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# Effects of medium polarization and pre-existing field on activation energy of enzymatic charge-transfer reactions

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**Abstract**

The highly organized spatial structure of proteins' polar groups results in the existence of a permanent intraprotein electric field and in protein's weak dielectric response, i.e. its low dielectric constant. The first factor affects equilibrium free energy gap of a charge-transfer reaction, the second (medium polarization effect) influences both equilibrium and non-equilibrium (reorganization) energies, decreasing the latter substantially. In the framework of the rigorous 'fixed-charge-density' formalism, the medium polarization component of the reaction activation energy has been calculated, both for the activation energy of the elementary act proper, and the effective activation energy accounting for the charges' transfer from water into a low-dielectric structureless medium. In all typical cases of reactions, the energy spent for charge transfer from water into structureless 'protein' is larger than the gain in activation energy due to the protein's low reorganization energy. Therefore, the low dielectric constant of proteins is not sufficient to ensure their high catalytic activity, and an additional effect of the pre-existing intraprotein electric field, compensating for an excessive charging energy, is necessary. Only a combined action of low reorganization energy and pre-existing electric field provides proteins with their high catalytic activity. The dependence of activation energy on the globule geometry has been analyzed. It is shown that, for each reaction, an optimum set of geometric parameters exists. For five hydrolytic enzymes, the optimum globule radii have been calculated using the experimental geometry of their active sites. The calculated radii agree satisfactorily with the real sizes of these macromolecules, both by absolute and by relative values. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Intraprotein charge transfer; Dielectric model of enzyme; Polarization of a protein globule; Globule optimum radius; Hydrolytic enzyme

## 1. Introduction

Any charge transfer reaction is affected substantially by the interaction of the charge being transferred with its polar surroundings. An enzymatic reaction occurs, as a rule, inside the protein globule, i.e. the enzyme itself is in a sense the medium of the enzymatic reaction [1]. Naturally, an analysis of the characteristic properties of this medium is of great importance for understanding the specific features of enzymatic reactions.

A self-evident property of proteins is their structural specificity making a given protein appropriate to perform a desirable function, and not the others. Therefore, a full analysis of an enzymatic process shall employ a detailed structural information on

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the protein. On the other hand, there is much common in behavior of many enzymes of rather different structures, and, therefore, we can expect that some substantial features of enzymes are due to some general principles of their architecture rather than to specific details of the structure of a concrete protein.

As we have stressed many times in our previous works (for review see, e.g. [2,3]), the specific feature of proteins as polar media is the high extent of their organization: there is the high concentration of protein's dipoles that are fixed in a rather rigid structure restricting drastically their ability to reorient under the action of an external electric field. This has two interconnected consequences [4]. First, due to fixation of dipoles in definite positions, there does exist inside a protein some permanent (averaged in time) structure-dependent field of dipoles set up prior to introduction of any extraneous charge. Therefore, the protein can be called a 'pre-organized medium'. The pre-existing electric field influences substantially the equilibrium energetics of enzymatic reactions. Second, in spite of a high concentration of strong dipoles, proteins display a weak dielectric response (low dielectric constants) due to a strongly restricted mobility of dipoles. This affects both equilibrium and non-equilibrium (reorganization energy) parameters of reactions.

The considerations given above show that proteins can be defined as *highly-polar low-dielectric* media. This definition stresses the principal difference between proteins (and, possibly, some other macromolecular substances) and low-molecular solvents. In the latter, due to practically free rotation of their molecules, 'polarity' and dielectric constant change in parallel. In proteins, 'polarity' (dipole moment and concentration of polar groups) and dielectric constant are not interconnected in such a direct way. From this point of view, it is no sense to oppose the notions of 'highly-polar medium' against 'low-dielectric medium' as applied to proteins. They are both at the same time, and these different (and, at the first sight, contradicting) properties manifest themselves predominantly in different phenomena.

The activation energy of the elementary act of electron or proton transfer $\Delta G^{\neq}$ is determined, according to a well-known Marcus equation, by two parameters, namely the reorganization energy $\lambda$ and the free energy of the elementary act $\Delta G$ (see e.g. [5–7])

$$\Delta G^{\neq} = \frac{(\lambda + \Delta G)^2}{4\lambda} \quad (1)$$

Both these parameters can be considered as a sum of some structural-dependent contributions ($\lambda_i$ and $\Delta G_{str}$) and of some component due to medium polarization ($\lambda_s$, $\Delta G_{pol}$).

According to Marcus, in the simple case of the charge transfer in a homogeneous medium $\lambda_s$ is proportional to so-called coupling constant $C = 1/\varepsilon_o - 1/\varepsilon_s$, where $\varepsilon_o$ and $\varepsilon_s$ are optical and static dielectric constants.

The low dielectric constant of proteins affects strongly the reorganization energy of any intraprotein charge transfer. For the common solvents having high or medium dielectric constants, $\varepsilon_s \gg \varepsilon_o$, and hence $C$ varies only slightly with a change of $\varepsilon_s$. On the contrary, transition to a medium with a very low dielectric permittivity changes the value of $\lambda_s$ drastically. For water, with $\varepsilon_o = 1.8$ and $\varepsilon_s = 78$, $C = 0.54$. For proteins, $\varepsilon_o \approx 2.5$ (the usual value for liquid amides about 2 corrected, according to Clausius–Mossotti equation, for higher density of proteins) and $\varepsilon_s \approx 4$, hence $C \approx 0.15$, i.e. about three times smaller. Therefore, a low reorganization energy is typical of proteins as the media of charge transfer reactions [8,9] (see also [10]). This is one of the main physical reasons of their catalytic effect. The same conclusion was inferred also from qualitative microscopic considerations: the dipoles inside the globule are rather rigidly fixed and cannot undergo a noticeable reorientation, hence the corresponding reorganization energy is low [4]. Quantitative molecular dynamics simulations for several biological charge transfer reactions confirmed low reorganization energies of particular proteins considered in these simulations [11–21].

In this paper, we will show that $\lambda_s$ for the intraprotein charge transfer in a heterogeneous (protein/water) system is much lower than for the similar process in aqueous solution. Furthermore, we will show that, for each charge transfer reaction, an optimum set of geometric parameters, in particular globule radii, exists. For several hydrolytic enzymes, the calculated optimum globule radii (based on the experimental geometry of their active sites) agree sat-

isfactorily with the real sizes of these macromolecules. Employing the model of a structureless dielectric, we will show that the decrease in reorganization energy (due to a low protein's static dielectric constant) never overweighs the energy expenditure necessary to transfer the charged reactant(s) from water into a low-dielectric medium. The corresponding energy loss should be compensated for by the effect of the intraprotein electric field.

The results of this study were reported in part at some conferences [22–24].

## 2. Charge transfer in a dielectric globule

### 2.1. The model of a dielectric sphere

For most enzymes, the active site is situated more or less close to the protein surface, and hence the charge transferred can interact substantially with the high-dielectric aqueous surroundings. In our previous works [8,9,25,26], we have analyzed such a heterogeneous system using the so-called 'fixed-displacement-field' technique. Here we will employ the contemporary rigorous 'fixed-charge-density' formalism [27–29].

In this section, we will consider simple enzymes represented by globular proteins. We will approximate the globule by a smooth sphere; that seems to be an appropriate model to simulate the general trends in the behavior of enzymes. Our model is depicted in Fig. 1A: two spherical reactants immersed into a spherical globule.

We will consider the electric field as the field of the point charges situated inside the globule. This means that we neglect the field distortion due to the difference of dielectric permittivities of the reactants' $\varepsilon_r$ and of the surrounding protein $\varepsilon_p$. This seems to be a reasonable approximation because the optical permittivities of reactants and proteins are practically coinciding, while the effective static values are rather close ($\geq 2$ and $\sim 4$), especially as compared to the external surroundings ($\sim 80$).

The solution of an electrostatic problem for a spherical globule can be represented in the form of a series of the Legendre polynomials [30]. With account of the boundary conditions, i.e. the continuity of the potential and the normal component of the

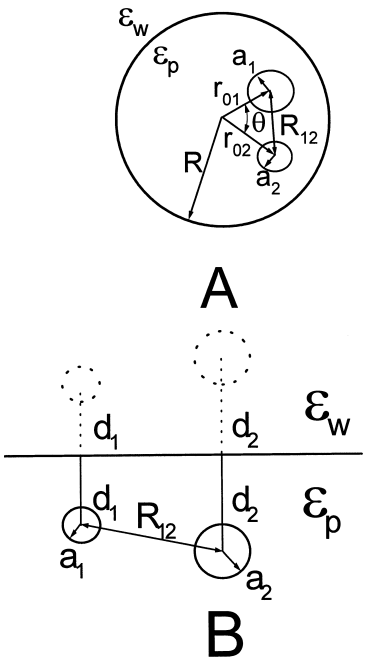



Fig. 1. The models used in calculations; the geometric and dielectric parameters are shown. (A) Two spherical reactants inside a spherical globule. (B) The model of a flat boundary; dotted spheres show positions of the charges' images.

dielectric displacement at the uncharged boundary, we obtain for potential at any point $r$ inside the globule

$$\varphi_p(r) = \frac{q}{\varepsilon_p|\boldsymbol{r}-\boldsymbol{r_0}|} + \sum_{n=0}^{\infty}\left(\frac{r_0^n}{R^{n+1}}\right)$$

$$\frac{(n+1)(\varepsilon_p-\varepsilon_w)}{\varepsilon_p[n\varepsilon_p+(n+1)\varepsilon_w]}\left(\frac{r}{R}\right)^n P_n(\cos\theta) \quad (2)$$

Here $q$ is the charge situated at the point $\mathbf{r_0}$, $P_n(\cos\theta)$ is the Legendre polynomial, the meaning of all geometric parameters is shown in Fig. 1A.

With this expression, we can find the charging energies (including both purely 'Bornian' charging of each ion and the energy of their interaction) in two media, optical and static ones; the difference of these two quantities represents the reorganization energy [27–29]

$$\lambda_s = e^2\left(\frac{1}{\varepsilon_p^o}-\frac{1}{\varepsilon_p^s}\right)\left(\frac{1}{2a_1}+\frac{1}{2a_2}-\frac{1}{R_{12}}\right)+$$

$$A_1^o + A_2^o - A_3^o - A_1^s - A_2^s + A_3^s \quad (3)$$

Here the first term that is identical with Marcus equation gives reorganization energy in an infinite protein, the quantities $A_{1,2}$ are the contributions of images of charges in the aqueous surroundings to charging energies of each reactant separately, $A_3$ to their interaction energies, the quantities calculated for both media having optical ($A_i^o$) or static ($A_i^s$) dielectric constants. The corresponding expressions are

$$A_1 = \frac{e^2}{2}\sum_{n=0}^{\infty}\left(\frac{r_{01}}{R}\right)^{2n}\left(\frac{1}{R}\right)\frac{(n+1)(\varepsilon_p-\varepsilon_w)}{\varepsilon_p[n\varepsilon_p+(n+1)\varepsilon_w]} \quad (4a)$$

$$A_2 = \frac{e^2}{2}\sum_{n=0}^{\infty}\left(\frac{r_{02}}{R}\right)^{2n}\left(\frac{1}{R}\right)\frac{(n+1)(\varepsilon_p-\varepsilon_w)}{\varepsilon_p[n\varepsilon_p+(n+1)\varepsilon_w]} \quad (4b)$$

$$A_3 = e^2\sum_{n=0}^{\infty}\frac{r_{01}^n r_{02}^n}{R^{2n+1}}\frac{(n+1)(\varepsilon_p-\varepsilon_w)}{\varepsilon_p[n\varepsilon_p+(n+1)\varepsilon_w]}P_n(\cos\theta) \quad (4c)$$

In Eqs. 4a and 4b, the equality $P_n(1)=1$ has been accounted for.

The proper combination of static terms will be used also to calculate the equilibrium charging energy (a sum or difference of charging energies with or without interaction energy).

For the sake of comparison, we will consider also the model of two semi-infinite dielectrics separated by a flat boundary (Fig. 1B); the effective charge of images is $e'=Ke$, where $K=(\varepsilon_p-\varepsilon_w)/(\varepsilon_p+\varepsilon_w)$. The new expressions for $A_i$:

$$A_1 = \frac{e^2K}{4d_1} \quad (5a)$$

$$A_2 = \frac{e^2K}{4d_2} \quad (5b)$$

$$A_3 = \frac{e^2K}{(R_{12}^2+4d_1d_2)^{1/2}} \quad (5c)$$

Eqs. 5a–c transform, naturally, to Eqs. 4a–c at $R\to\infty$ (under condition $R-r_{0i}=d_i=\text{const.}$)

### 2.2. The reorganization energy for intraglobular charge transfer

Some results of calculations for one set of geometric parameters typical, by the order of magnitude, of many enzymatic charge transfers are presented in Fig. 2. They give much lower (by e.g. 95–120 kJ/mol) reorganization energies for an enzymatic charge transfer than for the same reaction in an aqueous solution (cf. curves 1–4 and the upper horizontal line a). A drastic decrease in reorganization energy obtained here is not a result of some improper choice of the model geometry. For several real enzymes discussed in Section 2.4, the calculations employing the geometric parameters taken from the X-ray structure data result in a decrease of reorganization energy by 70–130 kJ/mol. So, this effect is quite substantial, and it presents a very important factor in the high catalytic activity of enzymes.

It should be mentioned here that a substantial lowering of reorganization energy is inherent not only to globular enzymes, but also to any intraprotein charge transfer. Such an effect we have found, for instance, in intramembrane electron transfer in the photosynthetic reaction center: for the primary charge separation, electron transfer to quinone, etc. [31].

The physical reason for the reorganization energy decrease is quite clear: some part of the solvent in the vicinity of reactants is replaced by protein, i.e. the low-reorganization medium, and that decreases the total reorganization energy. The screening by protein reduces the interaction of the charge being transferred with aqueous surroundings decreasing thereby the reorganization of the latter. The better the screening, the lower is the reorganization energy.

It should be noted here that our previous calculations [25,26] carried out in the framework of the non-rigorous 'fixed-displacement-field' formalism resulted in a similar shape of $\lambda_s$–$R$ curves, but with a substantial higher reorganization energies (for the model parameters as in Fig. 2, by about 34–62 kJ/mol). In other words, our previous calculations have underestimated the catalytic effect of the protein's dielectric screening. This stresses the necessity of revisiting the problem using the rigorous formalism, not only to get the refined quantitative data, but also to obtain a stricter foundation for the relevant qualitative conclusions.

In Fig. 2, the lower horizontal line b presents the reorganization energy that would characterize the model reaction in an infinite protein. We see that the actual reorganization energies are markedly higher: this is due to two effects of the highly polar

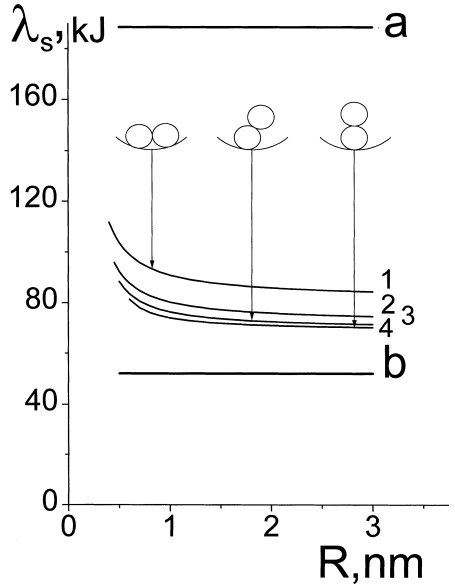



Fig. 2. Medium reorganization energy as a function of the globule radius at different geometries of the active site. Geometric parameters: $a_1 = a_2 = 0.2$ nm, $R_{12} = 0.4$ nm, $R - r_{01} = 0.2$ nm, $R - r_{02}$ varies and equals to 0.2 nm for curve 1, 0.3 nm for 2, 0.4 nm for 3, and 0.6 nm for 4. Typical configurations corresponding to curves 1, 3 and 4 are shown schematically in the upper part of the figure. In calculations, instead of optical, purely electronic $\varepsilon_o$ of water ($\varepsilon_o = 1.8$) the value of a so-called 'quantum' dielectric constant $\varepsilon_* = 2.1$ is employed that accounts for the high-frequency vibrational modes possessing a quantum behavior [7]. The upper horizontal line a marks the reorganization energy of the same reaction in an infinite homogeneous aqueous medium, the lower horizontal line b presents this quantity for reaction in an infinite homogeneous protein.

aqueous surroundings: the reorganization of the aqueous phase itself and the reorganization of the protein phase under the action of redistribution of images in the aqueous surroundings; calculations give the ratio of these two effects as approximately 2:1 (not shown).

The interaction of charges with protein and water depends, naturally, on the geometry of the system. Initially, the value of $\lambda_s$ diminishes fast with an increasing globule radius, but later this decrease slows down substantially. Indeed, at first an increase in globule radius results in a drastic increase of the charge screening by protein from the aqueous phase, but gradually more and more distant solvent layers are excluded of the play, and, as the interaction of these distant layers with the charge is rather weak, the further increase of the globule size does not give an appreciable gain in reorganization energy. This kind of dependence should be quite general, the differences for various enzymes can be of quantitative, but not qualitative, character.

The extent of screening affects also the dependence of $\lambda_s$ on the orientation of reactants relative to the globule surface: the maximal $\lambda_s$ corresponds to the charge transfer parallel to the surface, it decreases with the increased angle between transfer direction and the surface becoming almost constant at the angle of 20–30° (cf. curves 1–4 in Fig. 2; curve 3 corresponds to the angle 30°). Similar considerations can be applied to other variations of geometric parameters. For instance, $\lambda_s$ diminishes with increasing distances of ions from the globule surface, but then becomes practically constant at the immersion depth of 0.7–0.8 nm (at globule radii $R > 1$ nm).

### 2.3. *The activation energy for intraglobular charge transfer and the globule geometry*

The charge–medium interaction affects both reorganization energy and the reaction free energy. The latter consists, as we have mentioned before, of some component dependent on the structures of both reacting groups ($\Delta G_i$) and protein (namely, the intraprotein electric field) $\Delta G_{str} = \Delta G_i + \Delta G_{ef}$, and of a 'polarization' component $\Delta G_{pol}$. At the present stage of our discussion, we will consider the 'structural' component as a parameter.

Both the electrostatic part of $\Delta G_{str}$ (interaction with the pre-existing intraprotein field) and $\Delta G_{pol}$ depend, generally speaking, on the geometry of the system, but these dependencies are different. The component $\Delta G_{str}$ depends on microscopic structure, i.e. on coordinates of all protein's atoms, while the polarization contribution $\Delta G_{pol}$, due to the general dielectric response, depends only on so to say 'macroscopic' factors (size and shape of the globule and the reactants, disposition of the latter); in other words, this part of energy can be analyzed in the framework of the model of a structureless dielectric.

The charge–polarization interactions manifest themselves in the influence of the system geometry on the Bornian charging and on the charge–charge interaction energies. This leads to a new effect, absent in homogeneous systems, namely the dependence of the reaction energy on the orientation of reactants with respect to the interface [32].

Depending on the reaction type, these effects will affect the $\Delta G$ differently. Let us consider first the process of the exchange of charge positions (charge shift), e.g.

$$A^+ + B = A + B^+$$

The 'polarization' component for this reaction is

$$\Delta G_{\mathrm{pol}} = \frac{e^2}{\varepsilon_{\mathrm{p}}}\left(\frac{1}{2a_B} - \frac{1}{2a_A}\right) + A_B^{\mathrm{s}} - A_A^{\mathrm{s}} \qquad (6)$$

Let us consider the case when $B$ is closer to the globule surface than $A$, i.e. the charge is transferred from inside the globule towards its surface. In this case, the charge interaction with the aqueous phase increases making the charge transfer in this direction favorable. The influence of geometric parameters on $\Delta G_{\mathrm{pol}}$ is qualitatively similar to their influence on $\lambda_{\mathrm{s}}$. Therefore, the dependencies of the activation energy on the globule radius, reactants' orientation, etc., are similar to the corresponding dependencies of $\lambda_{\mathrm{s}}$ (cf. Figs. 2 and 3).

In any catalytic process, the system must return to its initial state after completion of the catalytic cycle, i.e. the charge transfer from $A$ to $B$ should be followed by a step with the reverse transfer direction. Therefore, at any disposition of the reactants, we have to consider both possible directions of the charge transfer. The charge transfer outwards is favorable, but in the opposite direction is not. The comparison of curves 1–4 of Figs. 3 and 4 shows that the activation energy for outward charge transfer is lower than for the inward direction.

In Figs. 3 and 4, we give sets of curves corresponding to different $\Delta G_{\mathrm{str}}$, namely 0, +41.8 and −41.8 kJ/mol (0 and ±10 kcal/mol). These values are close by order of magnitude to those for the different steps of enzymatic catalysis (e.g. formation and decay of a tetrahedral intermediate in hydrolysis of esters and amides). As it would be expected, the activation energy for the endergonic transfer even in the outwards direction remains higher than for the inward exergonic process (cf. curves 1′–4′ in Fig. 3 and 1″–4″ in Fig. 4). Therefore, the outward endergonic process is here the rate-determining one. However, if one of the reactants is substantially larger than the other, the lower charging energy of the larger ion makes the charge transfer to this particle preferable, even in the case of its deeper immersion.

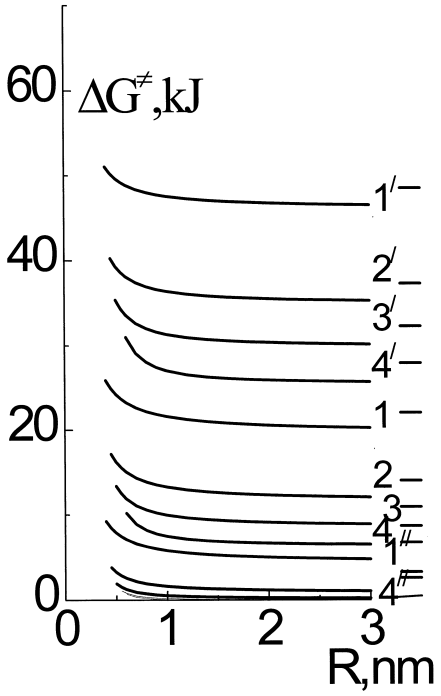



Fig. 3. Activation energy for the charge shift (charge displacement) reaction in a spherical globule with the same geometry of reactants as described in Fig. 2; dependence on $R$ is given up to $R = 3$ nm, and then the value corresponding to $R = \infty$ (flat boundary) is shown. In the initial state, reactant 1 is neutral, reactant 2 is charged: charge shift from inside the globule outwards (curves 2–4, for curves 1, charge shifts parallel to the globule surface). For curves 1–4, $\Delta G_{\mathrm{str}} = 0$, for curves 1′–4′, $\Delta G_{\mathrm{str}} = +41.8$ kJ, for 1″–4″, $\Delta G_{\mathrm{str}} = -41.8$ kJ (energoneutral, endergonic and exergonic reactions in an infinite medium, correspondingly).

We can also conclude that the charge transfer at an acute angle to the globule surface is favorable: under this condition, the activation energy is rather low, and a relatively small additional decrease that could be achieved by the charge transfer almost perpendicular to the surface would be connected with much deeper immersion of the initial ion into the globule, the immersion demanding, as a rule, a large energy expenditure.

An important conclusion follows from the form of the dependence of the activation energy for the rate-determining step on the globule radius. We observe a strong initial decrease followed by a rather weak dependence. As is shown in Fig. 3, for the curves corresponding to the optimum reactants configurations

(curves 3, 4, 3′ and 4′), at the globule radius about 0.8–1.0 nm $\Delta G^{\neq}$ is only 2 kJ higher than at $R = 3$ nm. Therefore, the radius close to 1 nm can be considered in a sense as an optimum. Indeed, its further increase would require a large additional consumption of protein (proportional to $R^3$) but will not bring about an appreciable gain in the reaction rate (only by about 2.2 times). The optimal size of the globule depends, of course, on the size of the reactants, depth of their immersion, interreactant distances, etc. (some examples will be given in Section 2.4).

Similar in a general shape dependencies were also obtained for other types of reactions, e.g. for a charge separation in presence of the third ion. For a simple reaction

$$A + B = A^+ + B^-,$$

$\Delta G_{pol}$ is substantially positive because it is unfavorable to create charged particles inside a low-dielectric medium. However, this unfavorable effect can be largely compensated for, e.g. by the presence of the third pre-existing ion $C$, closer to an oppositely charged reactant. Not dwelling on details of calculations (calculation of the pre-existing field effect was done according to [33]), we will notice here that the field of the third ion decreases drastically $\Delta G^{\neq}$ for charge separation: for the set of parameters similar to those of Figs. 2–4, to the level of 15–20 kJ as compared to the minimum value of 55 kJ in the absence of the third ion. As to optimum orientation of the three particles in respect to the globule boundary, the situation is in principle similar to the case considered above: an obtuse-angled triangle $ABC$ with at least one of reactant immersed somewhat deeper than other(s) (the effective shift of the charge gravity center outwards at an acute angle to the surface).

Our analysis revealed a substantial influence of the globule radius on the reaction activation energy. We believe that the effect of globule size plays an important functional role. It is well known that the dimensions of the active site region are usually substantially smaller than the size of the globule. The results described above (and many other similar model computations which we have performed) show that the seemingly excessive dimensions of the enzyme globule are necessary for the effective screening of the charge being transferred from the interaction with the surrounding water. This results in a low activation energy, i.e. in a substantial increase of the enzyme's catalytic activity. The effect is really great: for instance, the decrease in activation energy in comparison to reactions in water for the optimum parameters of the model depicted in Fig. 3 equals to 30–35 kJ/mol, the similar effects were obtained for other models too.

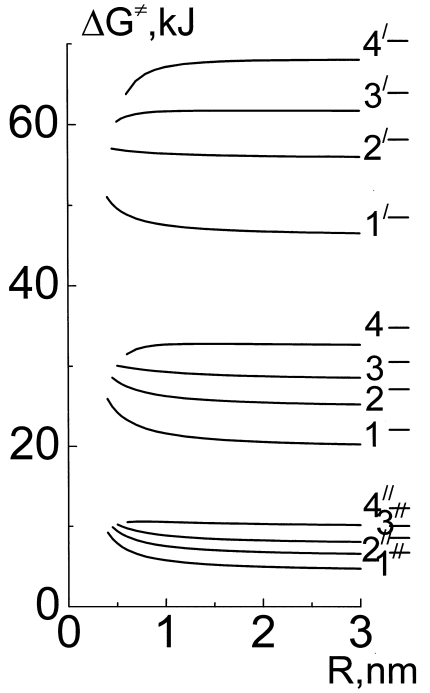



Fig. 4. Activation energy for the charge shift (charge displacement) reaction in a spherical globule with the same geometry of reactants as described in Fig. 2. In the initial state, reactant 2 is neutral, reactant 1 is charged: charge shift from the outer part of the globule inwards (curves 2–4, for curves 1, charge shifts parallel to the globule surface). All designations are as in Fig. 3.

We are quite aware that the effect of charge screening by the protein cannot be the single factor influencing the optimal size of any enzymatic globule. For instance, some additional amino acids can be necessary to maintain a fixed structure of the active site. However, many physiologically active polypeptides (e.g. inhibitors or hormones) having quite definite spatial structures are substantially smaller than typical enzymes.

One more factor that can influence the globule size, is the necessity to create an essential (and highly 'structured') intraglobular electric field, set up, first of all, by polar peptide bonds (see discussion in Sec-

tion 3). Our calculations have shown, on the example of the active site of α-chymotrypsin, that a marked (and sometimes even predominant) contribution to this field originates not only from a few closest but, in some cases, from a large number of more distant (up to ~1 nm) peptide groups [34,35]. However, this distance remains substantially smaller than the globule diameter. So, the proper organization of the intraglobular field may demand a rather large globule, but, at least for the case studied, viz. α-chymotrypsin, smaller than the globule necessary to provide the optimum dielectric screening (cf. Section 2.4). In any case, it is clear that the screening effect should exert its influence in any system, and this is the rationale for its special analysis.

In our calculations, we have accepted the structural component $\Delta G_{str}$ as some parameter, constant for the series of the curves. If we compare the optimum radii for $\Delta G_{str} = 0$ and $\Delta G_{str} = +41.8$ kJ, i.e. practically in a full range of the rate-determining endergonic steps, we will see that the difference between them is relatively small. For example, for curves 3 and 4 in Fig. 3, the optimum radii can be estimated as equal to 0.9–1.0 nm, while for the strongly endergonic case (curves 3′, 4′) the corresponding values are 0.8–0.9 nm. Therefore, we can conclude that the relative figures obtained with our method are not very sensitive to the value of the structural component $\Delta G_{str}$. This is due to the fact that $\Delta G_{str}$ is strongly dependent on the protein structure while $\Delta G_{pol}$ and $\lambda_s$ are connected with the protein's general dielectric response, much less sensitive to structural details. That can serve as an intuitive rationale to analyze separately the influence of polarization and structural factors.

### 2.4. The globule size of real enzymes

The aim of this subsection is to estimate the optimum radii of several real enzymes and to compare them with their sizes. To our best knowledge, there is no attempt in the literature (except our earlier papers [8,9,25,26]) to find any factor determining the size of enzymes.

Remaining in the framework of the model depicted above, we will discuss here only soluble enzymes; we will not consider enzymatic complexes carrying out several reactions, or enzymes possessing some regulatory site(s) influencing the general geometry of the protein. We have to exclude considering enzymes with many ionized groups in the active site: most probably, in that case, the local dielectric constant is markedly higher, and the electrostatic problem demands a more sophisticated treatment.

Our approach will be as follows: we will start with the experimentally established reaction mechanism in order to find out what groupings are involved in charge transfer, and what type of the process takes place. Then, on the basis of X-ray crystallographic data, we will define the main geometric parameters of the active site: the size of reacting groups, their mutual disposition and the distances between them. The reactants in their exact geometry will be positioned inside a spherical globule. The distances of the reactants from the globule surface will be also estimated from the structural data using the averaged distances between the reactant and some closest surface atoms. This parameter will be the approximate one because the surface of a real globule is neither spherical nor a smooth one. Therefore, we will vary these distances within reasonable limits consistent with the crystallographic data.

In our calculations, we accepted the following effective ionic radii: $O^-$ 0.16 nm, $CO_2^-$ 0.2 nm (center of charge in the middle between two O atoms), $ImH^+$ 0.26 nm (center of charge between two N atoms), $S^-$ 0.19 nm, $O^+$ in saccharide ring 0.15 nm. Variation of these parameters by 0.02–0.04 nm affected the absolute values of activation energies, but did not practically influence the estimates of the optimum globule radii.

In considering the size of the globule (and, correspondingly, the distances between reacting groups and the globule surface), the problem arises that the dimensions of an enzyme–substrate complex depend on the size of the substrate, being substantially different for, say, complexes with a protein and a small peptide. Under physiological conditions, an enzyme should be effective both for reactions of macromolecules as well as their small fragments. An increase in the globule size should lead, as a rule, to an increase in its catalytic activity. Therefore, we will analyze the complexes of enzymes with their minimal substrates.

As described before, we will vary also $\Delta G_{str}$. The values of this parameter were chosen in such a way

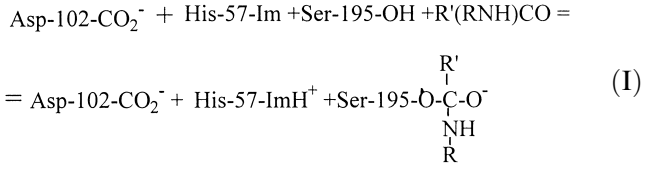
to maintain the rate-limiting step endergonic or, at least, thermoneutral, and to keep the calculated activation energy in limits corresponding to the usual range of about 5–15 kcal (we prefer the values around 10 kcal).

For the enzymes considered, each step of the process presents a charge transfer, in most cases, the proton transfer. In our previous studies of the kinetic isotope effect in chymotryptic and tryptic hydrolysis, it was shown that the main pathway of proton transfer consists in its tunneling from the ground state [36–38]. That means that the reaction activation energy is not affected substantially by the energy of stretching of the hydrogen covalent bond that behaves quantum-mechanically, and hence can be treated in the framework of the theory of medium reorganization. We can expect that this conclusion should be valid for a broad class of proton transfer reactions in which the movement along the classical coordinates connecting nucleophilic and electrophilic atoms rather than the movement along the H atom quantum coordinate contributes substantially into reaction activation energy.

For all reactions considered, the charge transfer proceeds in the presence of the third ion. The inspection of the structural data revealed that in all these cases, three charged particles form an obtuse-angled triangle with at least one of ions immersed deeper than others. This experimental fact confirms with the conclusions drawn above.

### 2.4.1. Optimum radii of hydrolytic enzymes

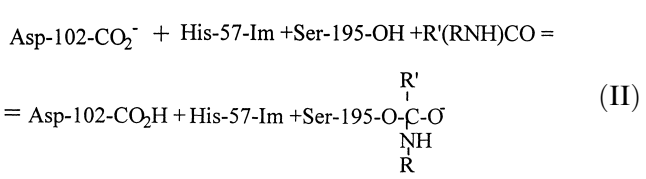
*2.4.1.1. α-Chymotrypsin.* For chymotryptic hydrolysis, two possible mechanisms have been considered. The first is the charge separation in the presence of the third ion (Asp-102–$CO_2^-$):



$$\text{Asp-102-CO}_2^- + \text{His-57-Im} + \text{Ser-195-OH} + \text{R'(RNH)CO} = \text{Asp-102-CO}_2^- + \text{His-57-ImH}^+ + \text{Ser-195-O-C(R')(NH-R)-O}^- \quad \text{(I)}$$

In this reaction, one proton is transferred from Ser-195–OH to imidazole ring of His-57 with a simultaneous nucleophilic attack of substrate's carbonyl C by serine's oxygen. As a result, imidazole ring becomes charged positively, and oxygen of the tetrahedral intermediate negatively.

The other formulation of the mechanism of this step implies a concerted two-proton transfer (one from Ser to Im and the second from the other side of Im to Asp):



$$\text{Asp-102-CO}_2^- + \text{His-57-Im} + \text{Ser-195-OH} + \text{R'(RNH)CO} = \text{Asp-102-CO}_2\text{H} + \text{His-57-Im} + \text{Ser-195-O-C(R')(NH-R)-O}^- \quad \text{(II)}$$

Reaction II is the charge shift from Asp-102–$CO_2$ to C–O. Mechanism I is considered, at least for small substrate molecules, as the most probable one (for more detailed discussion see [39–41]).

The geometric parameters of α-chymotrypsin were obtained from the X-ray data on the enzyme complex with turkey ovomucoid [42] (pdb 1cho), the most part of the last protein was discarded to leave coordinates of only few atoms corresponding to a minimal substrate. The position of O atom in tetrahedral intermediate (in the oxyanion hole) was approximated as this of the ovomucoid's Leu-18 carbonyl oxygen.

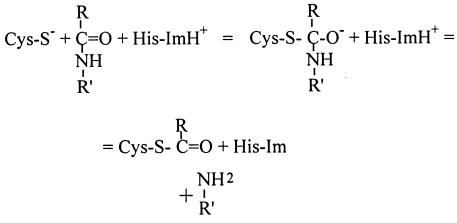
The shape of the $\Delta G^{\neq}$–$R$ relationship is analogous to that discussed above. Assuming conditionally that, for the optimum globule size, the excess over the minimal $\Delta G^{\neq}$ is equal to 2.0 kJ we obtain, for mechanism I, the optimum radii of 1.5–1.8 nm; the interval corresponds to somewhat differing estimates of the depth of reactants immersion and to different $\Delta G_{str}$ accepted in these calculations. For activation energy closer to experimental values of ~10 kcal, the optimum should be 1.65–1.8 nm. For mechanism II, the optimum radii were calculated to be substantially smaller, viz. 1.2–1.4 nm. Thus, the globulae optimal for mechanism II are too small for mechanism I, and hence only larger globulae can provide a low enough activation energy for all substrates (the larger radius is shown in Fig. 5).

*2.4.1.2. Subtilisin Carlsberg.* The reaction mechanism is the same as for chymotrypsin. Structural data for subtilisin complex with eglin-C were taken from [43] (pdb 1cse). As the position of oxygen in the oxyanionic hole the coordinates of the eglin's Leu-45

carbonyl oxygen were accepted. The relationships here are similar to those of chymotrypsin.

*2.4.1.3. Serine carboxypeptidase II (wheat).* The enzyme contains the same catalytic triad Asp–His–Ser, and the most probable reaction mechanism is the same as for chymotrypsin. The calculations were done on the basis of structural data [44] (pdb 3sc2), the O coordinates of tetrahedral intermediate were represented by those of $H_2O$ occupying the oxyanion hole [44].

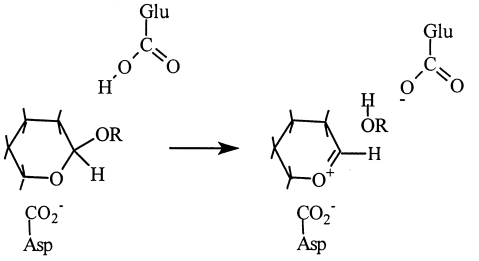
*2.4.1.4. Papain.* The reaction mechanism can be formulated as follows [45]:




(III)

The first step (designated later as IIIa) is the process of the charge shift in the presence of the third ion ($ImH^+$) favoring the appearance of the negative charge at the oxygen atom. This step is preceded by Cys–SH dissociation, the pre-equilibrium dependent on the static dielectric constants of surroundings. The second step (IIIb), presents the charge neutralization process. In calculations of its activation energy, the pre-equilibriums of Cys–SH dissociation and tetrahedral intermediate formation were accounted for. In the real enzyme, there is one more ion in vicinity of the active site, viz. Asp-158, and its field was also taken into account. Coordinates of the complex papain–leupeptin representing a transition-state analog were taken from [46], O atom of inhibitor's Arg-303 was considered as O of the tetrahedral intermediate. For reaction IIIb, the probable interval of optimum radii was estimated as 1.45–1.5 nm (at $\Delta\Delta G^{\neq} = 2.0$ kJ) while in the case of the first step of the process IIIa being rate determining the corresponding values are 1.65–1.7 nm. As the globule size should provide favorable conditions for all reaction steps, we accept the larger value as the final one.

*2.4.1.5. Lysozyme.* The rate-determining step is the charge separation in the presence of the third ion stabilizing the positive charge on the saccharide ring [47].




(IV)

The coordinates were taken for the complex of enzyme with tri-*N*-acetylchitotriose according to [48] (pdb.1hew).

*2.4.2. Comparison with the experimental data*

Here, we will compare the optimum radii calculated above with the experimental dimensions of the corresponding enzymes. The effective 'experimental' radii of spheres corresponding to the real globulae of enzymes were calculated using enzymes' molecular masses and accepting for all of them the specific gravity equal to 1.33. In Fig. 5, these effective globule radii are plotted as abscisae, and the ordinates are the estimated optimum radii. The intervals of estimated values are presented, the most probable (corresponding to activation energies close to 10 kcal) shown as thick vertical bars, and the full range of estimates shown as thin bars.

In Fig. 5, the optimum radii corresponding to $\Delta G^{\neq} - \Delta G^{\neq}_{min} = 2.0$ kJ are presented. We have also tried other values. The general picture is qualitatively similar though there are some quantitative differences; e.g. at $\Delta G^{\neq} - \Delta G^{\neq}_{min} = 1.5$ kJ, $R_{opt}$ are by about 0.5 nm larger.

Fig. 5 clearly shows that the estimates of the globulae optimum radii are in a satisfactory agreement with the real size of enzymes' molecules. It should be noted here that the accepted excess of activation energy over its possible minimal value is some conditional figure, and there is no warranty that precisely the same difference is optimal for all enzymes. One could speak here rather on order of magnitude of this excess, and therefore consider these results not as a strict quantitative evaluation of the optimum radii, but as some semi-quantitative estimate of their values. As a matter of fact, one could not expect a better accuracy keeping in mind the assumptions

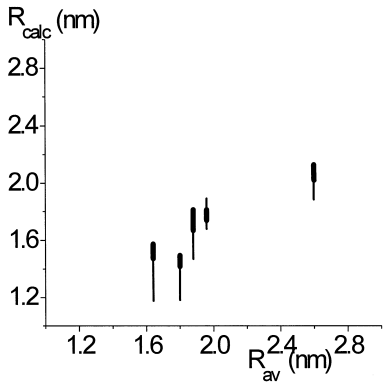



Fig. 5. The calculated optimum radii plotted against averaged radii of enzyme's globule. Thick bars correspond to parameters resulting in activation energy of order of 10 kcal, thin bars show the full range of calculated values. The enzymes (from left to right): lysozyme, papain, α-chymotrypsin, subtilisin, carboxypeptidase II. The last enzyme is a dimer of two identical subunits having active sites disposed close to opposite surfaces of dimer and acting independently. In calculation of its effective 'experimental' radius, the mass of one subunit was employed.

used, and a minimum information employed in these calculations. Nevertheless, in spite of all approximations, our analysis has led us to two important results:

1. the calculated optimum radii have a correct order of magnitude close to the experimental sizes of real enzymes' globulae; it is worth mentioning here that our earlier calculations [26] carried out in the framework of a non-rigorous formalism resulted in the radii almost twice smaller.
2. the relative values of the calculated radii agree with the relative dimensions of the real enzymes.

In the last aspect, especially interesting are the data on the typical representative of three different classes of serine peptidases: α-chymotrypsin, subtilisin, and carboxypeptidase II. These enzymes are a well-known example of a convergent evolution: they have no common in their sequence and general fold, but have the same catalytic triad Asp–His–Ser [49]. The disposition of these active residues in the globule is rather similar in α-chymotrypsin and subtilisin, but substantially different in carboxypeptidase II; in the latter enzyme, His and, especially, Asp are buried in the globule markedly deeper than in two others [44]. These differences in the geometry of the active site proved to be enough to result in a substantial increase in radius (and, therefore, molecular mass) for carboxypeptidase II, while α-chymotrypsin and subtilisin, similar in the active site geometry, have almost coinciding dimensions.

It should be stressed that the agreement between calculated optimum radii and the experimental ones has been achieved here without any adjustable parameter, only on the basis of the reaction mechanism and the geometry of the active site. This shows that the effect of the medium (protein and water) polarization really plays a very important role in determining the enzymes' dimensions.

## 3. The medium polarization and pre-existing field effects: comparison of two contributions

Eq. 1 describes the activation energy of the elementary act of charge transfer proper, the so-called 'ideal' or 'true' activation energy. However, to compare the reaction kinetics in an aqueous phase and in enzyme, it is necessary to consider also the free energy of the reactants transfer from water into protein, $\Delta G_{tr}$. The sum of these components gives us the effective activation energy

$$\Delta G^{\neq}_{eff} = \Delta G_{tr} + \Delta G^{\neq} \quad (7)$$

$\Delta G_{tr}$ depends both on the general dielectric response and on structural factors. It includes a large positive contribution due to transfer of ions from water into a low-dielectric protein. The protein's low dielectric constant causes two opposite trends (in $\lambda_s$ and $\Delta G_{tr}$), and hence a special analysis is necessary to find out what of the tendencies is the prevailing one.

In our previous paper [50], we have analyzed the problem in the framework of a non-rigorous 'fixed-displacement-field' formalism. As shown in Section 2, the correct 'fixed-charge-density' approach results in the reorganization energy substantially lower than that estimated earlier. Therefore, it becomes necessary to reconsider the whole problem. The new and more correct analysis of this paper confirms the principal qualitative conclusions of the previous work.

### 3.1. Medium polarization effects

As described above, in the 'fixed-charge-density'

formalism [27–29], the reorganization energy can be calculated as the difference of total charging energies ($W$) in two different (o and s) media

$$\lambda_s = W^o - W^s \quad (8)$$

$$W^j = B_1^j + B_2^j - C^j \quad (9)$$

Here $B$ is the energy of charging of each particle separately, $C$ is the absolute value of their interaction energy, all corresponding quantities should be calculated using $\varepsilon_o$ and $\varepsilon_s$ ($j$ = o or s).

In the following, we will try to consider the problems in a most general and straightforward form. Only in the case where we will need some quantitative examples, we will use the calculations in the framework of the planar boundary model (Fig. 1B). They are representative enough, because, as shown in Section 2, the results for globulae of optimum radii are close to this simple limiting case. Furthermore, this model describes, in a good approximation, the intra-membrane charge transfers proceeding closer to one side of the membrane.

For the reorganization energy, Eqs. 8 and 9, hold true for any kind of charge transfer reactions. At the same time, the electrostatic component of the reaction equilibrium energy depends on the type of the process. Therefore, let us consider some of the typical cases separately.

### 3.1.1. Charge separation

In the charge separation process, the charge transfer between two initially neutral particles results in charging them to $+e$ and $-e$ (involving, of course, their interaction)

$$\Delta G_{pol} = B_1^s + B_2^s - C^s \quad (10)$$

Index s is used here and later on because the equilibrium reaction energy is determined by the equilibrium static value of $\varepsilon_s$. Substituting Eqs. 8–10 in Eq. 1 we obtain

$$\Delta^{\neq} = \frac{(W^o + \lambda_i + \Delta G_i)^2}{4\lambda} \quad (11)$$

We see that the numerator of this expression does not contain any term depending on charging in the static medium, i.e. it is independent of $\varepsilon_s$. This result was obtained earlier for a homogeneous [51] and an arbitrary inhomogeneous [31] medium.

Let us compare activation energies for two cases: (1) reaction in a protein contacting an aqueous phase, i.e. in an inhomogeneous medium (we will mark the corresponding quantities by a subscript p); and (2) the same reaction in an infinite aqueous solution (subscript w).

The inner-sphere component of the reorganization energy $\lambda_i$ is the same in both cases. Let us suppose initially that the same is true for the 'structural' component $\Delta G_{str}$ (that means, we neglect here the effect of the intraprotein electric field $\Delta G_{ef}$ and suppose $\Delta G_{str} = \Delta G_i$; we will turn to this point in Section 3.2).

For proteins, $\varepsilon_o$ is somewhat higher than for water. Therefore, $W_p^o$ can be expected to be a little bit smaller than $W_w^o$ (but not in a simple proportion to $\varepsilon_o$: note positive correction terms for inhomogeneous medium, e.g. in Eqs. 5a–c due to $K_o > 0$; for the infinite aqueous medium, these corrections are absent). So, the numerator of Eq. 1 is somewhat smaller for $\Delta G_p^{\neq}$ than for $\Delta G_w^{\neq}$. At the same time, the denominator of this expression is substantially smaller for the process in protein: $\lambda_p < \lambda_w$ because, at rather close values of $W_p^o$ and $W_w^o$, $W_p^s \ll W_w^s$ due to a great difference of static values of $\varepsilon_s$ ($\sim$4 and 78). This is a general feature of proteins, and therefore we can conclude that, in the framework of the model describing proteins as a structureless dielectric, the activation free energy for charge separation in proteins should be essentially higher than in water.

### 3.1.2. Charge separation in the presence of the third ion

The reaction free energy of the charge separation may be made more favorable if a third charged particle is present in a definite position so that the field of this ion assists the charge separation. In this case

$$\Delta G_p^{\neq} = \frac{W_1^o + \lambda_i + \Delta G_{str} + C_i)^2}{4\lambda_p} \quad (12)$$

where $C_i$ is the interaction energy of the third ion with two ions forming in the course of the charge separation process

$$C_i = \frac{e^2}{\varepsilon_p}\left[\frac{1}{R_{13}} - \frac{1}{R_{23}} + Kf(d_1, d_3, R_{13}) - Kf(d_2, d_3, R_{23})\right] \quad (13)$$

Here $R_{13}$, $R_{23}$ are the distances between corresponding ions, the last two terms present symbolically the image charge correction: $K$ reflects the difference of dielectric constants (e.g. in the case of a planar boundary, $K=(\varepsilon_p-\varepsilon_w)/(\varepsilon_p+\varepsilon_w)$), $f$ is the function of geometric parameters. With $R_{13} > R_{23}$, i.e. with a favorable position of the third ion, $C_i < 0$, and, therefore, the situation is quite possible when $\Delta G_p^{\neq} < \Delta G_w^{\neq}$ (in aqueous solution we will not consider the interaction with the third ion, taking into account a low probability of the triple collision as compared to collision of two particles and the weak electrostatic interaction in the high-dielectric medium).

The picture becomes quite different when we consider the effective activation energy including the energy spent for the transfer of the third ion from water into protein, i.e. the energy necessary to create the favorable conditions for the elementary act of the charge separation proper.

$$\Delta G_{tr} = e^2\left(\frac{1}{\varepsilon_p^s} - \frac{1}{\varepsilon_w^s}\right)\frac{1}{2a_3} + Kf(d_3) \approx \frac{e^2}{\varepsilon_p^s 2a_3} + Kf(d_3) \quad (14)$$

The first half of this expression is a large positive quantity always exceeding the last term, and therefore $\Delta G_{tr} > 0$, and the effective $\Delta G_{eff}^{\neq}$ increases substantially. This increase makes $\Delta G_{eff}^{\neq} > \Delta G_w^{\neq}$. Let us illustrate this by an example.

We will use an approximate form of Eq. 1 valid under usual condition $|\Delta G| \ll \lambda$

$$\Delta G^{\neq} = \frac{\lambda}{4} + \frac{\Delta G}{2} \quad (1a)$$

In our case,

$$\Delta G_p^{\neq} \approx \frac{\lambda_p}{4} + \frac{\Delta G_{str} + W_p^s + C_i}{2} \quad (12a)$$

Therefore, to find the influence of the third ion on the effective activation energy, we have to calculate the sum $\Delta G_{tr}+C_i/2$.

Let us perform these calculations using the planar boundary model with all three particles situated on the same normal to the boundary. For the simple case of $a_1 = a_2 = a_3 = a$ we obtain

$$\Delta G_{tr} + \frac{C_i}{2} = \frac{e^2}{\varepsilon_p}\left(\frac{3}{8a} + \frac{7K}{240a}\right) \quad (15)$$

The sum under interest is a large positive quantity; for instance, at $a = 0.2$ nm and $\varepsilon_p^s = 4$ it equals to 0.62 eV. This is substantially larger than $\lambda_{sw}/4 \approx 0.45$ eV. The effective activation energy in protein exceeds the true activation energy in water by

$$\Delta G_{eff}^{\neq} - \Delta G_w^{\neq} = \frac{\lambda_{sp} - \lambda_{sw}}{4} + \frac{W_p^s - W_w^s}{2} + \Delta G_{tr} + \frac{C_i}{2} \quad (16)$$

As $\Delta G_{tr}+C_i/2 > \lambda_{sw}/4$ and $W_p^s > W_w^s$, we conclude that $\Delta G_{eff}^{\neq} > \Delta G_w^{\neq}$. Similar results were obtained with a different geometry of the system. Therefore, we can conclude that the energy expenditure for the transfer of the third ion from water to protein exceeds largely the gain in activation energy due to its favorable interaction with the charges formed in the course of reaction. Hence, the charge separation process in the presence of a third ion is effectively less favorable in protein (more precisely, in a structureless dielectric with the permittivity of protein) than in water.

### 3.1.3. Charges' recombination

The ideal activation energy

$$\Delta G_j^{\neq} = \frac{(W_j^o - 2W_j^s + \lambda_i + \Delta G_{str})^2}{4\lambda} \quad (17)$$

The activation energy in protein is, as a rule, lower than in water as $W_p^o \leq W_w^o$, and $W_p^s \gg W_w^s$. However, if we take into account the preceding ions' transfer from water to protein, i.e. if we add to $\Delta G_p^{\neq}$ the value $W_p^s - W_w^s$, the effective activation energy increases drastically. Using Eq. 1a one can obtain

$$\Delta G_{eff}^{\neq} - \Delta G_w^{\neq} = \frac{W_p^o - W_w^o}{4} + \frac{W_p^s - W_w^s}{4} \quad (18)$$

The first term here presents a small negative quantity, while the second is a large positive one. Therefore, in this case again the effective activation energy for the reaction in protein substantially exceeds the activation energy in water.

*3.1.4. Charge translocation*

Charge is shifted from one point to the other. As only one charged particle exists, the equilibrium energy does not involve the charge–charge interaction, only difference of charging energies B in two positions, 1 and 2. We will consider here the rate-determining step which should prefer the energetically favorable direction of the charge transfer, i.e. $B^s_{(1)p}-B^s_{(2)p}\leq 0$. The ideal activation energy in protein is lower than in water, $\Delta G^{\neq}_p < \Delta G^{\neq}_w$ (see, e.g. estimates in Section 2).

The effective value $\Delta G^{\neq}_{eff}$ is higher by the difference of charging energies of the initial reactant in globule and in water $B^s_{(2)p}-B^s_{(2)w} > 0$.

$$\Delta G^{\neq}_{eff}-\Delta G^{\neq}_w \approx \frac{\lambda_p-\lambda_w}{4}+\frac{B^s_{(1)p}+B^s_{(2)p}-B^s_{(1)w}-B^s_{(2)w}}{2} \tag{19}$$

Taking into account Eqs. 8 and 9, we obtain the other form of this equation

$$\Delta G^{\neq}_{eff}-\Delta G^{\neq}_w \approx \frac{W^o_p-W^o_w}{4}+\frac{1}{4}$$

$$(B^s_{(1)p}+B^s_{(2)p}-B^s_{(1)w}-B^s_{(2)w})-\frac{C^s_p-C^s_w}{4} \tag{19a}$$

The first term is small and negative, but both the second and third terms are positive and much larger than the first one. Therefore, the effective activation energy in protein is always higher than in water.

In the last two examples considered, we have not taken into account the possible decrease of the ideal activation energy by the third ion. As it is clear from considerations similar to the previous ones, the additional energy of the transfer of this ion from water into protein makes the effective activation energy even higher.

### 3.2. The role of the pre-existing intraprotein electric field

We have considered above practically all principal cases of the charge transfer processes in enzymes as compared to similar reactions in aqueous solutions. Describing the protein as some structureless dielectrics, we have found that the ideal (or true) activation energy in this medium is lower than in aqueous solution (the only exception is the charge separation between two initially neutral reactants). However, the effective activation energy (i.e. the activation energy of the enzymatic reaction proper plus the energy of the transfer of charged particles from water into protein) is always higher than the activation energy of the same reaction in aqueous solution.

The last conclusion, contradicting seemingly the experimental fact of the high catalytic activity of enzymes, is based on the structureless dielectric model, i.e. it does not account for the effect of the intraprotein field, both in the equilibrium reaction free energy and in the energy of ion transfer. As revealed by several calculations based on the known crystallographic structures of corresponding proteins, the unfavorable loss of solvation energy in low-dielectric proteins as compared to water is largely compensated for by the effect of an intraprotein electric field (for reviews see, e.g. [2,3,52–56]). This compensation cannot take place at any point inside the globule, but at definite reactants' positions inside the active site of enzyme, hence the effect is strongly structure-dependent.

In living nature, an intricate catalytic complex with a rather fixed structure is necessary to overcome the entropy restraints, in particular, to put together two or more reactants in a proper orientation, to ensure the high specificity of binding, etc. However, such a complex is inevitably fairly large in size, and, due to its rigidity, has a rather low dielectric constant. The latter results in a low reorganization energy, and, as discussed above, this factor demands for an additional increase in the globule size that provides a better charge screening. However, all this leads to an additional energy consumption in transfer of charged particles into the macromolecule. The medium preorganization, i.e. the internal electric field is necessary to compensate for this unfavorable effect.

The intraprotein electric potential may compensate the loss of the Born solvation energy to various extents. An exact compensation is not necessary: in the case the equilibrium $\Delta G$ of the elementary act of reaction becomes comparable with the corresponding value in aqueous solution, a substantial acceleration will already take place due to decrease in the reorganization energy. An additional acceleration can be

achieved by decreasing $\Delta G$ to the level lower than in water. However, there should be some limit for lowering the energy of the charge transfer: when electric field produces a very strong stabilization of a charged state then its subsequent transformation (or release as a free ion) will be strongly retarded. Therefore, solely the pre-existing intraprotein field cannot result, in general, in a very high acceleration of the total process because there should be a limit for lowering of the charged state equilibrium energy. However, a substantial decrease in activation energy can be achieved due to low reorganization energy. So, we see that only the combined action of two factors, viz. the pre-existing field and a low reorganization energy (low dielectric constant), is the most effective way to ensure enzymes' high catalytic activity.

The combination of high polarity of monomeric units and their fixation inside a relatively rigid structure distinguishes proteins from other types of polymers, like polyhydrocarbons or polycarbohydrates. Thus we can hypothesize that precisely this feature of proteins gave them an advantage over some other types of biopolymers in the natural selection of the 'construction material' for enzymes.

A special case presents a quite different class of biological catalyst, namely the ribozymes (for review, see, e.g. [57]). These ribonucleic acids catalyze effectively transesterification and hydrolysis, i.e. reactions accompanied by a substantial charge transfer. In spite of their chemical difference, some analogy can be found between RNA and proteins: the backbone of RNA contains a regular arrangement of highly polar phosphodiester groupings, and the polar sidechains of purine and pyrimidine bases are linked to this backbone. Recently, the first X-ray crystallographic structure of one domain of the *Tetrahymena* ribozyme has been obtained showing a rather dense packing of these structural elements [58]. So, in ribozymes we may meet with the same principle as in proteins: a high concentration of strongly polar groups substantially restricted in their orientational mobility. With the further accumulation of the relevant structural information, it would be extremely interesting to analyze the ribozymes function on the same physical basis as outlined above for the proteinaceous enzymes.

## 4. Conclusion

We have considered here some factors influencing the activation energy of enzymatic charge-transfer reactions. The specific property of proteins as polar media is the highly organized and relatively rigid spatial structure of the proteins' polar groups resulting in the existence of a permanent intraprotein electric field and in a protein's low dielectric constant. The first factor that is very structure-sensitive affects equilibrium free energy gap of a charge-transfer reaction. The second (medium polarization effect) influences both the equilibrium and non-equilibrium (reorganization) energies.

In this paper, we have shown that, for a heterogeneous system protein–water, the charge-transfer reorganization energy is substantially lower than in aqueous solutions. It was shown that the medium polarization effects largely determine the geometry of the globular enzymes, in particular, a range of optimum globule radii ensuring the almost lowest activation energy and, at the same time, a minimal expenditure of 'the construction material', i.e. protein. For five hydrolytic enzymes with a known charge-transfer mechanism, the optimum globule radii have been calculated using the experimental geometry of their active sites. The calculated radii: (1) agree by their order of magnitude with the real sizes of these macromolecules; (2) agree satisfactorily with the relative dimensions of the globulae. It is worth mentioning that for three serine proteinases, viz. chymotrypsin, subtilisin, and carboxypeptidase II, having the same catalytic triad, but in different orientations relative to globule surface, both the calculated and experimental globule's dimensions differ substantially.

A low static dielectric constant, and hence a low reorganization energy, provide the true activation energy of many intraprotein charge transfers lower than for the same reaction in water. However, the effective activation energy accounting for the energy necessary to transfer the charged particle(s) from water into a low-dielectric structureless medium is always higher than for the process in purely aqueous surroundings. This unfavorable effect should be compensated for by the action of the pre-existing intraprotein electric field. The general principle of protein

structure, namely a rather rigid fixation of many highly polar groups, results in the existence of a permanent intraprotein electric field lowering the equilibrium reaction free energy, and in a low dielectric constant ensuring low reorganization energy. Only a combined action of these two effects results in a substantial decrease of activation energy, and this presents one of the important general physical reasons for the high catalytic activity of enzymes.

## Acknowledgements

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