Sorption of Proteins by Slightly Cross-Linked Polyelectrolyte Hydrogels: Kinetics and Mechanism

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Sorption of proteins such as cytochrome *c*, lysozyme or protamine by slightly cross-linked poly(acrylic acid) and bovine serum albumin by slightly cross-linked poly(*N*,*N*-dimethyl-*N*-ethylaminoethyl methacrylate bromide) hydrogels in salt-free and saline aqueous solutions was studied. The polyanionic hydrogel uptakes the proteins at pH below their isoelectric points while polycationic one at pH above them. As a result highly swollen original hydrogel transforms into relatively compact cross-linked polyelectrolyte−protein complex. Sorption of proteins by slightly cross-linked polyelectrolyte hydrogels is a chemically drawn diffusion process. The driving force of the process comes from the gain in the free energy of the interpolyelectrolyte coupling reaction between the protein and oppositely charged segments of the polyelectrolyte network. Apparently the mechanism of protein uptake is similar to that earlier proposed for linear polyelectrolytes. It involves a "relay-race" transfer of protein molecules from one fragment of polyelectrolyte network to the other without radial mixing via interpolyelectrolyte exchange reaction. As a result "core−shell" constructs consisting of an outer weakly swollen complex shell and a highly swollen hydrogel core are formed at intermediate stages of the process. The rate of sorption is determined by the rate of the interpolyelectrolyte exchange reaction, that is the rate of the formation of free fragments of polyelectrolyte network (vacancies) on the inner complex−hydrogel boundary. The amount of vacancies depends on the area of this boundary. Kinetic curves of protein sorption by hydrogels in neutral salt-free solutions could not be fitted under the terms of Fickian diffusion but can be expressed in terms of the kinetic equation derived for a frontal heterogeneous reaction. At the same time kinetics of protein sorption obeys to Fick's diffusion law when the salt concentration increased.

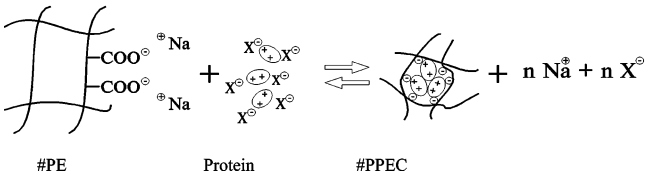
Introduction

Sorption of proteins[1−7] as well as linear polyions[8−10] by oppositely charged slightly cross-linked highly swollen polyelectrolyte hydrogels (#PE) from water solutions is driven by cooperative electrostatic binding of sorbate species to polyionic fragments of the gel network.

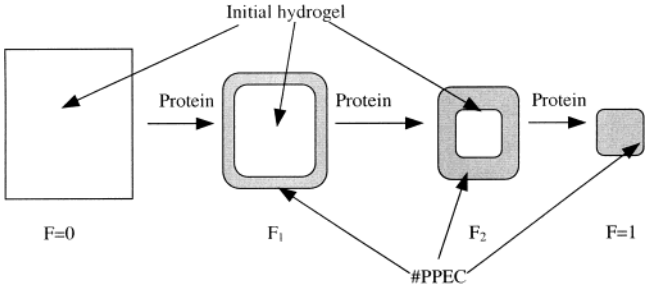
Scheme 1 represents some essential features of such an interaction demonstrated by uptake of a globular protein exhibiting a net positive charge at pH below its isoelectric point (pI), by a cross-linked anionic hydrogel

In other words the process is nothing but a cooperative interpolyelectrolyte reaction resulting in formation of salt bonds between the polymeric reagents and release of an equivalent number of simple counterions originally neutralizing the protein molecules and the hydrogel network. It is precisely the increase in entropy of released counterions that provides the gain in free energy, which drives formation of the cross-linked protein−polyelectrolyte complex (#PPEC). By and large the process represents a heterogeneous reaction propagating inward the piece of gel. Its mechanism was established in our early studies.[1,5,8,10] At intermediate stages of such a reaction, if it proceeds in the absence of an external salt, the sample remains heterogeneous, consisting of a newly formed compact #PPEC shell separated by the sharp boundary from the unreacted highly swollen original hydrogel core as represented by Scheme 2. Here $F = N_P/(N_P)_\infty$ is extent of transformation of the original hydrogel into #PPEC, N_P is the current mol amount of sorbed protein, and $(N_P)_\infty$ is the maximum mol amount of the protein which the hydrogel sample can uptake at given experimental conditions. $F = 1$ corresponds to the state when the interpolyelectrolyte reaction in Scheme 1 is completed.

SCHEME 1



SCHEME 2



The picture represented by Scheme 2 could be observed directly on the slices of the reacting hydrogel samples at intermediate degrees of conversion.

The above-mentioned previous studies on sorption of proteins by oppositely charged #PE hydrogels were of a phenomenological character. In this paper, we attempted to quantify some important regularities of such processes in particular their kinetics and effect of external salts.

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Experimental Section

The monomers, acrylic acid (AA) from Kargin Polymer Institute (Dzerzjinsk, Russian Federation) and *N*,*N*-dimethylaminoethyl methacrylate (DMAEM) from Institute of Synthetic Rubber (Yaroslavle, Russian Federation), were purified by vacuum distillation. *N*,*N*′-Dimethyl-*N*-ethylaminoethylmetacrylate bromide (DMEAEMB) was synthesized by alkylation of DMAEM with ethyl bromide (EtBr): DMAEM was added dropwise to 5-fold excess of EtBr on stirring and ice-cooling. DMEAEMB formed as a white precipitate was washed by ethyl ether and dried in a vacuum. Hydrogels of cross-linked poly(acrylic acid) (#PAA) and poly(*N*,*N*′-dimethyl-*N*-ethylaminoethylmetacrylate bromide) (#PDMEAEMB) were synthesized by free-radical copolymerization of the corresponding monomers with 1 mol % of *N*,*N*′-methylenebis(acrylamide) as a cross-linker in 10 or 20 wt % aqueous solution of AA or DMAEMA correspondingly. A redox system composed of potassium persulfate and sodium metabisulfite was used as an initiator to the extent of 0.25 wt % of the monomer content. The copolymerization was performed in sealed ampules contained 10 mL of the comonomers mixture in the absence of air during 1 day at 40 °C. Voluminous hydrogel samples were dialyzed against distilled water to purify from the residual monomers and sol fraction. Then #PAA was completely neutralized by NaOH, i.e., transformed into poly(sodium acrylate) (#PANa). The water contents measured in arbitrary chosen volume elements of the obtained hydrogel samples were found to be equal indicating a macroscopically uniform distribution of cross-links. The synthesized #PE hydrogels had in average not more than one cross-link per 100 monomer units. So the average contour length of chain fragments between two neighboring cross-links in #PE networks deliberately the exceeded diameters (*d*) of the studied protein molecules.

Cytochrome *c* from a horse heart (Sigma) according to the literature had molecular mass $M = 12\,400$ (sphere $d = 30$Å) and pI = 10.3. It is known that a molecule of cytochrome *c* contains 13 carboxyl groups and 23 amine groups.[11] Lysozyme from a chicken egg white (Sigma) had $M = 14000$ (ellipsoid $d_1 = 35$ Å, $d_2 = 45$Å) and pI = 11.0. It is known that a lysozyme molecule contains 7−10 carboxyl groups and 17−19 amine groups.[12,13] Protamine sulfate salt (isolated and kindly given to us by Dr. V. K. Rybin) was represented by a 3:1 (wt/wt) binary protein mixture from Acipenser stellatus gonads. Each component in this mixture contains 20 amine groups per one protein molecule but differs in the content of arginine and lysine units and one extra carboxyl group. The average molecular mass of protamine was $M = 4400$ (fibrillar protein), pI = 11.5.[14] Bovine serum albumin (BSA) (Sigma) had molecular mass $M = 69000$ (ellipsoid $d_1 = 150$ Å, $d_2 = 40-45$ Å)[13] and pI = 4.9.[15] One BSA molecule contains 143 carboxyl and 101 amine groups.[13]

The swelling behavior of the original gels and the products of their reaction with the proteins was studied by weighting technique and characterized by the equilibrium degree of swelling $H = (m_1 - m_2)/m_2$ in pure water. Here m_1 and m_2 are the masses of the swollen and dry samples, respectively. The *H* values for #PANa and #PDMEAEMB equaled 1000 and 600, correspondingly.

The molar concentrations of proteins in solutions were determined by UV/vis spectrophotometry using a Hitachi model 150-20 spectrophotometer (Japan) at wavelength $\lambda = 409$ nm for cytochrome *c* (the extinction coefficient $\epsilon = 110\,000$ L/(mol·cm)), at $\lambda = 281$ nm for lysozyme ($\epsilon = 35\,000$ L/(mol·cm)), at $\lambda = 230$ nm for protamine ($\epsilon = 6000$ L/(mol·cm)), and at $\lambda = 282$ nm for BSA ($\epsilon = 36\,000$ L/(mol·cm)). The pHs of the solutions were measured with a PHM 83 Autocal pH-meter (Radiometer) using glass electrode.

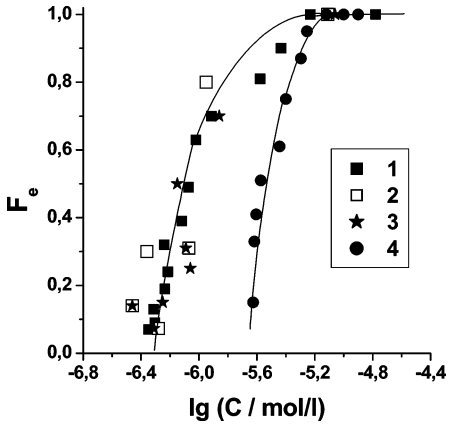


Figure 1. Sorption isotherms of cytochrome *c* by #PANa hydrogel from salt-free aqueous solution at 20 (1), 7 (2), and 37 °C (3) and 0.08 mol/L NaCl solution at 20 °C (4); pH 7. The mass of the initial gel sample was 0.6 g.

The sorption of proteins from dilute aqueous solutions by cubic (0.3−1.0 g) equilibrium-swollen samples of the neutralized #PE hydrogels was studied at pH values established so that the proteins were oppositely charged relative to the hydrogel.[1−6] In particular, sorption of cytochrome *c*, lysozyme, and protamine by #NaPA was carried out at pH < pI while sorption of BSA by #PDMEAEMB was performed at pH > pI. The hydrogel sample was immersed in the protein solution with a predetermined concentration (10^{-5} to 2×10^{-4} mol/L). The volume ratio of an environmental solution to an original gel sample varied in the range from 10 to 20. Sorption isotherms were obtained measuring a residual amount of protein in solution at equilibrium. Each point in the isotherm was controlled by 7 days incubation of the system at established constant concentration of proteins in environmental solution. The extent of sorption F_e was expressed as equilibrium value of $N_P/(N_P)_\infty$ established at protein concentration *C* in environmental solution. The used macroscopic hydrogel samples were also convenient to observe visually their evolution in course of Scheme 1.

The number of salt bonds formed between a protein molecule and #PE network was determined from the amount of the counterions released into environmental solution as the result of Scheme 1. The concentration of Na^+ ions was measured by flame photometry using a Flopho photometer and that of sulfate ions by ion-exchange chromatography using a Biotronic SIC-800 chromatograph.[1]

Results

Isotherms of cytochrome *c* sorption by #PANa hydrogel are represented in Figure 1. It is seen that the protein uptake occurs only at *C* exceeding a certain threshold value. It is also seen that even in rather dilute saline solution this value is much higher than in the absence of added salt. Moreover, the equilibrium in Scheme 1 shifts from right to left at higher salt concentrations so that preformed #PPEC dissociates releasing absorbed protein. At the same time, the data of Figure 1 show that the equilibrium in Scheme 1 does not depend on temperature, at least in the range where the protein retains its native tertiary structure indicating that sorption enthalpy is close to 0. Enthalpy values close to 0 are also typical for other earlier studied coupling reactions between oppositely charged polyelectrolytes if electrostatic factor dominates.[16]

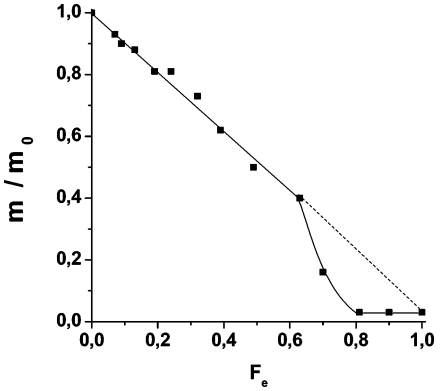


Figure 2. Dependence of the ratio of current mass of the sample (m) to the mass of the initial hydrogel (m_0), on the extent of conversion upon sorption of cytochrome *c* by #NaPA; pH 7, 20 °C.

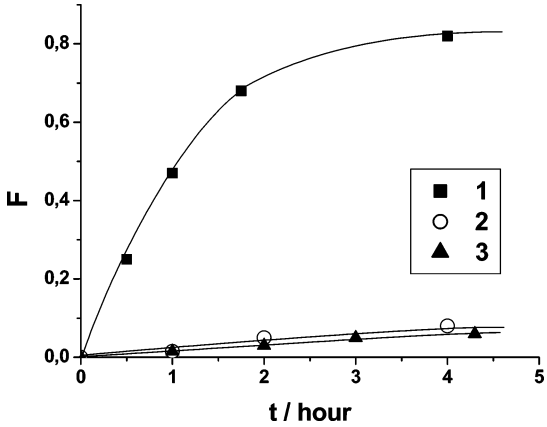


Figure 3. Kinetics of sorption of cytochrome *c* (curve 1) and lysozyme (curve 2) by #NaPA hydrogel and cytochrome *c* by #NaPA initially treated with lysozyme to reach $F = 0.2$ (curve 3) in neutral salt-free solution, 20 °C.

Figure 2 shows the relative mass, m/m_0, of the gel reacting in the salt-free solution as a function of F_e. It is seen that in a wide range of conversion of #PE hydrogel into #PPEC m/m_0 decreases in proportion to F_e. The total volume decrease at complete transformation of original hydrogels into #PPECs depends on the nature of reacting components, pH, and salt concentration in environmental solutions and reaches 2 orders of magnitude in the absence of an external salt in agreement with the data published in refs 2−4.

The data summarized in Table 1 show that the ultimate #PPEC composition at $F_e = 1$ also depends on pH and salt concentration that agrees with refs 1 and 6.

The real pictures taken from the slice of #NaPA hydrogel partly transformed into its complex with cytochrome *c* in the absence of an external salt fully correspond to Scheme 2 indicating the two phases, which coexist within the sample at $0 < F < 1$. The red colored shell and colorless core phases were separated mechanically by cutting and then analyzed. It was found that the collapsed shell actually contains all of the protein molecules sorbed at that stage. Moreover, the composition of the separated shell does not depend on its thickness and is equal to the ultimate PPEC composition at $F = 1$. Importantly the highly swollen core and the weakly swollen shell are kept separated by the sharp boundary if the sorption experiment was interrupted at the intermediate stage. Neither mixing of shell and core phases nor even erosion of the sharp core−shell interface was observed for the whole observation time (up to 2 months). However, the situation drastically changes if the partly transformed hydrogel sample is stored in 0.08 mol/L saline solution. In such case the sharp boundary between the two phases gradually disappears so that uniform distribution of the protein is established upon storing the partly transformed reaction sample for ca. 4 weeks.

The kinetic curves of sorption of cytochrome *c* and lysozyme by #NaPA from salt-free water solutions are compared in Figure 3 (curves 1 and 2). It is seen that cytochrome *c* is sorbed much faster than lysozyme. However, #NaPA hydrogel sample originally treated with lysozyme up to $F = 0.2$ to form lysozyme containing #PPEC shell then sorbs cytochrome *c* with the low rate characteristic for lysozyme (curve 3). Importantly, formation of red cytochrome *c* containing #PPEC shell proceeds without noticeable radial mixing of the proteins. The preformed opaque lysozyme-containing layer moves inward into the hydrogel sample followed by a gradually thickening red shell.

Discussion

In the absence of external salts in the environmental solution, the equilibrium in Scheme 1 always shifts from left to right upon increasing protein concentration C within the pH range where #PE and protein are oppositely charged.

The proportionality of the m/m_0 decrease to F_e increase is in accord with Scheme 2, indicating that contraction of the reacting sample actually occurs in the interface between an already formed #PPEC and still unreacted #PE hydrogel core. In other words, the decrease of the volume of the reacting sample is totally due to collapsing of the #PPEC shell (local collapse[2,4]). The linearity breaks down only at F_e exceeding 0.6−0.7, when sharp contraction is observed. Probably this break can be treated in terms of conventional collapse theories.[17]

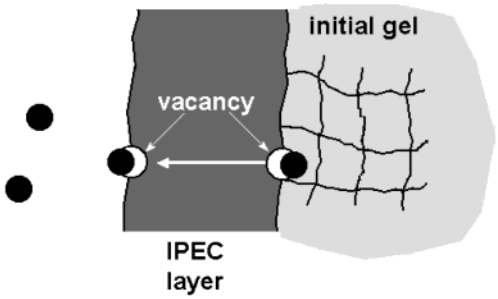
Local collapse observed at $1 < F_e < 0.7$ most probably corresponds to equilibrium states of the system. It can be understood assuming that uniform distribution of protein molecules, i.e., ionic cross-links within the hydrogel sample, would result in decrease of the total volume of the sample if it

TABLE 1. Ultimate Composition of #PPECs Prepared under Various Conditions

system	[NaCl]/M	pH	#PE units per 1 protein molecule	salt bonds per 1 protein molecule	#PE capacity (g of protein/1 g of dry #PE)
	0	3.0	70		2.5
	0	4.5	35		4.9
#NaPA−cytochrome *c*−NaCl	0	7.0	8−10	7.2 ± 0.6[b]	16.5−13.2
	0.04	7.0	19 (19[a])		6.9
	0.08	7.0	28 (27[a])		4.7
#NaPA−lysozyme	0	7.0	9−10		16.6−14.9
#NaPA−protamine	0	7.0	20	12.3 ± 1.0[c]	2.3
#PDMEAEMB−BSA	0	7.0	55−60		4.7−4.3

[a] #PPEC originally prepared in salt-free media then equilibrated against saline solution [b] From three independent measurements [c] From six independent measurements

SCHEME 3



is compared with the integrated volume of the collapsed shell and highly swollen core. Then the gain in entropy of mixing at homogeneous protein distribution may not compensate for an increase in osmotic pressure of simple counterions within the #PE partly transformed into PPEC, so that the system in the local collapse state has a lower free energy. The equilibrium degree of swelling of the original #NaPA hydrogel, i.e., the core of the partly transformed hydrogel sample in 0.08 mol/L NaCl, is ca. 6 times smaller than in salt-free water solution, while the formed PPEC is 70% higher. Therefore, uniform distribution of ionic cross-links now can be reached with a smaller additional contraction of the whole sample, so that the gain in entropy of mixing dominates. A similar transformation from local collapse to uniform distribution occurred upon sorption of cytochrome *c* from salt-free solution if #NaPA hydrogel was replaced by less swollen slightly cross-linked copolymer of sodium acrylate and acrylamide (14−20 mol % of sodium acrylate).[5]

The mechanism of transportation of protein molecules from the environmental solution in toward the reacting hydrogel sample through the already formed #PPEC shell is of primary importance. On the basis of a whole series of representative qualitative observations, we proposed earlier a general scheme apparently applicable to a wide range of slightly cross-linked hydrogels interacting with oppositely charged polyelectrolytes or micelle-forming surfactants.[8−10]

The data of Figure 3 suggest that this scheme adapted to transportation of globular protein species can be pictured as shown in Scheme 3.

The rate-determining step is formation of a vacancy at a shell−core interface as a result of thermal fluctuation, which is nothing but exchange with a protein polyion between two fragments of the hydrogel network. Such vacancies neutralized when simple counterions diffuse through the shell layer backward to the solution−shell boundary where they react with new protein molecules. Then if the numbers of vacancies born in a time unit at shell−core interface and reached solution−core boundary are equal, the protein uptake should proceed as a frontal relay race propagating a heterogeneous reaction. The above picture is qualitatively confirmed by the following experiment. Generally, consecutive cooperative electrostatic sorption of different species by polyelectrolyte hydrogels is a basis to design and construct polyfunctional multilayer materials.

Figure 4 reproduces the earlier published kinetic data on sorption of cytochrome *c*, lysozyme or protamine by #NaPA and BSA by #PDMEAEMB hydrogels from neutral salt-free aqueous solutions.[1,6] The kinetic measurements of protein sorption by macroscopic samples of oppositely charged hydrogels were always carried out in inner diffusion regime. To make sure it was found that the rate of sorption did not depend on the protein concentration in environmental solutions exceeding 10^{-5} mol/L.

Here, using these data, we attempted to give a quantitative kinetic description of the above intriguing phenomena.

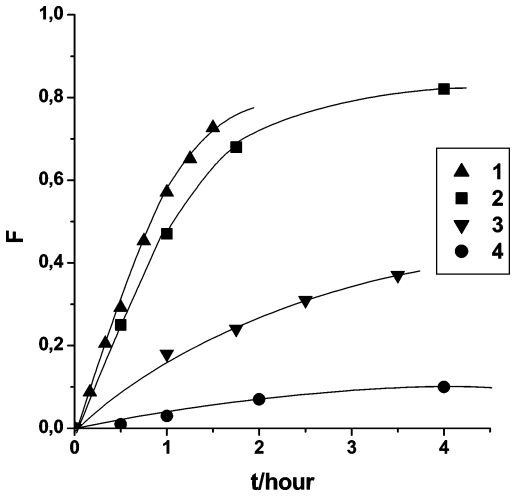


Figure 4. Kinetics of proteins uptake by #PANa hydrogel (0.7 g): (1) protamine (4.5×10^{-4} mol/L), (2) cytochrome *c* (4.2×10^{-5} mol/L), (4) lysozyme (5×10^{-5} mol/L), and (3) BSA (4.1×10^{-5} mol/L) by #PDMEAEMB hydrogel (0.9 g); from salt-free solutions at pH 7, 20 °C.

As the original cubic shape of hydrogel samples remains nearly unchanged in the course of protein sorption, the original hydrogel sample could be presented as a cube with a side l_o. Then the degree of conversion of the reacting sample is expressed as

$$F = 1 - (l/l_o)^3 \tag{1}$$

where l is the side of the cubic core still nontransformed at time t. According to Scheme 3, the rate of sorption is determined by the rate of formation of vacancies at the shell−core interface, dN/dt, if it equals the rate of their release at the solution−shell boundary. In turn dN/dt is proportional to the surface of shell−core interface S decreasing in the course of protein uptake, i.e.

$$dN/dt = kS = 6kl^2 \tag{2}$$

and

$$dN = \alpha dS = 12\alpha l dl \tag{3}$$

Solution of the system of equations given in eqs 2 and 3 gives

$$\ln\frac{l}{l_0} = -\frac{1}{2}K_s t \quad \text{or} \quad \left(\frac{l}{l_0}\right)^3 = e^{-\frac{3}{2}K_s t} \tag{4}$$

where $K_s = k/\alpha$ is the first-order rate constant of protein uptake.

Combining (1) and (4), one finally obtains

$$\ln(1 - F) = -\frac{3K_s}{2}t \tag{5}$$

The kinetic data of Figure 4 are plotted in Figure 5a in coordinates of eq 5. It is seen that the plots are linear in rather broad ranges of F. The K_s values calculated from the kinetic data are given in Table 2. Comparing the data of Tables 1 and 2 one can see that the rate constant values directly correlate neither with the protein molecular masses that contrasts to protein diffusion in neutral hydrogels, nor the numbers of salt bonds formed between a protein molecule and #PE network. In fact K_s may be strongly dependent on the disposition of the protein surface charged groups involved in formation of salt bonds. Indeed, earlier it was shown that the rate constant of polyion exchange reaction proceeded in water solution contain-

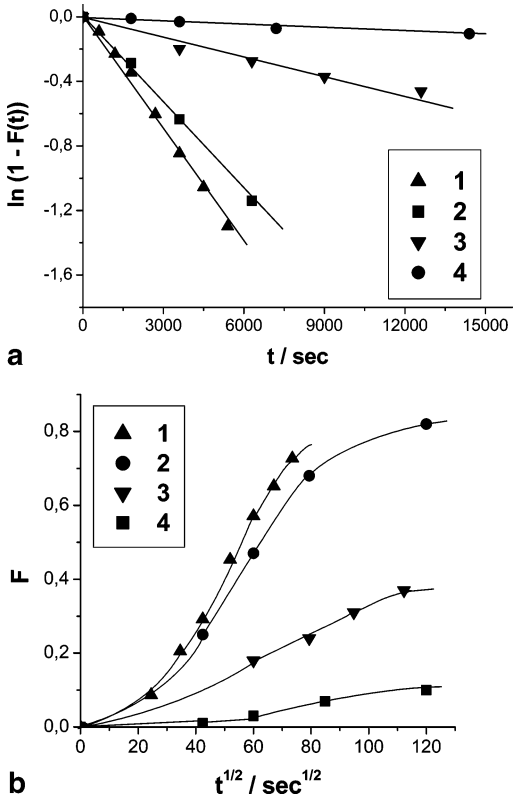


Figure 5. Data of Figure 5 plotted in eq 5 (a) and Fickian (b) coordinates.

TABLE 2. Rate Constant Values Calculated from the Kinetic Data of Figure 6a

system	M	K_s, s^{-1}
#NaPA−lysozyme	14 000	2.29×10^{-6}
#PDMEAEMB−BSA	69 000	1.23×10^{-5}
#NaPA−cytochrome *c*	12 400	5.10×10^{-5}
#NaPA−protamine	4400	6.80×10^{-5}

ing two oppositely charged linear polyelectrolytes drastically increased with decrease in charge density of one polyelectrolyte component despite its molecular mass increase.[18] The effect of surface charge distribution on interaction of proteins with linear polyelectrolytes was observed in ref 19. Clarification of protein structural characteristics actually affecting K_s is an attractive subject for future research.

Figure 5b represents the same kinetic curves of Figure 4 plotted in coordinates of the Fick diffusion equation, which obviously does not fit to the sorption experiments performed in the absence of an external salt.

However, the situation reverses if protein is sorbed by the same hydrogel but from saline solution. Figure 6a represents the kinetic curve of cytochrome *c* uptake from 0.08 mol/L NaCl.[6] Figure 6b shows that this is just the case when diffusion kinetics follows the Fick law, while eq 5 does not fit. The reason for switching from non-Fickian to Fickian kinetics most likely relates to the degree of surface contraction and time correlation between formation of vacancies at shell−core interface and their release on the surface of the reacting sample exposed to environmental solution. Both factors are minimized when proteins are sorbed from saline solutions.

Conclusions

Two different mechanisms of chemically activated sorption of proteins by oppositely charged slightly cross-linked polyelectrolyte hydrogels were established. In one case it is a frontal

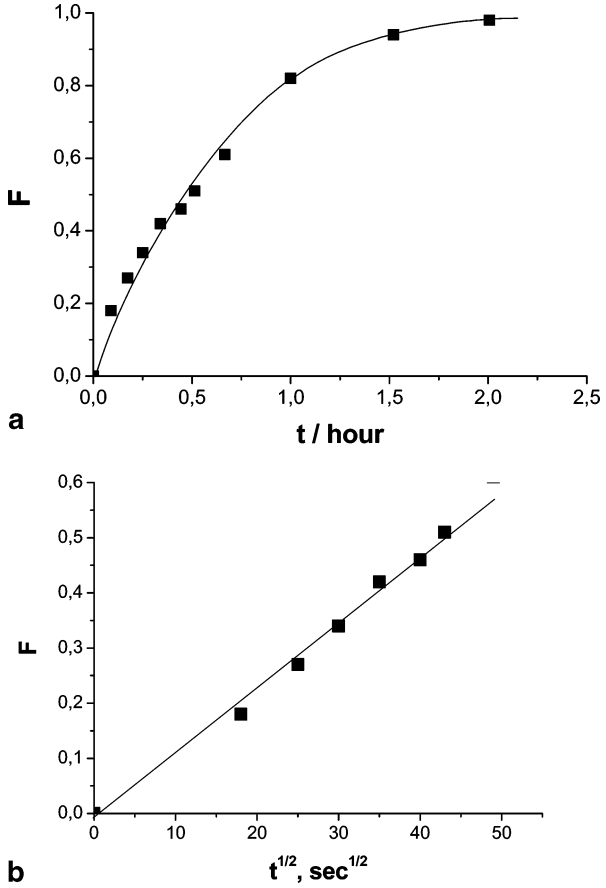


Figure 6. Kinetics of cytochrome *c* uptake by #NaPA hydrogel from 0.08 mol/L NaCl at pH 7, 20 °C (a) and the above kinetic curve plotted in Fickian coordinates (b).

heterogeneous reaction under which a sharp boundary between thickening #PPEC shell and diminishing highly swollen original hydrogel core remains until the end of the whole process. The kinetic equation was derived which permitted to quantify the above mechanism. In the second case, the kinetics of protein diffusion follows Fick law, and the process is accompanied by blurring of the shell−core interface. Both mechanisms confirmed by direct observation of the reacting macroscopic hydrogel samples and kinetic analysis. The first mechanism can be transformed to the second by replacing sorption from salt-free solution by that for saline environmental solution.

The results obtained may be applied for controlled design of environmentally sensitive protein-containing multilayer polycomplex composites.

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References and Notes

(1) Karabanova, V. B.; Rogacheva, V. B.; Zezin, A. B.; Kabanov, V. A. *Polym. Sci.* **1995**, *37A*, 1138.

(2) Skobeleva, V. B.; Kovrigin, D. I.; Rogacheva, V. B.; Zezin, À.B. *Vest. Moscow State Univ. (Russ.), Chem., Ser. 2* **1998**, *39* (3), 201.

(3) Skobeleva, V. B.; Zinchenko, A. V.; Rogacheva, V. B.; Zezin, À.B. *Vest. Moscow State Univ. (Russ.), Chem. Ser. 2* **1998**, *39* (4), 268.

(4) Skobeleva, V. B.; Rogacheva, V. B.; Zezin, A. B.; Kabanov, V. A. *Dokl. Acad. Nauk (Russ.)* **1996**, *347* (2), 207.

(5) Skobeleva, V. B.; Zinchenko, A. V.; Rogacheva, V. B.; Zezin, A. B.; Kabanov, V. A. *Polym. Sci.* **2001**, *43A*, 315.

(6) Zezin, A.; Rogacheva, V.; Skobeleva, V.; Kabanov, V. *Polym. Adv. Technol.* **2002**, *13*, 919.

(7) Nagamatsu, Shigeki; Nabeshima, Yoshikini; Hoffman, Allan S. Polym. Prepr. **1992**, *33* (2), 478.

(8) Zezin, A. B.; Rogacheva, V. B.; Kabanov, V. A. *Macromol. Symp.* **1997**, *126*, 123.

(9) Kabanov, V. A.; Zezin, A. B.; Rogacheva, V. B.; Prevish, V. A. *Macromol. Chem.* **1989**, *190*, 2211.

(10) Chupyatov, A. M.; Rogacheva, V. B.; Zezin, A. B.; Kabanov, V. A. *Polym. Sci.* **1994**, *36*, 169.

(11) Lemberg, R.; Barrett, J. *The Cytochromes*; Academic Press: London, 1973; p 122.

(12) Lehninger A. L. *Biochemistry*; Worth: New York, 1972.

(13) Tristram, G. In *Amino acid composition of proteins. Vol. 1: The proteins. Chemistry, biological activity and methods*; Neuram, H., Bailey, K., Eds.; Academic Press: New York, 1953−1954, p 244.

(14) Yulikova, E. P.; Rybin, V. K.; Silaev, A. B. *Bioorgan. Khim.* **1979**, *5*, 5.

(15) White, A.; Handler, Ph., Smith, E.; Hill, R.; Lehman, R. *Principles of Biochemistry*; McGraw-Hill, Inc.: New York, 1978; Vol. 1, p 125.

(16) Michaels, A. S. In *Encyclopedia of Polymer Science and Technology*; Wiley: New York, 1969; Vol 10, p 765.

(17) Khokhlov, A. R.; Starodubtsev, S. G.; Vasilevskaya, V. V. *Adv. Polym. Sci.* **1993**, *109*, 123.

(18) Bakeev, K. N.; Izumrudov, V. A.; Kuchanov, S. I.; Zezin, A. B.; Kabanov, V. A. *Macromolecules* **1992**, *25*, 4249.

(19) Xia, J.; Dubin, P. L.; Kim, Y.; Muhoberac, B. B.; Klimkowski, V. J. *J. Phys. Chem.* **1993**, *97*, 4528.