhibition of hemopoiesis and lymphopoiesis, to morphological charges in the thymus and to cell depletion in the T- and B-dependent zones of the spleen. Lithium ions markedly enhance the mitogenic effect of lymphocytes on lipopolysaccharides. Zinc salts injected to mice increase the immune response to SRBC, stimulate the migration and proliferation of stem cells and change their differentiation in the direction of erythropoiesis. $FeCl_3$ markedly increases the cooperation of T- and B-lymphocytes. Water-soluble and insoluble PMC were obtained by simple mixing of solutions of PE (poly-N-vinylimidazole, poly-4-vinyl-N-alkylpyridinum derivatives, polyacrylic acid, polycanidine and copolymers: acrylic acid + 2-methyl-5-vinylpyridine, acrylic acid + N- vinylpyrrolidone, N-vinylimidazole + N-vinylpyrrolidone) and Cu^{2+} ions at neutral pH and their immunomodulating activity investigated recently [13,20,38,41–43,58]. A comparison of immunological data with the results of physico-chemical analyses revealed that the nature of changes in the immunological activity of PMC depends critically on the structural and chemical transitions of binary copolymers within the composition of coordinate polyionic Cu^{2+} -containing complexes. At the initial steps of Me binding the latter predominantly coordinate the neighbouring links of the polymeric chain, as a result of which the polyion acquires a surplus positive charge, eventually resulting in the unfolding of the macromolecular coils. Within this ürange of metal concentrations the value of the stimulation index of the given immune response increases. A further rise in local concentration of Cu^{2+} leads to the compactization

of the polyion. At these ratio values the adjuvant effect of PMC diminishes and the complexes cease to "operate". At the relatively higher concentrations of metal ions they act as crosslinking agents not only towards the same macromolecule but also towards different macromolecules of PMC, in which they induce coil aggregation and, correspondingly, increase the apparent molecular mass of PMC together with the expansion of the working surface of individual molecular associates manifesting themselves as the rise of the local concentaration of essential groups of the polymer, as a result of which the immunomodulating activity of the polymer increases.

Data from physico-chemical analyses of the PE behaviour in aqueous solitions containing low molecular weight complex-forming "additives" (SAS, transient metal ions) as well as immunological data provide compelling evidence that in all the systems under study the mechanisms underlying the immunomodulating effects of PEC are universal, being coupled with the structural and chemical transitions and conformational state of PEC.

Polyelectrolyte-hapten. The first artificial antigen constructed on the basis of a synthetic PE is the electrostatic complexes of trinitrophenol (TNP) with poly-2-methyl-5-vinyl-pyridine and poly-4- vinylpyridine with molecular mass 10^5 [69]. To determine the immunogenic properties of these complexes, animals were immunized with different doses of these compounds for determination of the production of AFC and nestteforming cells (RFC) and the hemaglutinin titres to them using trinitrophenylated sheep red blood cells. It was found that PE picrate possessed marked antigenic properties. It should be noted that immunization of the animals with haptens on protein carriers (BSA, globulin, atc.) leads to the development of a marked immune response only on joint injection with adjuvants stimulating the immune reactions (e.g. complete Freund's adjuvants, etc.). The introduction of complexes ensures the development of the immune response in the absence of adjuvants. In addition, experiments on B mice showed that they are timus independent antigens.

The next case is the covalent bind dinitrophenol (DNP) with water soluble nonionogenic polymer-polyethylene oxide (PEO) [70,71]. Thymus- independent immunogenicity *in vitro* of the divalent antigen DNP-PEO has been found. Mono-and dihapten derivatives of polyethyleneglycole (PEG) with DNP and TNP have been prepared for immunologically studies. It has been shown that both DNP and TNP derivatives of PEG have a weak immunogenecity. The inclusion of the additional dimethylene spayser in the structure of the antigens increased the immunogenecity of the conjugate antigens.

Polyelectrolyte-protein (or polysaccharide). Antoher group of artificial immunogens based on nonnatural PE carriers includes polyelectrolyte complexes (or conjugates) of proteins, polypeptides and polysaccharides antigens [12,19,20,29,33,35,36–58]. It has been shown that the binding of weakly immunogenic antigens of different origin to synthetic PE intensifies the immune response of organisms to the test antigens. Complexes (or conjugates) which are fairly stable under phpysiological conditions exhibit the highest immunological activity. Such systems include complexes stabilised by cooperative electraostatic and hydrophobic interactions between fragments of the PE and antigen molecules- and conjugates, where the functional groups of the components are linked by covalent bond. These immunogens (PE: serum albumins, globulins, T-G-A-L polypeptide, α-phetoprotein, O-antigen from salmonella etc.) are able to stimulate the production of antigen-specific antibodies all by themselves but are also independent of the control of the thymus or of the Ir-gene control of the immune response. Some PEC display opposite immunological proporties: immunogenic activity of the complexes of gammaglobulin with copolymers of acrylic acid and 2-methyl-5-vinylpyridines is much lower than that of free protein antigens [46]. With lowered immunogenicity characterized the star covalent conjugates of proteins and low molecular weight (M=5 kDa) nonionogenic polyethylene glycol [72–88]. But the similar conjugates with ionogenic PE-polyacrylic acid (M=5 kDa) are exhibit the highest immunological

activity [20].

The use of PE as carriers for microbial and viral antigens (Mycobacterium tuberculosis protein antigens, the surface antigens of the influenza virus- hemagglutinin and neuraminidase or M protein, the main antigens of salmonellae-0-antigen (lipopolysaccharide of the wall of the bacteria) and H-antigen (protein of the flagella apparatus of the bacteria) whose components are firmly linked together to form a stable complex (or conjugate) made it possible not only to increase by several orders of magnitude the immune responsivness of the organism but also afforded effective immune protection which, in turn, opened the way to the construction of artificial vaccines against the yet unconquered infections [58,20]. The another way of binding oligosaccharide (or polysaccharide) antigens to PE is the copolymerization of allylglycosides of the specific oligosaccharides with hydrophylic monomers (acrylamide, etc.) [90,91]. Further developments in the field led to the formulation of general principles whose significance can hardly be overestimated, such as the principle of phenotypic correction of genetic control over the immune response as well as an entirely new principle underlying the creation of artificial immunogens. Moreover, physico-chemical criteria for the construction of artificial immunomodulators and ummunogens on the basis of PEC and some practicable approaches to the transition from artificial antigens to synthetic vaccines were laborated [10,20].

The future of synthetic polymers in clinical or veterinary medicine will reside in close anttention to biodegradability and/or control of molecular weight, particularly for the case of the polyanions or polycations used systemically.

Electrostatically bound complexes of protein antigens with nontoxic PE- copolymers N-vinylpyrrolidone-acrylic acid and N-vinylpyrrolidone-maleic anhydride, of relatively low molecular mass (20-50 kDa), have been obtained by the introduction of small amounts of complex-forming "additives" (transient metal ions and SAS), without significant alteration of the chemical structure of the carrier polymer (or antigen). These water-soluble ternary complexes of various protein antigens with nontoxic copolymers which in the presence of very small amounts of metal ions induce pronounced immunogenicity and immunological protection and manifest the properties of T-cell-independent artificial vacines [20,41,42]. Thes polycomplexes can be obtained by technologically simple procedures-in a single step by mixing solutions of the selected components. The macromolecular structure of the complexes formed does not depend significantly on the inhomogeneity of the composition and the molecular mass of the polymer chain and the nature of the protein antigen. These results open the way for the creation of universal polymenic carriers which could be used to bind a wide range of polymeric substance to various antigens by means of different metal ions, thus enabling various artificial vaccines to be created. another approach to this problem is to fabricate biodegradable polymers that can retain their biologically active structure long enough to perform their task, and then are converted to harmless by products that are readily eliminated.

One of the possible methods for the construction of biodegradable polymers is the polymerization of vinyl monomers by redox systems [92,93]. The reducing agent may be alcohol [94], polyol [95,96], ketone [97], aldehyde [98], amine [99], carboxylic acid [97,100], hydroxy acid [101]. The resulting polymer was suggested to have corresponding chain ends this method was used for the preparation of block copolymer of polyacrylonitrile and polyethylene glycol and polyoxypropylene[103–105]. Starch [106] and cellulose [107] were the most used polymers for grafting. Graft copolymer of modified starchpolyacrylamide [107] was also prepared with this method. Combine of such end group containing polymers with each others by known methods gives the possibility of creation of biodegradable products.

In recent years by redox polymerization acrylamide polymerized in the presence of hydroxy [108–110] and amino acids (phenylalanine, aspartic acid, serine, glycine, alanine, methionine), gives amino acid end-group containing polyacrylmide (Mn=20 kDa) [111–113]. Such polymers can bind from end-groups resulting

high molecular macromolecules which weakly bound to each other and easily degradable.

It has been shown that amino acid end-group containing polyacrylamides with M_w=20 kDa form a stable water-soluble polymer-metal and ternary polymer-metal-protein complexes with immunostimulating activity [114–115] which opened the possibility to the construction of artificial polymer containing vaccine for the clinical or veterinary medicine.

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