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ENZYMATIC MECHANOCHEMISTRY: A NEW APPROACH TO STUDYING THE MECHANISM OF ENZYME ACTION

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Summary

1. Covalent binding of model enzymes, chymotrypsin and trypsin, to elastic polymer supports, nylon and viscose (cellulose) fibers, human hair, methacrylate rubber, has been effectuated. On mechanical stretching of the fibers, the catalytic activity of the enzymes bound to them decreases, and when they relax, it increases to the initial level. The data obtained by us fit the concept that the effect is due to reversible deformation of the bound enzyme molecules induced by fiber stretching.

2. Analysis of the dependence of the catalytic activity of the enzymes chemically bound to the fiber on the degree of fiber deformation shows that the reversible inactivation of the enzymes induced by support stretching occurs even if the deformation of the enzymes' molecules is as small as 0.5 Å.

3. The deformation of the enzyme molecules induced by fiber stretching entails a change in the substrate specificity of the biocatalysts, i.e. the activity towards "good" substrates decreases, and towards "poor" substrates increases.

4. The deformation of the enzyme molecules induced by fiber stretching results in a decrease of the specific catalytic activity of the biocatalyst, whereas its thermal stability increases.

5. The results obtained allowed a new, mechanochemical, approach to be suggested for studying major problems of enzymatic catalysis.

Introduction

The key problem of enzymology is the structure-function relationship of an enzyme. The logical way to tackle this problem would be to subject an enzyme

protein globule to a certain mechanical deformation without changing the environment, i.e. to alter the structure of the biocatalyst and then see how the enzymatic function has been affected. However, this can hardly be realized because enzyme molecules are too small compared to any macroscopic level through which mechanical action is effectuated (e.g. compared to man's fingers).

Another approach is yet possible. Let us imagine that we have succeeded in attaching an enzyme's molecule to a macroobject which can readily be deformed mechanically, e.g. to an elastic fiber. It may be expected that, if the fiber is stretched, the attached molecules of the enzyme should also be deformed. Then, comparing the enzymatic properties of the native and deformed (to a given degree) molecule of the biocatalyst, one may obtain unique information about the mechanism of its action. This is the principle which was laid as the basis for the mechanochemical approach to studying enzymatic catalysis developed in this laboratory.

This work is actually an application of this new approach to chymotrypsin and trypsin, which are most comprehensively studied enzymes. Nylon and viscose fibers, human hair and methacrylate rubber films were used as elastic supports to which the enzyme molecules were covalently attached.

Experimental

Materials

Bovine α-chymotrypsin and trypsin used were products of the Olaine chemical reagents plant (U.S.S.R.). The active site concentration in the enzyme preparations, determined by spectrophotometric titration as described for chymotrypsin [1] and for trypsin [2], was 70 and 53%, respectively.

Nylon fibers (140 0.03 mm filaments plaited together) used as elastic support for the enzymes were obtained from the All-Union Chemical Fibers Institute, type No. 107; also used: fair human hair (see Acknowledgement), viscose (cellulose) fibers (150 0.02 mm filaments woven together), a kind gift of the All-Union Institute of Rubber Industry, type 5.55; methacrylate rubber was kindly given to us by Dr. E.V. Zimin (All-Union Research Institute of Synthetic Rubber), type SKS-30 No. 1289.

The agents for the binding of the enzymes to supports were glutaraldehyde (25% solution, Merck), 1-cyclohexyl-3 (2-morpholinoethyl)-carbodiimide metho-*p*-toluene sulfonate (Sigma), polyalanine (mol. wt. > 3000, Reanal), hexamethylenediamine, sodium metaperiodate and 10 M HCl (all commercial preparations of Reakhim).

The components of the buffer solutions and other reagents employed are analytical grade Reakhim products.

Substrates for trypsin were *N*-tosyl-L-arginine methyl ester or *N*-benzoyl-L-arginine ethyl ester (both from Reanal); for chymotrypsin, *N*-acetyl-L-tyrosine ethyl ester (Reanal). The enzymes were titrated by *N*-trans-cinnamoyl imidazole (Serva) and *p*-nitrophenyl *p'*-guanidinebenzoate (synthesized as described in [2]). Phenylmethyl sulfonylfluoride (Serva) and *p*-nitrophenyl trimethylacetate (synthesized as described in Ref. 3) were the acylating agents.

N-Acetyl-D-tryptophan, a substrate-like inhibitor for chymotrypsin, was a commercial Reanal preparation.

Enzyme-support binding

The covalent binding of the enzymes to nylon fibers was performed according to Sundaram and Hornby [4], where the binding of urease to nylon tube is described. A nylon fiber 6 m long was folded into a ring and treated for 1.5 h with 3 M HCl at 45° C with stirring. Then the partially hydrolyzed fiber was thoroughly washed with water, 0.1 M $NaHCO_3$ and again with water, after which at pH 9.4 (0.2 M $NaHCO_3$) and 4° C it was modified for 15 min with 2.5% glutaraldehyde, which forms Schiff's bases with NH_2 groups of the support. The fiber obtained as a result of all these manipulations is rather elastic, it can be reversibly stretched by approx. 30%. The enzymes were bound both to unstretched and stretched (by 25%) fiber. To this end, after glutaraldehyde had been washed off with water, the fiber was treated for 1.5 h at 4° C with 0.1% enzyme solution at pH 8.0 (0.03 M KH_2PO_4), which via its NH_2 groups binds to the carbonyl groups of the support-bound glutaraldehyde. Non-covalently bound enzyme was washed off with water, 1 mM HCl, 0.1 M $NaHCO_3$, 1 M NaCl and again with water. The resulting enzyme-carrying fibers were stored in water at 4° C.

We also bound chymotrypsin to nylon fiber via a long spacer of polyalanine of a molecular weight of >3000. Carboxy groups of the partially hydrolyzed fiber (with the acid, see above) were activated with 3 mg/ml aqueous solution of 1-cyclohexyl-3(2-morpholinoethyl)-carbodiimide metho-*p*-toluene sulfonate at pH 4.65 for 1 h. After washing, the carboxy groups of the support were "blocked" through treatment of the fiber with 0.16 M glycine methyl ester at pH 8.4 (10^{-2} M borate) for 1 h. Then the washed fiber was treated with glutaraldehyde (see above) and incubated for 18 h with 0.1% alcohol solution of polyalanine. The polyalanine-modified fiber was then treated with carbodiimide (see above), hexamethylenediamine (this procedure resembled that with glycine methyl ester), then with glutaraldehyde and, finally with the enzyme.

The covalent binding of the enzymes to human hair was performed as follows. A 40-cm long strand of hair (approx. 20 hairs) was treated with glutaraldehyde and then, after washing, incubated with a solution of the enzyme (see above).

Covalent binding of the enzymes to viscose (cellulose) fiber was carried out in accordance with the procedure described by Flemming et al. [5], somewhat modified. 2.5 m of viscose fiber was wound around a glass rod and incubated, with stirring, for 30 min at room temperature in a 0.4 M aqueous solution of $NaIO_4$. This procedure results in the cleavage of a part of the saccharide rings, aldehyde groups being formed. Then the fibers, washed first with water, then with a 3% solution of $Na_2S_2O_3$ and again with water, were incubated at 4° C for 18 h in a 0.1% solution of the enzyme at pH 8.5 (0.2 M H_3BO_3). The non-covalently bound enzyme was washed off as descibed above.

Covalent binding of the enzymes to methacrylate rubber was carried out as follows. A 2 mm × 3 mm × 50 cm band was cut out of a rubber sheet; the carboxy groups on its surface were activated by carbodiimide (see above). Af-

ter washing with water, the band was incubated at 4°C for 16 h in a 0.1% enzyme solution at pH 8.0 (0.03 M KH_2PO). The non-covalently bound enzyme was removed (see above). The ends of the band were connected with a metal clip, and it was ready to be used in mechanochemical experiments *.

Determination of the activity of support-bound enzymes

The enzymatic activity of the support-bound chymotrypsin and trypsin were followed by the steady-state rate of hydrolysis of the specific substrates in a pH-stat (Radiometer TTT 1c). 40—60 cm enzyme-carrying fibers were wound around a special stretching device. This device consists of a rod with right-hand threading on the upper half and left-hand threading on the lower. Both halves have matching rollers with the corresponding threading. When the rod rotates the rollers move along it in different directions, now towards and now from each other. If fiber is wound between the rollers it is stretched, the degree of stretching being determined by the distance between the rollers.

The stretching device with an enzyme-carrying fiber wound around it was placed in a thermostated cuvette of a pH-stat, containing 30 ml of $6 \cdot 10^{-3}$ M solution of the substrate (*N*-acetyl-L-tyrosine ethyl ester for chymotrypsin; *N*-tosyl-L-arginine methyl ester or *N*-benzoyl-L-arginine ethyl ester for trypsin) in 0.1 M KCl. The acid liberated as a result of the enzymatic reaction was titrated with $2 \cdot 10^{-2}$ M KOH. The reaction was performed at pH 8.0 and 25°C.

Study of the kinetics of interaction of nylon fiber-bound enzymes with acylating agents

We studied the effect of stretching of nylon fiber on the kinetics of acylation of bound chymostrypsin by phenylmethyl sulfonylfluoride and *p*-nitrophenyl trimethylacetate. The chymotrypsin-carrying fiber was incubated in $5 \cdot 10^{-5}$ M aqueous solution of the acylating agent (+1% isopropanol) at pH 7.0 (0.02 M KH_2PO_4) and 25°C. After a certain period of time the fiber was taken out of the solution, thoroughly washed with water, and the relative enzymatic activity of the unstretched fiber was determined in a pH-stat.

Results and discussion

Regulation of catalytic activity of enzymes chemically bound to a polymer fiber induced by its mechanical deformation

(A) Enzymes bound to unstretched fiber. The stretching of nylon fiber induces an approx. 3-fold decrease in the enzymatic activity of covalently bound chymotrypsin (see Fig. 1a). After relaxation of the fiber, which is almost instantaneous, the activity of the enzyme bound to it increases and reaches the initial level. This "stretch-relax" procedure may be repeated many times with the "decrease-increase" of the enzymatic activity being retained. Similarly, the catalytic activity of trypsin bound to nylon fiber may be regulated by mechanical action (Fig. 1b). By varying the concentration of the substrates (*N*-acetyl-L-

* The rubber band proved to be too rigid and we failed, with our stretching device, to evolve correctly the dependence of the activity of the rubber-bound enzyme on the degree of the rubber stretching.

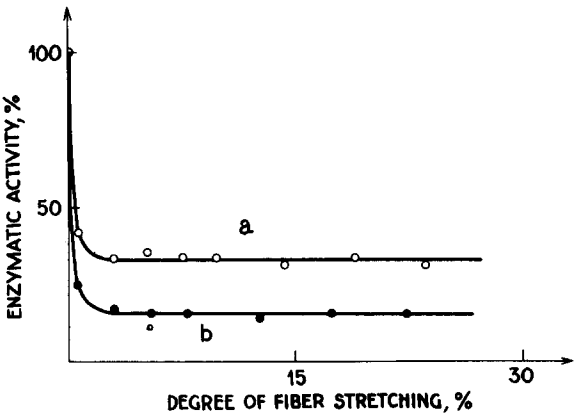

Fig. 1. Dependence of relative enzymatic activity of chymotrypsin (a) and trypsin (b) covalently bound to unstretched nylon fiber, on the degree of the fiber stretching. Substrates: (a) *N*-acetyl-L-tyrosine ethyl ester; (b) *N*-tosyl-L-arginine methyl ester and *N*-benzoyl-L-arginine ethyl ester. For conditions, see Experimental.

tyrosine ethyl ester in the case of chymotrypsin and *N*-tosyl-L-arginine methyl ester in the case of trypsin), we have found that, when fiber is stretched, the Michaelis constants of the enzymatic reactions do not change, but maximum rates decrease.

The result obtained cannot be accounted for by diffusion: we have purposely shown that the mechanochemical effect does not depend on the following factors.

(a) On the rate of stirring (during measurement of the catalytic activity of the enzymes bound to the fiber). Had this process been controlled by external diffusion (i.e. by the diffusion of the substrate from the solution to the surface of the fiber), an increase in the rate of stirring would have eliminated diffusion inhibition; see review [6].

(b) On the temperature in the 15—35°C range. Thereby the activation energy of the enzymatic process catalyzed by chymotrypsin both on the stretched and unstretched fiber, is 11 kcal/mol. And it is known [6] that external diffusion-controlled processes in water have an activation energy of 4—5 kcal/mol.

(c) On the concentration of the substrate, as on stretching of the fiber, V changes, but $K_{m,app}$ does not. It is known, however [6], that diffusion limitations affect $K_{m,app}$, rather than V.

(d) On the quantity of filaments woven together in the fiber. The change in this parameter may be actually viewed upon as a change in the size of immobilized enzyme particle which, as indicated in [6], is an adequate diffusion test.

(e) On the activity of the fiber-bound enzyme (on its being changed six-fold). Alteration of this parameter should necessarily alter the degree of diffusion control of the reaction [6].

Thus, the mechanochemical effect does not depend on these factors, the change in each of which should have unfailingly altered its character both quantitatively and qualitatively, had it been diffusion-dependent (see review, [6]).

Another explanation of the effect (Fig. 1) may be a change in the properties of the microenvironment (ionic state, hydrophobicity, dielectric constant

etc.)* of the enzymes bound to the fiber when the support is deformed [7—9]. This should also be ruled out for the following reasons. The presence and the value of the mechanochemical effect do not change (a) when the ionic strength is varied from 0.01 to 3 M KCl, which affects electrostatic interactions; (b) when pH is changed from 7.0 to 8.5; (c) on addition of isobutanol (up to 10%), which should have weakened hydrophobic interactions; (d) on changing of the character of the surface to which the enzyme is bound. The character of the surface of the nylon fiber was changed in the following way. Chymotrypsin bound to the fiber was totally inactivated by incubation in water at 100° C, then the fiber was treated by glutaraldehyde, washed, the enzyme bound to it again and this enzyme was thermoinactivated. This procedure was repeated three times. As a result, we had nylon fiber coated with inactivated protein. To this coating, chymotrypsin was bound with the help of glutaraldehyde; on stretching the fiber, the activity of the bound enzyme reversibly decreased (Fig. 2a). At the same time it is quite evident that in this case the microenvironment of the enzyme was quite different from when the enzyme was bound to the nylon fiber.

Finally, chymotrypsin was bound to other supports: human hair, viscose fiber, methacrylate rubber. In all these cases, when the support was stretched, the activity of the bound enzyme decreased, and when it relaxed the activity increased again (Fig. 2b and c).

On the basis of these data, one is bound to attribute the effect of regulation of the catalytic activity to stretching, which induces deformation of the molecules of the enzyme. The gist of the model, schematically represented in Fig. 3,

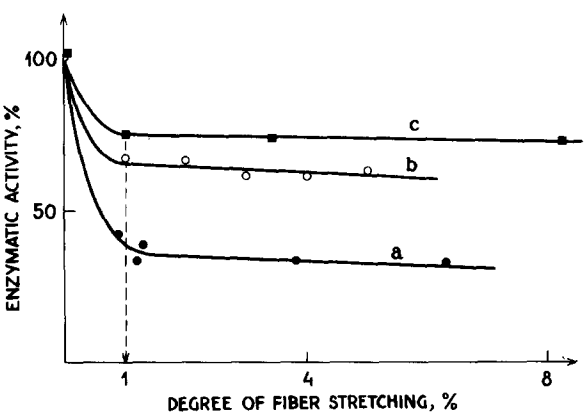

Fig. 2. Dependence of the relative activity of enzymes covalently bound to different elastic supports on the degree of stretching of the supports: (a) chymotrypsin on protein-coated nylon fiber; (b) trypsin on human hair; (c) chymotrypsin on viscose (cellulose) fiber. For conditions, see Experimental.

* An additional mechanism suggested, that the accessibility of the active site of the fiber-attached enzyme for the substrate diminished when the fiber was stretched, cannot be accepted for the following reason. In terms of this mechanism, it should be expected that the deformation through stretching of the support should always result in nothing but a decrease in the enzyme's activity. What we observe in reality is that with certain substrates the activity of the enzymes does decrease when the supporting fiber is stretched (*N*-acetyl-L-tyrosine ethyl ester with chymotrypsin and *N*-tosyl-L-arginine methyl ester and *N*-benzoyl-L-arginine ethyl ester with trypsin; see Figs. 1,2). But in the case of other substrates (*p*-nitrophenyl trimethyl-acetate and phenylmethyl sulfonyl-fluoride with chymotrypsin and *p*-nitrophenyl *p'*-guanidinebenzoate with trypsin), the activity of the enzymes increases (see for example Fig. 6).

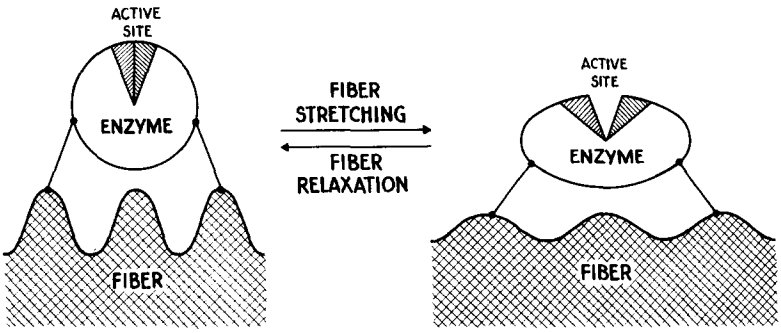

Fig. 3. Schematic representation of the deformation of the enzyme bound to a mechanically stretched elastic fiber.

is the following: if a molecule of the enzyme is attached to the support by more than one chemical bond, the distance between the points of attachment should increase on stretching; and the molecules of the bound enzyme should become deformed and inactivated. On relaxation of the fiber the initial distance between the points of attachment is restored and the enzyme renaturates.

(B) Enzymes bound to stretched fiber. If chymotrypsin (or trypsin) is bound to stretched nylon fiber which then is allowed to relax, the activity of the support-bound enzyme increases (Fig. 4a), and decreases again after stretching. Thereby, the curve of dependence of the enzymatic activity on the degree of the fiber deformation is similar to that of the dependence of chymotrypsin (or, respectively, trypsin) bound to unstretched fiber.

To explain this increase of catalytic activity of the enzyme bound to stretched fiber, on relaxation of the support, let us analyse the possible modes of enzyme-support binding. We shall proceed from the concept, now accepted [10,11], that under the action of thermal motion, the catalytically active (folded) and inactive (unfolded) conformations of the enzyme are in equilibrium in the solution. Hence, the molecule of the enzyme may become attached to the support (and, if the binding is multipoint, become fixed on it) both in its catalytically active (folded) conformation (Fig. 5,A) and catalytically inactive

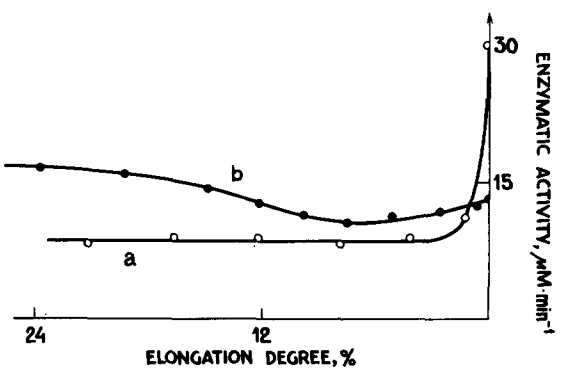

Fig. 4. Change in relative enzymatic activity of chymotrypsin covalently bound to a stretched nylon fiber, on its relaxation: (a) the enzyme is bound to a stretched fiber in a buffer solution; (b) the enzyme is bound to a stretched fiber in a buffer solution in the presence of 0.2 M *N*-acetyl-D-tryptophan, a substrate-like inhibitor of chymotrypsin. For other conditions, see Experimental.

(unfolded) conformation (Fig. 5,B). The probability of this latter fashion of binding is quite high, as the area on the surface of the fiber seized by the unfolded protein molecule (and hence the quantity of accessible potential binding sites) is greater than in the case of a folded molecule.

So let us assume that both fashions of binding (Fig. 5,A and B) are realized. If the enzyme was bound to unstretched fiber, which was subsequently stretched, the catalytically inactive molecules (Fig. 5,B) would, naturally be inactive all the time, because their unfolded structure would undergo further deformation when stretched. If the enzyme was bound to prestretched fiber, the activity of the molecules with a regular conformation (Fig. 5,A) would decrease on its being relaxed, while the activity of the molecules with irregular conformation would increase. It seems that it is predominantly the unfolded form that becomes attached and due to this, the overall effect is that of increase.

This model (Fig. 5,A and B) leads itself to experimental verification. Let us assume that we attach the enzyme to stretched fiber in the presence of a high concentration of an effective substrate-like inhibitor. The inhibitor should shift the equilibrium between the active and inactive conformers of the enzyme in the solution towards the former, as it binds with them only [10,11]. This means that the enzyme would bind predominantly in its active, folded conformation. This would result in (a) the initial activity of the bound molecules of the enzyme being higher than in the absence of an inhibitor; (b) a small increase in the activity of the bound enzyme as the proportion of the enzyme molecules bound in an unfolded conformation is rather low; (c) in the fact that, on relaxation of the fiber, the activity of the bound enzyme would even decrease, as its active conformation would be distorted.

We attached chymotrypsin to stretched nylon fiber in the presence of *N*-acetyl-D-tryptophan, a substrate-like inhibitor of the enzyme [12]. A study of the resulting preparation revealed that all the three predicted effects do take place (see Fig. 4a and b). This means that the experimental results obtained fit the model of the mechanochemical effect we previously suggested.

To sum up, external mechanical action may deform the molecules of a biocatalyst* and thus regulate the catalytic activity of the enzyme bound both to

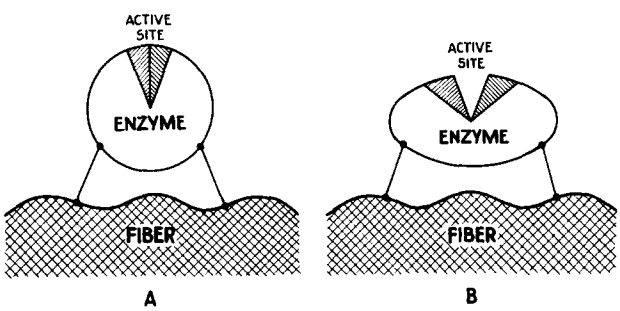

Fig. 5. Schematic representation of multipoint binding of a molecule of the enzyme to the support (A) in a regular, catalytically active, folded conformation; (B) in an irregular, catalytically inactive, unfolded conformation.

* Unfortunately, we failed to prove, because of the quantity of the support-bound enzyme being low, that stretching the fiber induces conformational change in the molecules of the bound enzyme, with the help of a direct physical method (i.g. by paramagnetic or fluorescent labels, etc.)

unstretched (Fig. 1 and 2) and stretched (Fig. 4) fiber.

Finally, mention should be made of one more fact. An analysis of the dependence shown in Figs. 1 and 2 makes one ask why the catalytic activity of fiber-bound enzymes decreases several-fold, but does not disappear on stretching of the fiber. This, in all likelihood, is associated with the fact that a part of the support-bound molecules hardly ever reacts to stretching of the fiber, being for example linked to the fiber with a single bond.

Change in substrate specificity of chymotrypsin bound to nylon fiber on its being stretched

It is shown in Fig. 1 that on stretching of the nylon fiber, the activity of the chymotrypsin bound to it towards *N*-acetyl-L-tyrosine ethyl ester, a specific substrate for the enzyme, decreases. This means that the deformation of the molecule of a biocatalyst results in its becoming inactive with respect to a specific substrate. In this connection it was very interesting to see how deformation of the structure of the enzyme (induced by fiber stretching) affected the catalytic activity of the enzyme with respect to a non-specific substrate.

We have studied the behaviour of fiber-bound chymotrypsin with quasi-substrates, *p*-nitrophenyl trimethylacetate [3] and phenylmethyl sulfonylfluoride [13], which only acylate the enzyme without subsequently deacylating it. It was revealed that when the fiber was stretched, the rate of acylation of the enzyme bound to it by the quasisubstrates increases approx. 2—3 times. Thus, when fiber-stretching induced deformation of a chymotrypsin molecule, its substrate specificity was altered, i.e. the activity towards a specific (good) substrate decreases, and towards non-specific (poor) substrate increases (Fig. 6).

This may probably be accounted for in the following way. In the case of a specific substrate when multipoint (in our case, three-point [14]) interaction with the active site of an enzyme takes place, congruence of all the regions of the active site to each other is indispensable. Even insignificant deformations of

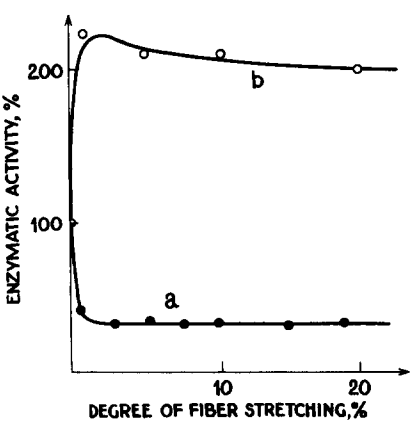

Fig. 6. The effect of stretching of a nylon fiber on the rate of reaction of chymotrypsin attached to it (a) with a specific (good) substrate, *N*-acetyl-L-tyrosine ethyl ester and (b) with a non-specific (poor) substrate, *p*-nitrophenyl trimethyl acetate. For conditions, see Experimental.

the structure of an enzyme may entail drastic decrease in its reactivity towards a good substrate. With a poor substrate, there is no need for the active site to be so congruent and a deformation in the molecule of an enzyme (Fig. 3) may result in the active site being more accessible for the substrate, i.e. the rate of reaction will increase.

Dependence of the catalytic activity of enzymes bound to elastic fiber on the degree of support stretching

It is obvious from Fig. 1 that both in the case of chymotrypsin and trypsin bound to nylon fiber, a change of enzymatic activity is fully realized with a very low degree of fiber stretching, i.e. less than 1%; as the degree of deformation of the support increases further, the activity of the bound enzyme remains constant.

If one assumes that the molecules of the enzyme deform to the same degree as the fiber is stretched and assuming the size of a chymotrypsin molecule to be about 50 Å [15], one may conclude that the effect of change in the catalytic activity of the enzyme is evident when deformation of the molecule is lower than 50 Å × 0.01 = 0.5 Å.

One may object that when nylon fiber is stretched, due to the properties of its surface, the molecules of the enzyme stretch to a higher degree than the polymer chains of which the fiber consists. To consider this possibility, we changed the surface of the fiber, having coated it with inactivated protein (see above). The enzyme was then made to bind this protein coat. On the modified fiber being stretched, the activity of bound chymotrypsin decreased, the effect of mechanochemical inactivation (or, on relaxation of the fiber, reactivation) being fully realized even if the fiber is deformed by less than 1% (Fig. 2a).

Finally, we bound chymotrypsin and trypsin to quite different elastic support: human hair and viscose (cellulose) fiber. In both cases, the effect of reversible inactivation of the enzyme is fully realized as early as when the fiber is stretched by only 1% (Fig. 2b and c, respectively). This means that the value of 1% is the upper limit of fiber deformation indispensable for the occurrence of the inactivation; this does not depend on the nature of the support and so is determined by the properties of the enzymes themselves.

It follows from the above views that even a slight stretching of the fiber will instantaneously induce deformation of the protein molecule and inactivation of the biocatalyst. But if molecules of an enzyme are attached to the fiber with the help of a long, moderately rigid, spacer to achieve a given degree of deformation of an enzyme, the degree of fiber-stretching would by higher than if the enzyme was bound without the spacer. We effectuated the binding with the help of a polyalanine spacer to find out that, if without the spacer for the mechanochemical effect to be realized, 1% stretching is necessary, with the spacer the required stretching is approx. 4% (Fig. 7a and b, respectively). This is another piece of evidence supporting our explanation of the essence of the mechanochemical effect.

Thus the results obtained by us demonstrate that rather small distortions in the structure of the enzyme (< 0.5 Å) induce dramatic changes in its catalytic properties. This is indicative of the fact that an enzyme molecule is an exceedingly well tuned instrument for catalysis. It should also be pointed out that

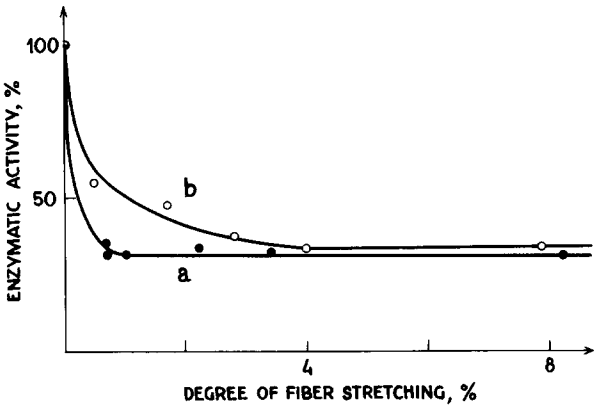

Fig. 7. Dependence of the relative enzymatic activity of chymotrypsin bound to a nylon fiber (a) without a long spacer and (b) via a long polyalanine spacer, on the degree of fiber stretching. For conditions, see Experimental.

the value of < 0.5 Å for conformational changes inactivating the enzyme obtained by the mechanochemical method throws doubt on the results of X-ray analysis (especially when no change is recorded in the structure of an enzyme in response to a given action). The resolution of X-ray analysis, which is a traditional method for studying conformational changes in proteins associated with their catalytic function, does not usually exceed 2 Å.

Change in the thermostability of chymotrypsin bound to nylon fiber on its being stretched

We have shown that, on stretching the fiber, the molecules of the enzyme attached to it undergo deformation. It was of interest to see how such deformation affects the properties of a biocatalyst. To this end, we studied the kinetics of thermoinactivation (at 40° C) of nylon-fiber bound chymotrypsin. It turned out that on stretching the fiber the initial rate of inactivation of the bound enzyme decreased by approx. 3-fold, i.e. the biocatalyst becomes more stable. Thus with the globule of the enzyme being stretched, the catalytic activity and thermal stability change in an adiabatic fashion, i.e. the former decreases whereas the latter increases.

Conclusion

As has been shown above, the mechanochemical approach developed by us allows one to study the intimate mechanisms of the functioning of the enzymes, to look from a different angle at the fundamental problems of enzymatic catalysis, such as the nature of high catalytic activity of the enzymes and their unique specificity, their ability to be regulated, their stability and many others which have not been touched upon in this paper. This permits us to consider the mechanochemical approach as being a valuable tool for studying enzymatic processes.

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