

Recognition of target colloid species by conjugates of a linear polyelectrolyte with a vector protein

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Redistribution reaction of quaternized poly-4-vinylpyridine polycations and their conjugates with α -chymotrypsin by oppositely charged latex particles is disclosed. The polycations are strongly adsorbed on the latex surface. Nevertheless, they are able to migrate between the latex species via occasional interparticle contacts. In the case of a homogeneous latex such interchange results in uniform distribution of polycations by latex particles. The distribution drastically changes, when α -chymotrypsin-polycation conjugates interact with a mixture of two latexes: one chemically modified by bovine serum albumin and the other one by specific protein inhibitor of α -chymotrypsin. In this case the interchanging polycations are finally fixed on the latex particles carrying the centres of specific binding of the enzyme vector, i.e. recognize them in the latex mixture. The obtained results are considered to mimic physico-chemical interaction and recognition of target supermolecular bio-objects by large macromolecules carrying relatively small molecular vectors.

Latex; Polyelectrolyte adsorption; Macromolecular interchange; Enzyme-polycation conjugate; Recognition phenomenon

1. INTRODUCTION

The mechanism and the driving force of recognition of a supermolecular object (such as a cell or a cell organelle, or a virus, etc.) by a large macromolecule carrying a relatively small molecular vector is of a great biological importance. In this paper the physico-chemical aspect of this mechanism was studied using the suspension of carboxylated and protein-modified latex particles as a cell-mimetic system, interacting with the high molecular mass linear polycations conjugated with the specific protein.

2. MATERIALS AND METHODS

The preparation and characterization of the CPS-latex have been described previously [1,2]. The PCEMA-latex was obtained by emulsion polymerization of chloroethylacrylate by analogy with the procedure described in [1]. The size of the latex particles was measured

by quasi-elastic laser light scattering technique using Autosizer 2C (Malvern, UK). The both latexes were found practically monodisperse with a particle diameter of 0.5 μm for CPS-latex, and 0.16 μm for PCEMA-latex. BSA, TIS, CT were obtained from Sigma (USA), BTNPE from BDH (UK).

The surface of PCEMA-latex was modified with BSA or with TIS via amination by protein amino groups using the following procedure. 0.5 ml of BSA aqueous solution, concentration 0.4 g/ml, or 0.5 ml of TIS aqueous solution, concentration 0.45 g/ml were added to 9.5 ml of latex suspension (ca 1.2% dry weight) in 0.01 M borate buffer, pH 8.5. The mixtures were incubated at 20°C for 10 h and then the latexes with the covalently bound proteins were separated from the free proteins by centrifugation, resuspended in 10 ml of 0.01 M borate buffer, pH 8.5, and washed for 0.5 h. The procedure was repeated until the absence of the proteins in solutions over the sediments was achieved, controlled by a fluorescence method according to [3].

The fluorescence method was also used to estimate the degree of covering of the latex surface by covalently bound protein molecular, α . The protein modified latex was treated by mercaptoethanol and *o*-phthalic aldehyde to produce the fluorescent tags on the attached protein globules. Then its fluorescence was measured. The number of protein molecules per one latex species was calculated using the calibration curve obtained for the corresponding tagged protein in aqueous solution. The α values were roughly estimated in assumption that the protein molecule occupies the area $S = d^2$, where d is the largest axis of the protein globule. They were found to be 0.7–0.8 and 0.9–1.0 for BSA- and TIS-modified latex, respectively.

The preparation of poly-4-vinylpyridine and its modification with ethylbromide and bromoacetic acid to prepare the polycationic copolymers have been described in [4] and [5], respectively. In this work the following copolymers were used: PEVP, $\bar{P}_w = 1000$ and PEAVP, $\bar{P}_w = 1000$. CT-PEAVP conjugate (one CT molecule per one PEAVP chain) was prepared in a hydrated reversed micelle system by the method described in [6].

Water was double-distilled and additionally purified using Milli-Q system (Millipore, USA).

The EPM of the latex particles was measured using a laser microelectrophoresis technique, Zetasizer 2C (Malvern, UK).

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Abbreviations: CPS-latex, carboxylated polystyrene latex; PCEMA-latex, polychloromethylmetacrylate latex; BSA, bovine serum albumin; TIS, trypsin inhibitor from soybean; CT, α -chymotrypsin; BTNPE, *N*-benzyloxycarbonyl-L-tyrosine-*p*-nitrophenyl ester; PEVP, (4-vinylpyridine/*N*-ethyl-4-vinylpyridinium bromide) copolymer (93:7); PEAVP, (4-vinylpyridine/*N*-ethyl-4-vinylpyridinium bromide/*N*-carboxymethyl-4-vinylpyridinium bromide) ternary copolymer (15:65:20); CT-PEAVP, conjugate of CT with PEAVP; EPM, electrophoretic mobility

The copolymer composition was determined by infrared spectroscopy [7]. The activity of CT was determined using BTNPE as a specific substrate of CT. The rate of enzymatic hydrolysis of the substrate was measured spectrophotometrically at $\lambda = 400$ nm (Hitachi by 150/20, Japan).

3. RESULTS AND DISCUSSION

Adsorption of the polycations on CPS-latex particles and the possibility of a polycation migration in the latex systems were studied using laser microelectrophoresis technique. The addition of PEVP solution in the latex particles suspension results in a drastic change of EPM of the latex species (Fig. 1). It is also seen that the points corresponding to the latex concentrations, which varied in the range of more than two orders of magnitude, are lying on the same curve. It means that the effective binding constant of the polycations on the oppositely charged latex surface is so high, that practically all polycations are bound in the studied concentration range.

In order to clarify whether strongly adsorbed PEVP chains can migrate from one latex species to another or not, the following experiments were carried out. PEVP was added to the original latex with $\text{EPM} = -4.1$ ($\mu\text{m/s}$) (V/cm) (Fig. 2a). As a result, EPM of the latex particles was changed up to 1.25 ($\mu\text{m/s}$) (V/cm) due to adsorption of the polycations (Fig. 2b). Then the polycation loaded latex was mixed with the same

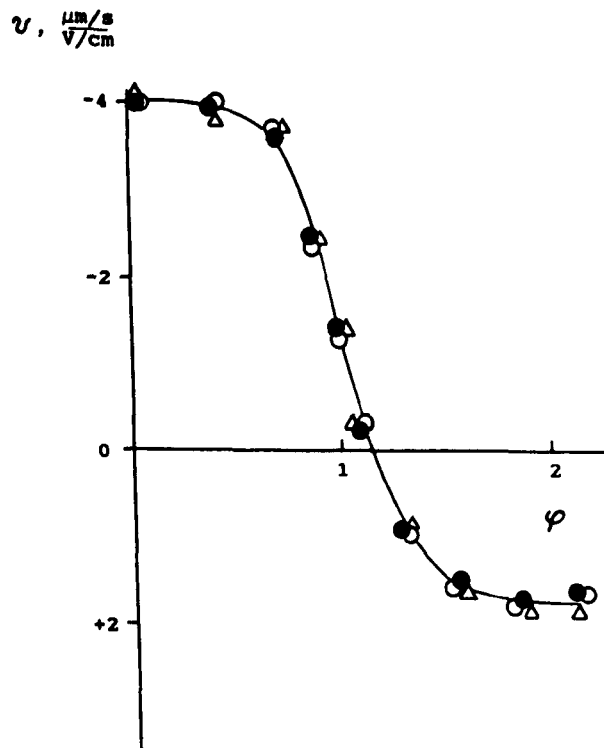


Fig. 1. The dependence of EPM of the CPS-latex particles, ν , on $\phi = [\text{surface-COOH}]/[\text{PEVP repeating units}]$, molar ratio. The latex concentration (l^{-1}): 1.8×10^{12} (\bullet); 1.8×10^{11} (\circ); 9.0×10^9 (Δ). Borate buffer 10^{-3} M; pH 9.18; 20°C .

amount of the original latex. After mixing 3 types of coexisting species characterized by different EPM values were observed (Fig. 2c). EPM values of two of them were close to those for pure and PEVP loaded latex species. The third one was characterized by an intermediate value of EPM. Gradually, the peaks corresponding to the initial components were decreased while the intermediate peak was increased. Finally, the peaks related to the original species disappeared, so that only one peak remained with $\text{EPM} = -1.6$ ($\mu\text{m/s}$) (V/cm) (Fig. 2d). The mean hydrodynamic diameter of latex particles remains equal to the initial

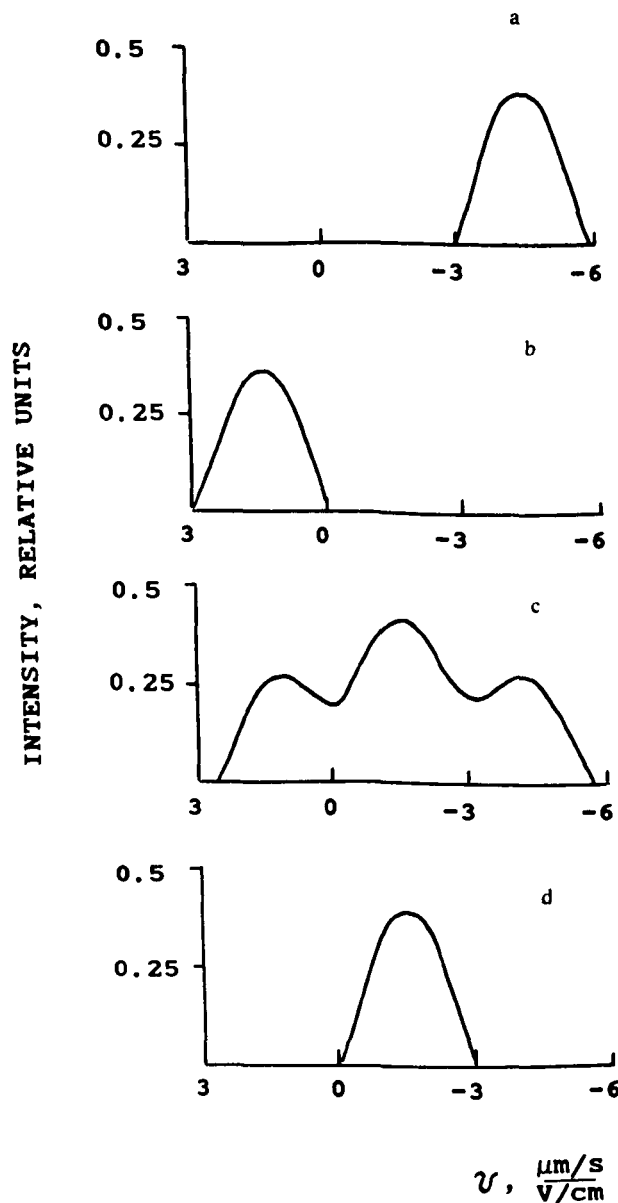


Fig. 2. Changing of EPM of the CPS-latex particles, ν , after addition of the PEVP aqueous solution; (a) original latex, (b) latex + PEVP (in equilibrium); (c, d) latex with adsorbed PEVP + original latex, 7 min (c) and 40 min (d) after mixing. Latex concentration $9 \times 10^9 \text{ l}^{-1}$, [PEVP repeating units] $= 1.5 \times 10^{-7} \text{ mol/l}$; borate buffer 10^{-3} M, pH 9.18; 20°C .

one. That corresponds to the uniform distribution of the polycations among the individual latex particles. This result should be considered as proof of redistribution of the electrostatically adsorbed polycations among the cell mimetic species, i.e. the possibility of their migrating from one species to another.

The contact mechanism of interchange seems to be more likely than the dissociation mechanism, if one takes into account the extremely low value of a dissociation constant of PEVP-latex complex. Actually, the equilibration time estimated in assumption that interchange proceeds via collisions of PEVP loaded latex particles was found to be in reasonable agreement with the experimental data. In the case of PEVP-latex dissociation mechanism, the calculated time should be much higher than that experimentally determined.

In order to mimic the target cells, we used PCEMA-latex particles carrying TIS covalently bounded to their surface (TIS-latex). The same PCEMA-latex particles, but modified by BSA (BSA-latex), were used to mimic other non-target cells. The CT-PEAVP conjugate was used as an vector-polyelectrolyte conjugate. TIS is a specific inhibitor of CT. Therefore, the TIS/CT pair can be considered as a mimicker of a receptor/vector combination.

The fact that complete binding of CT-PEAVP on the surface of both BSA- and TIS-latexes had occurred was demonstrated in the same way as was described above for the case of PEVP/CPS-latex system. The depend-

ences of EPM of polycation-latex complexes on the ratio of the number of added CT-PEAVP chains to the number of the latex species for various latex concentrations are shown in Fig. 3.

It is evident that recognition of the target species by the conjugate should result in inhibition of the enzyme. Therefore, this process can be followed simply by measuring of kinetics of cleavage of the specific CT substrate (PTNPE) added to the mimetic system.

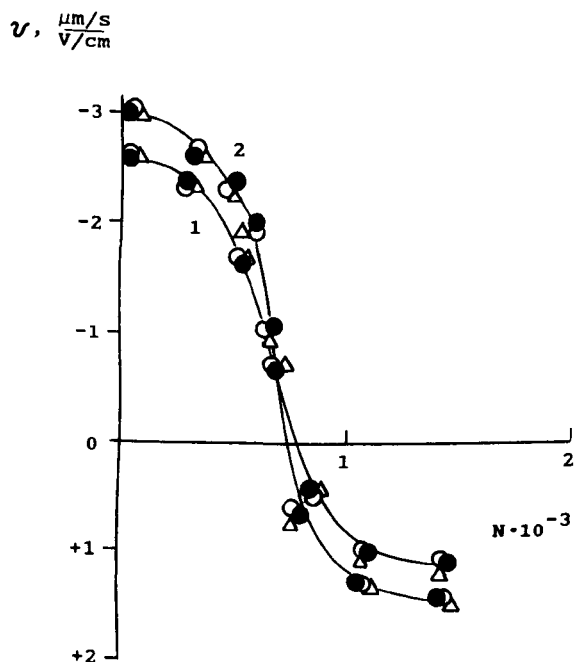


Fig. 3. The dependence of EPM of BSA-latex (1) and TIS-latex (2) particles, ν , on CT-PEAVP conjugate concentration. The latex concentration (l^{-1}): 5×10^{11} (Δ); 1.5×10^{12} (\circ); 1.5×10^{13} (\bullet). Borate buffer $10^{-3}M$; pH 9.0; $20^\circ C$. N is a ratio of conjugate molecule amount to latex particle amount in the system.

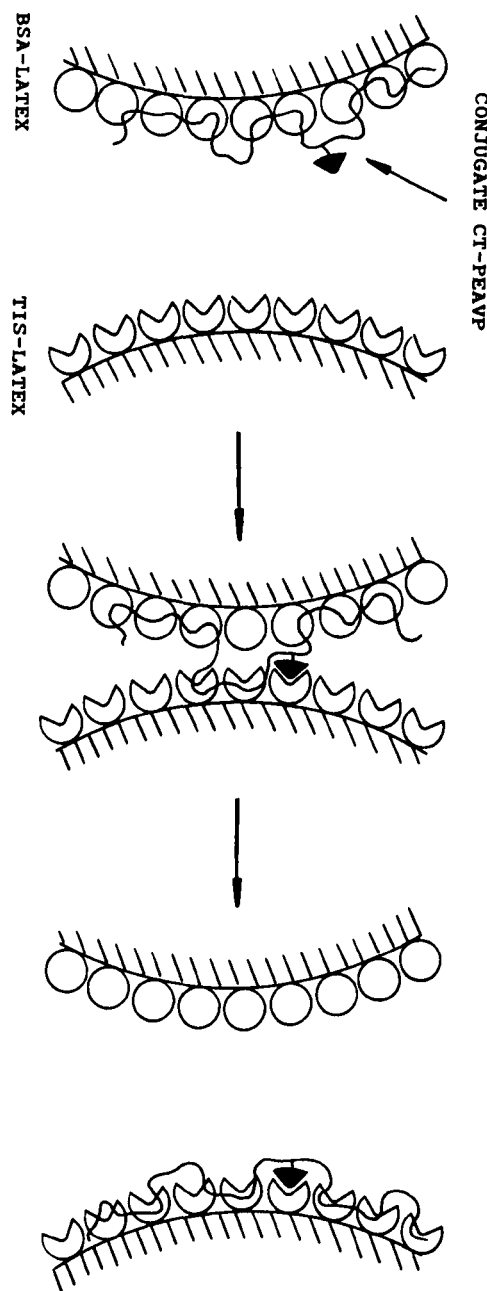


Fig. 4. The scheme of recognition of target species by macromolecules carrying molecular vectors.

The recognition test was carried out as follows. Firstly, CT-PEAVP conjugate (7.5×10^{-9} M aqueous solution in 10^{-3} M borate buffer, pH 9.0) was added to 1.5×10^{12} l $^{-1}$ BSA-latex so that about 30% of the surface of the latex species was occupied by adsorbed conjugate. It was found that adsorption of the conjugate on BSA-latex surface practically did not influence an enzymatic activity of conjugated CT. Then the system obtained was mixed with equal amount of TIS-latex, and it was found that an enzymatic activity of CT in the mixture completely disappeared after 10 min. The size of latex particles measured by quasi-elastic laser light-scattering and electron microscopy in the final equilibrated system remained equal to the original one. It shows that practically all CT-PEAVP conjugate species which were preliminarily adsorbed on the surface of BSA-latex migrated to the target TIS-latex and were fixed there due to specific interaction between TIS and CT.

Thus, the recognition phenomenon in the system, which mimics the behavior of a vector-conjugate in the vicinity of target and non-target cells, was actually observed. The roughness of the model makes the result even more significant. The most important point is that polyelectrolyte interchange is not kinetically restricted in spite of strong non-specific cooperative binding of polyelectrolyte chains with the surface of the cell mimetic species. However, if non-specific interaction is modulated by a specific one, the whole process becomes very selective, so that finally most protein vectors attached to the polyelectrolyte moiety find the corresponding receptors and fix the whole conjugate on the target species. The essence of the mechanism is as follows. At the first stage of the process a polymer conjugate was assumed to be adsorbed by any occasional cell. Then

the conjugate, thermally diffusing along a cell surface, migrates from one cell to another via permanent intercell contacts or random collisions until it reaches the target cell carrying the corresponding receptor. In this case a thermodynamic optimal contact between the cell and the conjugate is established. The process assumed can be illustrated by the following scheme (Fig. 4).

The value of such approach can be illustrated by certain examples. In order to explain the in vivo behavior of antigen-polyelectrolyte artificial immunogenes we proposed earlier [8,9] that they 'search' a proper clone of immune cells in a trial-and-error manner. Now this proposal has got a rather general physico-chemical foundation.

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