Adsorption of the Polypeptides on a Solid Surface. III. Behavior of Stiff Chains in a Pore

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Synopsis

A strict analytical theory has been developed describing the behavior of a model lattice polymer chain of arbitrary stiffness in a slitlike pore at polymer–adsorbent interaction energies $-\epsilon$. The thermodynamic characteristics of the system were calculated. It was shown that the transition of the macromolecule from the solution volume inside a pore occurs by the first-order phase transition with evolution of latent heat of adsorption. The transition point $-\epsilon = -\epsilon_c$ is determined by the chain stiffness and is independent of the pore width D. It is shown that in the precritical range, $-\epsilon < -\epsilon_c$, the free energy ΔF of the macromolecules in the pores is adequately described by the universal dependence $\Delta F = \Delta F(D^*/A)$, where D^* is some effective pore width depending on the value of $-\epsilon$, and A is the length of the Kuhn segment. At high attraction energies, $-\epsilon \gg -\epsilon_c$, the macromolecules are bonded to the pore walls by a great number of units and their free energy depends only on $-\epsilon$ and the chain stiffness, $\Delta F = \Delta F(A, \epsilon)$. Close to the critical energy $-\epsilon \simeq -\epsilon_c$ (transition range), ΔF is determined by both the stiffness of the macromolecule and the pore width D: $\Delta F \sim A^2 D^{-1}$ for fairly high values of A and D. The possibilities of using porous media as protein stabilizers are discussed, and the value of the stabilizing effect depending on the chain stiffness is estimated.

In previous papers[1,2] we considered the adsorption of helix-forming polypeptides on a planar solid surface. It was shown that the adsorption relationships for these molecules are determined by the thermodynamic chain stiffness (due to the presence of helical sequences in the chain), on the one hand, and by possible selectivity of the interaction of helical and random-coil units with the adsorbent surface, on the other.

It has been shown that if the bonding of helical units is energetically favorable, the increase in thermodynamic stiffness facilitates and sharpens the transition of the macromolecule into the adsorbed state. Simultaneously, drastic cooperative changes in the secondary structure take place.[2]

In this paper it will be assumed that the adsorbent is a slitlike pore formed by two parallel adsorbing planes at a distance D apart. Only the case of nonselective interaction, when the adsorption energies of helical and random-coil units coincide, $-\epsilon^h = -\epsilon^c = -\epsilon$, will be considered here.

It has been shown[2] that in this case, the cooperativity of structure affects adsorption mainly via the stiffness of the polymer chain. Hence, for simplicity, only noncooperative chains will be studied and the effect of ther-

modynamic stiffness on adsorption will be analyzed (see Ref. 1). The adsorption of flexible chains in a pore has already been considered.[3]

MODEL AND METHOD

The models and calculation technique used have already been described in detail.[1,2] Here only the recurrent equations describing the conformations of a five-choice model of the polymer chain (model I in Ref. 1) in a slitlike pore of width $(D + 1)$ (similar equations (1_I) and (2_I) in Ref. 1) are reported:

$$P_n{}^{(1)}(z) = gP_{n-1}{}^{(1)}(z + 1) + P_{n-1}{}^{(3)}(z + 1)$$
$$P_n{}^{(2)}(z) = gP_{n-1}{}^{(2)}(z - 1) + P_{n-1}{}^{(3)}(z - 1)$$
$$P_n{}^{(3)}(z) = 4P_{n-1}{}^{(1)}(z) + 4P_{n-1}{}^{(2)}(z) + (g + 2)P_{n-1}{}^{(3)}(z), \tag{1}$$
$$z \geqslant 1$$

Boundary conditions are given by

$$\left.\begin{array}{l} P_n{}^{(1)}(0) = e^{-\epsilon}[gP_{n-1}{}^{(1)}(1) + P_{n-1}{}^{(3)}(1)] \\[4pt] P_n{}^{(2)}(1) = P_{n-1}{}^{(3)}(0) \\[4pt] P_n{}^{(2)}(0) = 0 \\[4pt] P_n{}^{(3)}(0) = e^{-\epsilon}[4P_{n-1}{}^{(1)}(0) + (g + 2)\,P_{n-1}{}^{(3)}(0)] \end{array}\right\} \tag{2}$$

$$\left.\begin{array}{l} P_n{}^{(1)}(D) = 0 \\[4pt] P_n{}^{(1)}(D - 1) = P_{n-1}{}^{(3)}(D) \\[4pt] P_n{}^{(2)}(D) = e^{-\epsilon}[gP_{n-1}{}^{(2)}(D - 1) + P_{n-1}{}^{(3)}(D - 1)] \\[4pt] P_n{}^{(3)}(D) = e^{-\epsilon}[4P_{n-1}{}^{(2)}(D) + (g + 2)\,P_{n-1}{}^{(3)}(D)] \end{array}\right\}$$

Here $P_n{}^{(1)}(z)$, $P_n{}^{(2)}(z)$, and $P_n{}^{(3)}(z)$ are the nonnormalized probabilities of the arrival of the nth unit of the macromolecule into a layer z from above, from below, and from a side; g is the statistical weight of a *trans*-isomer (step forward); the statistical weight of the *gauche*-isomer is taken to be unity; and $-\epsilon$ is the adsorption energy acquired by a chain unit when it contacts the adsorbing surface. Similar equations can be written for other models, see Ref. 1.

Adsorption conditions described by Eq. (2) correspond to model I in Ref. 1. All energetic values are expressed in kT units, and the pore width D is expressed in lattice constants.

By applying the mathematical formalism developed in Ref. 1, an equation was derived [analogous to Eq. (15) in Ref. 1] that gives the equilibrium chemical potential of units in the system $\mu(\epsilon,D,g)$ (coinciding with the free energy per unit) for an infinitely long macromolecule in a pore. Omitting intermediate calculations, we write only the final form of this equation:

$$\text{Det}[W - E] = 0 \tag{3}$$

Here E is the unit matrix (4×4), and the matrix elements $W_{ij} = W_{ij}(\epsilon, D, g)$ are as follows:

$$W_{11} = W_{33} = q(\beta J_0 - \gamma J_1) + \frac{g}{\lambda}(\gamma J_0 - \beta J_1)$$

$$W_{12} = W_{34} = \frac{q}{\lambda}\left(-\frac{g}{\lambda}J_0 + J_1\right) + \frac{1}{\lambda}(\gamma J_0 - \beta J_1)$$

$$W_{13} = W_{31} = q\frac{4}{\lambda^2}J_{D-1} - \frac{4g}{\lambda^3}J_D$$

$$W_{14} = W_{32} = \frac{q}{\lambda}\left(J_{D-1} - \frac{g}{\lambda}J_D\right) - \frac{4}{\lambda^3}J_D$$

$$W_{21} = W_{43} = \frac{4q}{\lambda}\left(J_0 - \frac{g}{\lambda}J_1\right) + \frac{4g}{\lambda^2}\left(\frac{g}{\lambda}J_0 - J_1\right)$$

$$W_{22} = W_{44} = q\left[\left(1 + \frac{g^2}{\lambda^2}\right)J_0 - \frac{2g}{\lambda}J_1\right] + \frac{4}{\lambda^2}\left(\frac{g}{\lambda}J_0 - J_1\right)$$

$$W_{23} = W_{41} = \frac{4q}{\lambda}\left(J_D - \frac{g}{\lambda}J_{D-1}\right) + \frac{4g}{\lambda^2}\left(\frac{g}{\lambda}J_D - J_{D-1}\right)$$

$$W_{24} = W_{42} = q\left[-\frac{g}{\lambda}J_{D-1} + \left(1 + \frac{g^2}{\lambda^2}\right)J_D - \frac{g}{\lambda}J_{D+1}\right]$$
$$+ \frac{4}{\lambda^2}\left(\frac{g}{\lambda}J_D - J_{D+1}\right) \quad (4)$$

where

$$\beta = \frac{1}{\lambda}(\lambda - g - 2), \qquad \gamma = \frac{1}{\lambda^2}[4 + g(\lambda - g - 2)]$$

$$a = \frac{1}{\lambda^3}[(\lambda^2 + g^2)(\lambda - g - 2) + 8g]$$

$$f = \frac{2}{\lambda^2}[g(\lambda - g - 2) + 4]$$

$$c = a/f, \qquad q = 1 - e^\epsilon$$

$$J_k = \frac{1}{2\pi}\int_{-\pi}^{\pi}\frac{e^{-ik\alpha}\,d\alpha}{a + f\cos\alpha} = \begin{cases} -\dfrac{\sin[k\arccos c]}{\sqrt{f^2 - a^2}}, & |c| < 1 \\[2ex] \dfrac{1}{\sqrt{a^2 - f^2}}\left(\dfrac{1}{c + \sqrt{c^2 - 1}}\right)^k, & \\[1ex] & |c| > 1 \quad (5) \end{cases}$$

The largest root λ_1 of Eq. (3) gives

$$\mu_1(\epsilon, D, g) = -\ln\lambda_1 \quad (6)$$

When the macromolecule can leave the pore and pass into the solution,

where its free energy is $\mu_2 = -\ln \lambda_2 = -\ln(4 + g)$, the state of an infinitely long polymer chain is determined by the maximum of the two roots λ_1 and λ_2: at $\lambda_1 > \lambda_2$, it is inside the adsorbent pore; and at $\lambda_1 < \lambda_2$, it floats in solution.

When the macromolecule cannot leave the pore for some reason (e.g., if one of its ends is chemically crosslinked with the adsorbent), the largest root λ_1 determines the state of the chain at all values of the parameters. In particular, the degree of its adsorption θ, i.e., the fraction of units coming into contact with the pore surface, is given by

$$\theta = -\frac{\partial \ln \lambda_1}{\partial \epsilon} \tag{7}$$

and the adsorption energy (per unit) is given by

$$-E = -\epsilon\theta = \frac{\partial \ln \lambda_1}{\partial \ln \epsilon} \tag{8}$$

(under the condition that $-\epsilon$ is of a purely energetic nature)

RESULTS AND DISCUSSION

For convenience, the stiffness parameter g will be replaced by the length of the Kuhn segment A. For the five-choice scl model,

$$A = l_0 \frac{1 + \langle \cos \varphi \rangle}{1 - \langle \cos \varphi \rangle} = l_0\left(1 + \frac{g}{2}\right) \tag{9a}$$

and for the six-choice scl model,

$$A = l_0(2/3 + g/3) \tag{9b}$$

Here, φ is the angle formed by two successive steps on the lattice and l_0 is the lattice constant, taken to be unity.

Free Energy of Units

Figure 1 shows the dependence of free energy $\Delta F = \mu_1 - \mu_2$, calculated according to Eqs. (3)–(6), on the adsorption energy $-\epsilon$ for chains of various stiffness located in a pore of width D. The state of a random coil in solution is taken for the zero state. The free energy ΔF is an important characteristic of the system that can be measured experimentally. It determines the probability of the polymer chain's arriving from the solution to an adsorbent pore,

$$w \sim \exp(-\Delta FN)/[1 + \exp(-\Delta FN)]$$

(where N is the number of units in the macromolecule and the solution volume is taken to be equal to the pore volume).

In further discussion, our main attention will be devoted to the analysis of the dependence of ΔF on the parameters of the system: adsorption
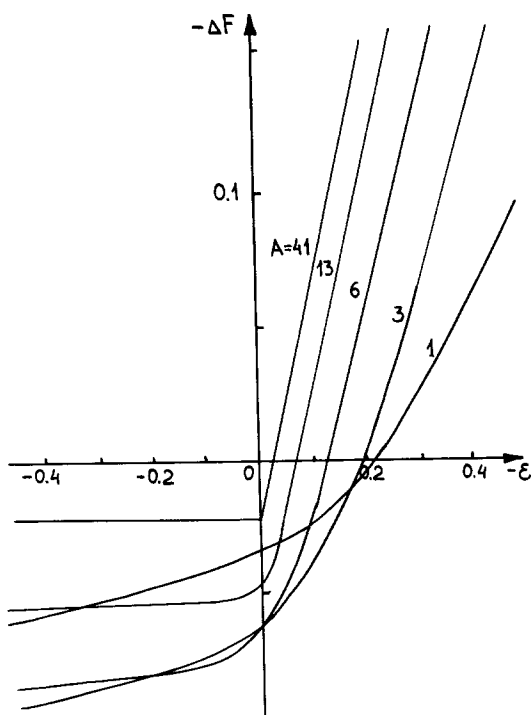
Fig. 1. Change in the free energy $-\Delta F$ (per unit) vs adsorption energy $-\epsilon$ for chains of various stiffness (values indicated) for a pore of width $D = 6$.

energy $-\epsilon$, pore width D, and chain stiffness A. As can be seen from Fig. 1, there is some value of the adsorption energy (called the critical value) at which $-\Delta F(-\epsilon_c) = 0$ and $-\epsilon_c = -\epsilon_c(A)$. If the macromolecule cannot leave the adsorbent pore, the critical energy $-\epsilon_c$ is not a special point, and all the thermodynamic and geometrical chain characteristics are continuous and have continuous derivatives at this point.

The situation is different if a long macromolecule ($N \to \infty$) can leave the pore and pass into solution. Then, the free energy of the system consists of two branches: $-\Delta F = 0$ at $-\epsilon < -\epsilon_c$ and $-\Delta F = -\Delta F(\epsilon)$ at $-\epsilon > -\epsilon_c$.

The critical energy $-\epsilon_c$ is the point of the first-order phase transition for infinitely long chains, as has been shown earlier for flexible chains.[4] Figure 1 shows that the character of the phase transition is independent of chain stiffness which determines the values of the critical energy $-\epsilon_c = -\epsilon_c(A)$ and free energy $-\Delta F$ only.

According to Ref. 5, the critical energy $-\epsilon_c(A)$ is determined only by the partition functions of chain units in solution, z_v, and on the surface, z_s. Hence, the value of $-\epsilon_c$ is given by Eq. (19$_1$) in Ref. 1 (see also Ref. 5).

For the five-choice lattice considered, applying Eq. (9) gives

$$-\epsilon_c = -\ln \frac{A + \sqrt{A^2 + 4}}{2(A + 1)} \tag{10}$$

The dependence of the critical energy on chain stiffness permits us to use adsorption methods for separating macromolecules according to stiffness: at a fixed adsorption energy $-\epsilon$, flexible chains, with $-\epsilon_c > -\epsilon$, remain in solution, whereas stiffer chains, with $-\epsilon_c < -\epsilon$, are adsorbed in pores or on the adsorbent surface (see Ref. 1 for further details).

Now the dependence $-\Delta F(\epsilon)$ will be considered for macromolecules located in pores at any value of $-\epsilon$ (Fig. 1). Tentatively, the entire range of changes in ϵ will be divided into three parts: (a) precritical range ($-\epsilon < -\epsilon_c$), (b) range of strong adsorption ($-\epsilon \gg -\epsilon_c$), and (c) transition range ($-\epsilon \simeq -\epsilon_c$). The relationships of adsorption in each range will then be analyzed. The characteristics of the transition of the macromolecule from solution into a pore will be considered in the analysis of the transition range.

Precritical Range ($-\epsilon < -\epsilon_c$)

First, the simplest case will be considered, namely, when all chain units are energetically indifferent to the adsorbent surface, $-\epsilon = 0$.

In this case, Eq. (3) gives the following for both models (five- and six-choice models on simple cubic lattice):

$$T_D(z_i) + \alpha_i\, T_{D-1}(z_i) = \beta_i \tag{11}$$

Here, $T_D(z) = (1 - z^2)^{-1/2} \sin(D \arccos z)$ is the Chebyshev polynomial of the second order, and the values α_i, β_i, z_i are related to λ and A. The character of this relationship is determined by the type of model ($i = \mathrm{I}$, II):

$$\alpha_i = \begin{cases} 0 & \text{(I)} \\ \dfrac{1}{\lambda}\left[1 + \dfrac{1}{4}(3A - 2)(\lambda - 3A - 1)\right] & \text{(II)} \end{cases}$$

$$\beta_i = \begin{cases} 1 + \dfrac{1}{2}(A - 1)(\lambda - 2A) & \text{(I)} \\ 1 + \dfrac{1}{4}(3A - 2)(\lambda - 3A - 1) & \text{(II)} \end{cases}$$

$$z_i = \begin{cases} \dfrac{[\lambda^2 + [4(A - 1)^2](\lambda - 2A) + 16(A - 1)}{\lambda[8 + 4(A - 1)(\lambda - 2A)]} & \text{(I)} \\ \dfrac{[\lambda^2 + [(3A - 2)^2 - 1](\lambda - 3A - 1) + 24(A - 1)}{\lambda[8 + 2(3A - 2)(\lambda - 3A - 1)]} & \text{(II)} \end{cases}$$

One can easily solve Eq. (11) for the special case of flexible chains where $A = 1$, corresponding to the random walk on the simple cubic lattice (four-choice walk in model I at $g = 0$ and six-choice walk in model II at $g = 1$). Equation (11) gives

$$\lambda_1 = \begin{cases} 1 + \sqrt{1 + 8 \cos[\pi/(D + 1)]} & \text{(I)} \\ 4 + 2 \cos[\pi/(D + 1)] & \text{(II)} \end{cases} \tag{12}$$

The second expression in Eq. (12) was obtained earlier by Rubin and Di-Marzio.[3]

In the general case, $A \neq 1$, the rigorous analytical solution of Eq. (11), valid for the whole range of parameters A and D, was not obtained. So, the approximate analytical solutions for two limiting cases $A \gg D$ and $A \ll D$ were found, and the numerical calculations for Eq. (11) in the whole range of parameters were carried out.

Let us consider first the case $D \gg A$. Evidently,

$$\lambda_1 = \lambda_2 \, e^{-\Delta F} \simeq \lambda_2 \, (1 - \Delta F) \tag{13}$$

provided ΔF is small ($\Delta F \ll 1$). Here λ_2 is the partition function of the chain unit in solution: $\lambda_2 = 4 + g = 2A + 2$ and $\lambda_2 = 5 + g = 3A + 2$ for models I and II, respectively. In the case $D \gg A$, the pore walls restrict the conformations of the coil as a whole, while the conformations of segments are slightly affected. Hence, $A\Delta F$ is small as well, where $A\Delta F$ is the change in free energy of the segment in the pore compared to that in solution. Using two conditions, $\Delta F \ll 1$, $A\Delta F \ll 1$, one obtains the following from Eq. (11):

$$\Delta F \simeq \frac{\pi^2}{6A} \frac{1}{(D/A + 1)^2} \tag{14}$$

The latter expression turns out to be valid for both models I and II. Moreover, the numerical coefficient $\pi^2/6$ in Eq. (14) coincides with that for the continuous model of polymer chains considered by Casassa in Ref. 6.

So, when the pore width D greatly exceeds the length of a stiff chain segment A, $D \gg A$, the change of free energy for segment $\Delta F A$ turns out to be the universal function of the D/A ratio, independent of the mechanism of flexibility. Note that at $-\epsilon = 0$ the values of A and D are the only linear scales in the system under consideration. The transition from $A = 1$ to $A > 1$ corresponds to the replacement of a regular zigzag to the more realistic statistical zigzag.[7]

The change in free energy per chain is given by

$$N\Delta F \approx \frac{\pi^2}{6} \frac{NA}{D^2} \simeq \pi^2 \frac{\overline{R^2}}{D^2} \tag{15}$$

Here, $\overline{R^2} = NA/6$ is the mean-square radius of the gyration of the chain. The $\overline{R^2}/D^2$ ratio characterizes the free energy of deformation of a Gaussian coil and is widely used in estimations of $N\Delta F$ in scaling theories.[8] The calculations of the present paper provide an opportunity of finding the numerical coefficient, which is approximately equal to 10.

Now consider the second case, $A \gg D \gg 1$. Using the conditions $\Delta F \ll 1$, $\Delta F A \sim 1$, one has the following from Eq. (11):

$$\Delta F \approx \frac{\gamma_i}{A} \left(1 - \gamma_i \frac{D}{A} \right) \tag{16}$$

where $\gamma_I = 1$, $\gamma_{II} = 2/3$. For this range of parameters, the pore walls disturb both the intracoil and intrasegmental distributions of the conformations. Thus, the dependence of $A\Delta F$ (or $N\Delta F$) on the D/A ratio loses its universality and is affected by the choice of the model, i.e., the mechanism of chain flexibility.

Figure 2(a) shows the dependence of $A\Delta F$ vs the D/A ratio for the five-choice model ($i =$ I) calculated according to rigorous formulas, Eqs. (3)–(6) (points), and approximate ones, Eqs. (14) and (16) (solid lines). As is seen from Fig. 2(a), the approximate formula (14) is valid in the whole range $D/A \gtrsim 1$ and can be used for quantitative estimations in experiments, provided the condition $A < D$ is fulfilled as a rule.

Figure 2(b) shows the dependence of ΔF vs A for various pore widths D. The nonmonotonic behavior of $\Delta F(A)$ (its maximal value is obtained at $A \simeq D$) can be explained by the following considerations. When $D/A \gg 1$, the increase in A is equivalent to the decrease in the pore width D, and ΔF increases. In the range of $D/A \ll 1$, $\Delta F \sim 1/A$ [see Eq. (16)]; so ΔF decreases with increasing A.

The approximate Eq. (14) was obtained for the case $-\epsilon = 0$. It was found, however, that this equation adequately describes the entire precritical energy range $-\infty < -\epsilon < -\epsilon_c$ if the real pore width D is replaced by some effective width

$$D^* = D - 2 + 2\frac{1 - e^{\epsilon_c}}{e^{\epsilon} - e^{\epsilon_e}} \approx D + \frac{2\gamma}{1 - \gamma} \qquad (17)$$

as has been done for flexible chains.[9] Here, $\gamma = \epsilon/\epsilon_c$, and the latter equation is approximately valid at $|\epsilon|$, $|\epsilon_c| < 1$. Then one has

$$\Delta F \simeq \frac{\pi^2}{6A}\frac{1}{(D^*/A + 1)^2} \qquad (18)$$

Figure 3 shows the dependence of the free energy of a segment ΔFA on the D^*/A ratio. The values of free energy for various chain stiffnesses, pore widths, and adsorption energies calculated according to the precise Eqs. (3)–(6) are plotted for this dependence (see legend to Fig. 3). It is clear that the approximate Eq. (18) universally describes the behavior of ΔF over the entire precritical range $-\infty < -\epsilon < -\epsilon_c$.

The deviations are observed only for high values of stiffness $A > D$ and also in the immediate vicinity of the critical point $-\epsilon \simeq -\epsilon_{c-0}$, where Eqs. (17) and (18) become inapplicable. [As can be seen from Eq. (17), approximation (18) yields $\partial\Delta F/\partial\epsilon \to 0$ at $-\epsilon \to -\epsilon_c$—which is wrong; see Fig. 1.]

High Adsorption Range ($-\epsilon \gg -\epsilon_c$)

At high energies of attraction, $-\epsilon \gg -\epsilon_c$, the macromolecule is bonded to the adsorbent surface by virtually all of its units and is a two-dimensional Gaussian coil. Evidently in this case, the free energy of the monomer unit is described by the equation
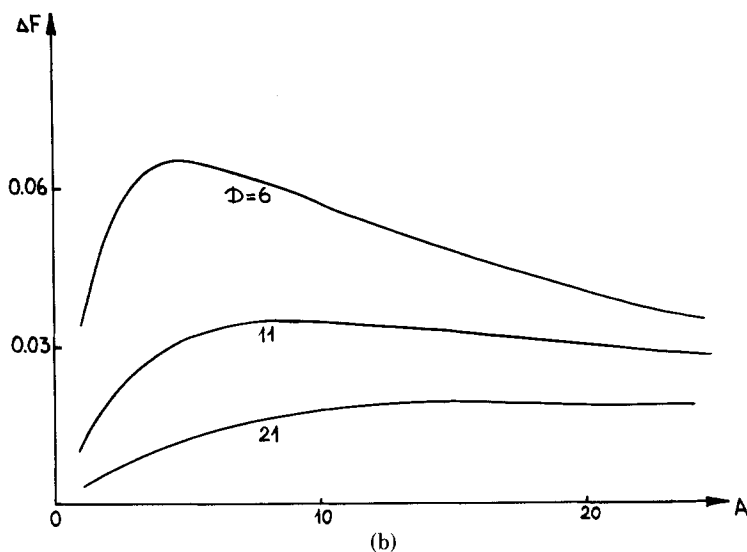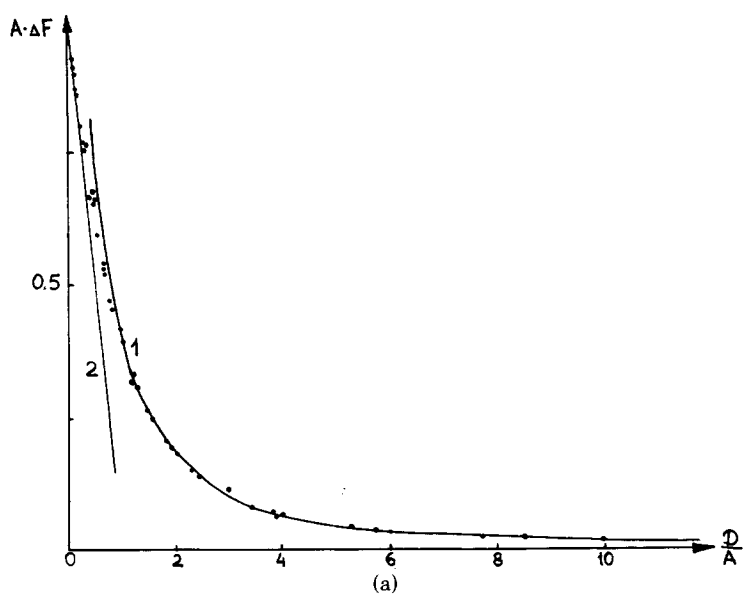
Fig. 2. Change in the free energy (a) per segment, $\Delta F A$ vs D/A ratio, and (b) per unit, ΔF vs the length of Kuhn segment A for various pore widths D (values indicated), $-\epsilon = 0$. Points in (a) correspond to the rigorous results obtained according to the Eqs. (3)–(6); the parameters A and D were varied in the following ranges: $A = 4$–76, $D = 4$–51. Solid lines in (a) correspond to the approximate equations (14) (1) and (16) (2).

$$-\Delta F \simeq -\epsilon + \ln(z_s/z_v) \qquad (19)$$

where z_s and z_v are the partition functions of the monomer units on the surface and in solution.

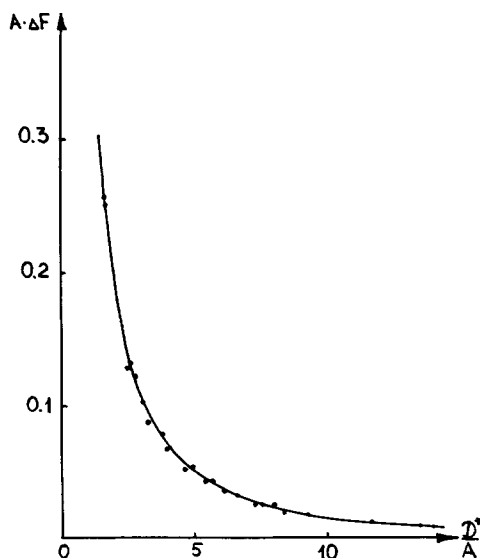In the five-choice model considered here, $z_s = 2 + g = 2A$, $z_v = 4 + g = 2 + 2A$ and the free energy

Fig. 3. Change in the free energy $A \Delta F$ (per segment) vs D^*/A ratio in the precritical range $-\epsilon < -\epsilon_c$ for various adsorption energies $-\epsilon$, pore widths $D = 6$–51, and stiffnesses $A = 1$–9. Solid line: approximate calculation according to Eq. (18).

$$-\Delta F \simeq -\epsilon + \ln[A/(A + 1)] \tag{20}$$

is independent of the pore width D and is determined only by the length of the Kuhn segment A and the adsorption energy $-\epsilon$. The value of D affects only the rate of approaching the asymptotic curve determined by Eq. (20) (see Fig. 4): the wider the pore and the stiffer the chain, the lower the values of $-\epsilon$ at which the free energy attains its asymptotic values (solid lines in Fig. 4).

Transition Range $(-\epsilon \gtrsim -\epsilon_c)$

First, the thermodynamic characteristics of the macromolecule at the critical point $-\epsilon = -\epsilon_c$ will be considered; in particular, we examine the degree of adsorption θ_c, since in the transition range

$$-\Delta F \simeq -\theta_c(\epsilon - \epsilon_c) \tag{21}$$

According to the concepts developed in Ref. 5, at the critical point, the units of the macromolecule uniformly fill the internal pore volume (except for the adsorbing surfaces), and each layer inside the pore contains $\sim N(1 - \theta_c)/D$ units. Then the critical degree of adsorption θ_c can be determined from the following considerations: θ_c is proportional to the number of chain arrivals from a layer adjacent to the adsorption layer and to the average length of the adsorbed sequence $(n_s)_c$ under critical conditions, i.e.,

$$\theta_c/(1 - \theta_c) \sim (n_s)_c/D \tag{22}$$
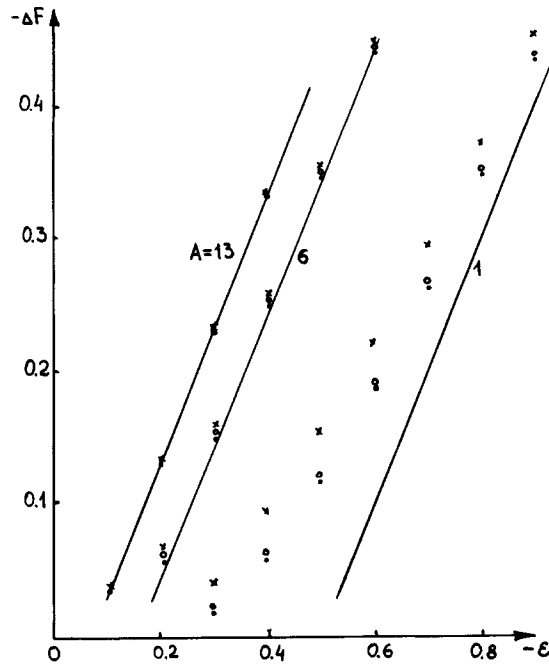
The following is used to calculate $(n_s)_c$:

Fig. 4. Free energy $-\Delta F$ vs adsorption energy $-\epsilon$ in the range of strong adsorption $-\epsilon \gg -\epsilon_c$ for various pore widths $D = 4$ ($\times$), 11 ($\bigcirc$), and 21 ($\bullet$), and various stiffness values (as indicated). Solid lines: approximate calculation according to Eq. (20).

$$(n_s)_c = -\frac{\partial \ln \Xi_s(A,\epsilon,\lambda)}{\partial \epsilon} \tag{23}$$

where $\Xi_s(A,\epsilon,\lambda)$ is the grand partition function of the adsorbed state (compare Ref. 5):

$$\Xi_s = \sum_{n=2}^{\infty} 4\lambda^{-n} e^{-\epsilon n} (2+g)^{n-2} = \frac{4e^{-2\epsilon}}{\lambda^2 - \lambda e^{-\epsilon}(2+g)} \tag{24}$$

Under critical conditions $(-\epsilon = -\epsilon_c)$, $\lambda_1 = \lambda_2 = 4 + g$ and

$$(n_s)_c = 2 + \frac{A}{2}\left(A + \sqrt{A^2+4}\right) \tag{25}$$

In a more general case (without choosing a specific model for the polymer chain), $(n_s)_c$ is adequately described by the equation[10]

$$(n_s)_c \simeq z_s^2/z_1 z_2 \tag{26}$$

Here, z_s is the partition function of a unit in the adsorbed sequence, and z_1 and z_2 are the partition functions of the first and the last units of the sequence, respectively. In our case, $z_s = 2 + g$, $z_1 = 4$, $z_2 = 1$, and $(n_s)_c \simeq (2+g)^2/4 = A^2$ [Eq. (25) gives $(n_s)_c \simeq A^2 + 3 + O(1/A^2)$].

Figure 5 is a plot of $\theta_c/(1-\theta_c)$ versus the average length of the adsorbed sequence $(n_s)_c$ for pores of different widths D. It is clear that the calculated
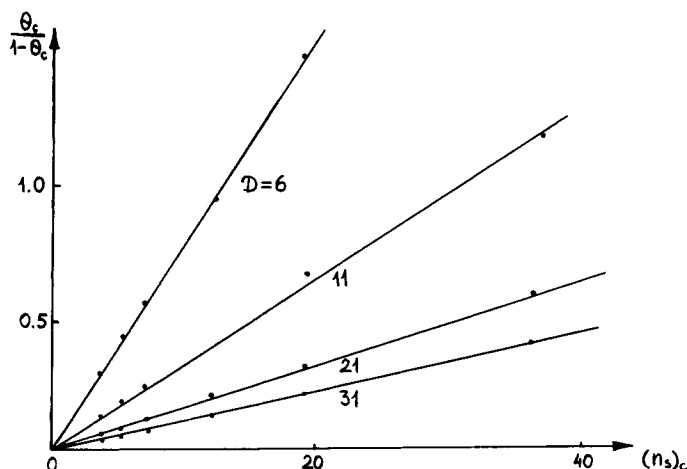
Fig. 5. Dependence of $\theta_c/(1 - \theta_c)$ on the average length of the adsorbed sequence $(n_s)_c$ under critical conditions for various pore widths (values at the curves).

values closely fit the straight lines predicted by Eq. (22) and their slope is inversely proportional to D (Fig. 6).

One can calculate the proportionality coefficient in Eq. (22) using the following considerations about unit distribution in a pore under critical conditions.

If θ_i is the fraction of units in one of the internal pore layers and n_i is the average length of random walk in it, it follows that

$$(\theta_i)_c/\theta_c = (n_i)_c/(n_s)_c \tag{27}$$

Hence,

$$\frac{\theta_c}{1 - \theta_c} \simeq \frac{1}{D} \frac{(n_s)_c}{(n_i)_c} \tag{27'}$$
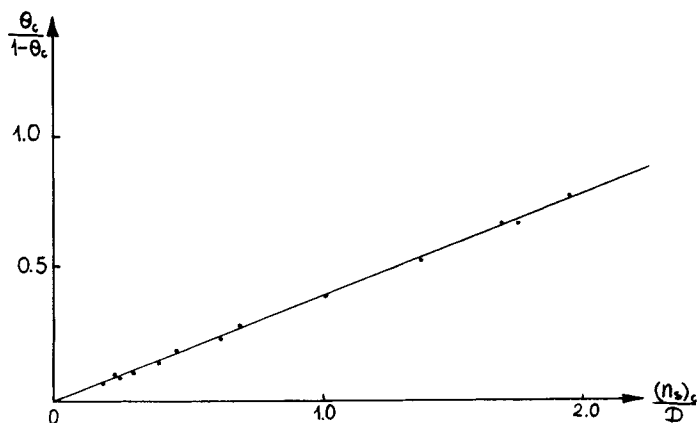

Fig. 6. Dependence of $\theta_c/(1 - \theta_c)$ on $(n_s)_c/D$.

It is easy to calculate $(n_i)_c$:

$$(n_i)_c = \frac{g}{g+4} + \sum_{n=2}^{\infty} n \frac{2}{g+4} \frac{4}{g+4} \left(\frac{2+g}{4+g}\right)^{n-2} = 3 \qquad (28)$$

It can be seen from Eq. (28) that in contrast to $(n_s)_c$, the value of $(n_i)_c$ is independent of the chain stiffness. Hence, the density distribution of units inside the pore and on its surface becomes increasingly asymmetrical with chain stiffening $(\theta_i)_c/\theta_c \sim A^{-2}$. This distribution of units of stiff chains differs greatly from that for flexible chains, for which $(n_s)_c \simeq (n_i)_c$. As is known,[3] the number of units of flexible chain on the adsorbing surface approximately coincides with that in any internal pore layer.

Figure 7 shows the plot of latent heat of adsorption $-\Delta E_c = -\epsilon_c \theta_c$ vs the D/A ratio generated when the macromolecule passes from solution into the adsorbent pore (when $-\epsilon$ is of an entirely energetic origin).

It is seen from Fig. 7 that at a high D/A ratio, the value of $-\Delta E_c$ is described by the universal curve and falls smoothly to zero at $D/A \to \infty$. At relatively low D/A ratios, $-\Delta E_c$ passes through a maximum. The narrower the pore, the greater is the value of this maximum. The nonmonotonous shape of the plot of $-\Delta E_c$ vs D/A is the result of two opposing factors: the decrease in θ_c with increasing D/A [according to Eq. (22)] and the increase in $-\epsilon_c$ with increasing chain flexibility (decreasing A).

Degree of Adsorption θ

Figure 8 shows the degree of adsorption θ calculated according to Eq. (8) versus the adsorption energy $-\epsilon$ for (a) chains of various stiffness and (b) various pore width D. It is clear that for both flexible and stiff chains in a pore, the value of θ is nonzero over the entire range of energies $-\epsilon$. θ increases continuously with increasing $-\epsilon$, in contrast to adsorption on a plane where θ exhibits a break at the critical point $-\epsilon = -\epsilon_c$ [broken line in Fig. 8(b)].
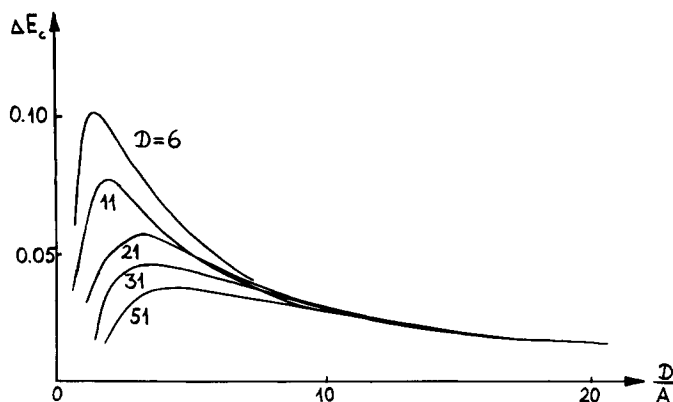

Fig. 7. Jump in energy ΔE_c occurring at the critical point $-\epsilon = -\epsilon_c$ vs the D/A ratio for various pore widths (values indicated).
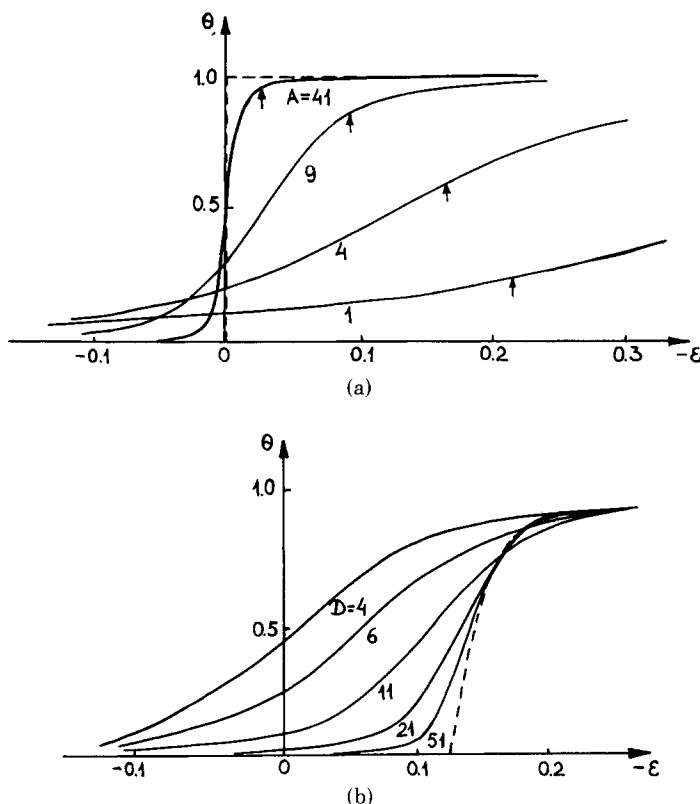
Fig. 8. Degree of adsorption θ vs adsorption energy $-\epsilon$ for chains with various pore widths (a) and stiffness values (b) (as indicated). The critical adsorption energy $-\epsilon_c$ is shown by arrows. Broken lines correspond to $A \to \infty$ (a) and $D \to \infty$ (b).

However, just as for adsorption on a plane, the stiffening of the chain makes adsorption curves $\theta = \theta(\epsilon)$ sharper. In the limit of absolutely stiff rods, $A \to \infty$, the bonding of the macromolecule to the pore walls will occur stepwise as soon as the energy of interaction between the units and the adsorbent exceeds zero [broken line in Fig. 8(a)].

The sharpening of the dependence $\theta(\epsilon)$ can also be caused by an increase in the pore width D, and for fairly wide pores, the adsorption curves are essentially the same as those for a plane [Fig. 8(b)].

The decrease of the transition region caused by the increase in the pore width and the chain stiffness is also indicated by the tendency of the dependence $\Delta F = \Delta F(\epsilon)$ to asymptotical behavior (Fig. 1).

CONCLUSION

In conclusion, consider the application of the concepts developed here to some problems of enzyme stabilization, in particular, to the increase in the stability of proteins inserted mechanically into the pores of a matrix that prevents their uncoiling and subsequent inactivation.

A protein, α-chymotrypsin, inserted into polyacrylamide gel has been investigated experimentally under conditions where the protein–polymer adsorption interaction was virtually absent $(-\epsilon \simeq 0)$.[11,12] It was found that when the polymer content in the swollen gel was relatively high, i.e., when the size of the gel pores was comparable to that of the native protein, the inactivation rate constant for α-chymotrypsin dropped sharply. This led the authors[11] to the conclusion that steric hindrances (caused by the polymer matrix) affect the thermodynamics of protein unfolding.

The magnitude of this effect can be estimated if it is assumed that during denaturation the protein passes from the globular state into the random-coil state, in which it can be modeled by a Gaussian coil of length L with the Kuhn segment A.

Then, the denaturation temperature in solution is given by

$$T_d = \Delta H_d / \Delta S_d \tag{29}$$

where ΔH_d and ΔS_d are the enthalpy and entropy of denaturation, respectively. When the protein is located in a matrix pore, its denaturation temperature is given by

$$T^*_d = \Delta H_d / (\Delta S_d - \Delta S^*) \tag{30}$$

where ΔS^* is the entropy loss for a coil in the pore compared to that in solution.

Assuming that $\Delta S^* \ll \Delta S$, we have

$$T^*_d \simeq \frac{\Delta H_d}{\Delta S_d} \left(1 + \frac{\Delta S^*}{\Delta S} \right) \tag{31}$$

Then,

$$\frac{\Delta T_d}{T_d} = \frac{T^*_d - T_d}{T_d} \simeq \frac{\Delta S^*}{k} \frac{k T_d}{\Delta H_d} \tag{32}$$

For the α-chymotrypsin studied in Ref. 12, T_d was $\sim 65°C$ and ΔH_d was ~ 180 kcal/mol. Equation (18) will be used for the evaluation of $\Delta S^*/k$. It should be noted that Eq. (18) was obtained for a slitlike pore. However, it also remains valid for cylindrical pores if $\pi^2/6$ is replaced by $\pi^2/3$.[9]

Since the pore shapes in the gel are closer to cylindrical, it will be assumed in further estimations that

$$\frac{\Delta S^*}{k} \simeq \frac{\pi^2}{3} \frac{LA}{(D + A)^2} \sim \frac{3LA}{(D + A)^2} \tag{33}$$

Let the Kuhn segment A contain n amino acid residues; then $A = nl$ and $L = Nl$ where l is the length of one amino acid residue ($l \sim 3.7$ Å) and N is their total number (for α-chymotrypsin, $N = 241$).

Evidently, the limitations of the conformational array of the chain due to the effect of the pore walls are great when the size of the unperturbed coil $2R_{coil}$ exceeds the pore size D. At the same time, it is necessary that the protein globule can enter the pores of the matrix and remain there in the undeformed state, i.e., the condition $2R_{gl} < D$ should be fulfilled.

Let us evaluate the pore range D over which the effect of protein stabilization by matrix walls should be pronounced. For the Gaussian coil, one has

$$R_{\text{coil}} \simeq \sqrt{LA/6} \qquad (34)$$

If A is taken to be 10–12 Å ($n \sim 3$), we have $2R_{\text{coil}} \approx 90$ Å. Hence, over the range 50 Å $< D <$ 100 Å ($2R_{\text{gl}} \sim 50$ Å), the stability of a protein located in the pore should greatly increase as compared to the same protein in solution. Thus, for pores 50, 100, and 200 Å in width, the evaluation of $\Delta T_d/T_d$ according to Eqs. (32) and (33) yields 2.5, 0.9, and 0.2%, respectively. It should be noted that if the random-coil protein chain is stiffer than was supposed ($n > 3$), the range of D becomes wider and the value of $\Delta T_d/T_d$ increases, i.e., the effect of chain stabilization increases for stiffer chains [under the condition that the change in the second factor in Eq. (32) is not too great].

Hence, the relative increase in the denaturation temperature T_d can be a few percent and the absolute shift T_d can reach 10°. Naturally the above considerations are valid for the case of complete decompactization of the protein globule during thermal denaturation. In the opposite case, the calculated effect will be less pronounced.

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