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The Role of Geometry and Elastic Strains in Dynamic States of Proteins

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Abstract. A theory is developed, where a linear macromolecule with geometrically constrained ends, elastically strained, exchanging energy with the solvent molecules through random collisions may provide a mechanism for the following specific functions in proteins: a) Induction of transient, oriented strains in substrates during transition between conformations. b) External variation of the rigidity and geometry of the active site. More generally, a macromolecule in solution possessing appropriate geometrical and elastic properties constitutes a machine, whose possible operations have common features with biological function such as passive transport, enzymatic catalysis and active transport. The theory suggests a quantitative law by which new information about the dynamical state of the protein molecule can be elucidated from the Arrhenius plot. It predicts a relationship between the rate of catalysis and the local viscosity of the solution.

Key words: Structural fluctuations – Conformation transitions – Molecular machine.

Introduction

The elastic properties of proteins have been discussed in relation to possible mechanisms for enzymatic catalysis. Koshland [1] suggested that the flexibility of the protein is important in the enzymatic specificity at maximal velocity of the catalysis by the occurrence of "induced fit" for productive binding between a substrate and the enzyme. Jencks argued [2] that "the substrate-induced change in the conformation of the enzyme may itself serve to induce strain in the substrate and facilitate reaching the transition state". The same is true for the product due to the rate acceleration in the reverse as well as in the forward direction of the reaction. In addition, the oscillation of an enzyme in its free or complexed state between two conformations has been suggested as an "ideal mechanism" for catalysis [2]. The energy source for

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such oscillations assumed [3] to be the thermal bombardment of the protein by the surrounding water molecules in the solution. Careri [4] suggested that time-correlated fluctuations of some relevant conformational variables are "the driving force which causes the enzyme-substrate complex to evolve along the chemical pathway". He proposed that the charge density fluctuations of the bath and conformation fluctuations of the protein induce the above correlated fluctuations.

Although structural properties like geometry and elasticity of a protein molecule (in its free or complexed state) are well defined in principle, an actual correlation between these properties and their contribution to the biological function of the protein has never been done. The problem is involved since the structure of the polypeptide chain is elastically inhomogeneous, and geometrically constrained by its three-dimensional folding.

The purpose of this paper is to suggest a qualitative and quantitative connection between the structural properties of the protein and its biological function, which is based on first principles.

1. The Model

The flexibility of a molecule is its property for distortion under the action of external forces without breaking its chemical bonds. A simple quantitative description of flexibility is possible only if the structural elements of the molecule i.e. angles between bonds and bonds' lengths, are weakly strained under the action of these forces. Under such conditions there is a linear relation between the magnitude of the external forces and the corresponding strains; this is Hook's law in its generalized form [5]. As the result of which, one can attribute elastic force constants to a structural element with respect to the type of the strain i.e. stretching, shearing and torsion.

The elastic force constant is a powerful concept, when it is used in a phenomenological way, since it has the advantage of relating strains in structural elements to energy changes. Therefore, it is unnecessary to know the nature of forces which contribute to these strains; e.g. purely mechanical forces, electric forces due to redistribution of charges, etc. A chemical process that involves bond-formation or bond-breaking in the molecule may be defined by this conceptual framework in situations, which are believed to include the protein. This problem is discussed in sec. 4 here.

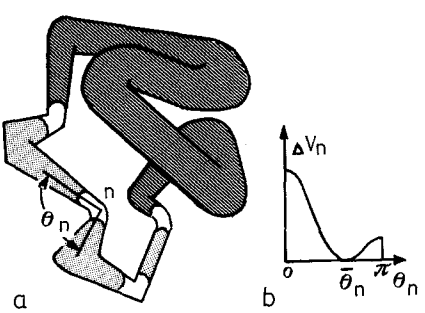

Fig. 1. a) Simplification of the protein's structure by dividing it into "working elements" according to their flexibility: *supporting part* (heavily shaded) and *rigid units* (lightly shaded) behave rigidly. The *elastic units* (unshaded) behave as angular springs. The last two types constitute the active part which is flexible as a whole. **b)** The assumed form of the potential energy of the *n*th elastic unit. The plotted function is given by Equation (A-1) in arbitrary units

Our basic assumption when building the model is that a protein in its free or complexed state, which achieves biological function may be treated effectively as a machine having a small number of "working elements". The latter being determined by the geometry and the flexibility of the protein's molecular structure. A model-experiment may probe these elements by measuring the response of a molecular structure to the action of local forces of small magnitude. Repeating the "experiment" at different loci on the structure enables us to simplify such structure by dividing it into rigid and flexible parts according to their relative response. External parameters like pressure, temperature etc., are held constant. Overall rotation and translation of the structure are excluded. Various works [6–16] together with the results of this model rationalize the choice of elements, whose definitions are given below with respect to the model-experiment (Fig. 1).

Supporting Part. A part of the structure which is not deformed and does not move. This should be related to those parts of the structure which are stabilized by internal and/or external interactions, thus exhibiting a "bulky" nature.

Active Part. A part of the structure which exhibits flexibility. Let us take the active part to be a linear array of structural elements, which is the most favourable structure to possess flexibility. This is expected to be a phenomenological description of some parts of a polypeptide chain, or of a substrate that is liganded to some residues in the active site. The active part may be subdivided into the following elements which appear alternately. *Rigid units* which behave rigidly i.e. they can move but are not deformed. *Elastic units* which are deformed under the action of properly oriented forces. This absolute division into rigid and elastic units is justified for an elastically inhomogeneous structure.

An active part includes N rigid units and $N+1$ elastic units. Let us assume the following: a) the bond between a rigid unit and an elastic unit includes an axis around which the rigid unit is free to rotate e.g. a σ-bond. This implies that the n'th elastic unit defines an angle θ_n between its neighbouring rigid units (Fig. 1a). b) The state of the n'th elastic unit is completely determined by θ_n. c) There is no interaction between rigid units not through elastic units. Since an elastic potential energy involves only deformable parts of a structure, the elastic potential energy V of the active part has the following form due to assumption (c).

$$V = \sum_n \Delta V_n \,, \tag{1}$$

where ΔV_n is the potential energy of the n'th elastic unit. ΔV_n is merely a function of θ_n due to assumption (b). Let us assume for simplicity as follows: d) $\Delta V_n(\theta_n)$ has a single minimum at $\theta_n = \bar{\theta}_n$ (Fig. 1b). Thus for $|\theta_n - \bar{\theta}_n| \ll 1$ we obtain the "harmonic approximation" of ΔV_n:

$$\Delta V_n = \tfrac{1}{2} C_{\bar{\theta}_n} (\theta_n - \bar{\theta}_n)^2 \,, \quad C_{\bar{\theta}_n} = \frac{d^2(\Delta V_n)}{d\theta_n^2}\bigg|_{\bar{\theta}_n} > 0 \,. \tag{2}$$

Geometrically, $C_{\bar{\theta}_n}$ is the curvature of ΔV_n at its minimum. An elastic unit may be visualized [according to Eq. (2)] as an angular spring having an elastic force constant $C_{\bar{\theta}_n}$. For $\theta_n = \bar{\theta}_n$ the elastic unit is free. For $\theta_n \neq \bar{\theta}_n$ it is strained. e) The size of

each of the elastic units is small enough such that the geometry of the active part is determined by the rigid units alone (see Fig. 2a).

For given rigid units the supporting part imposes geometrical constraints on the spatial form of the active part by mean of interdependence among the θ_n's. A given set of values for $\theta_1, \ldots, \theta_{N+1}$ may belong to a few spatial forms of the active part, all of them having the same potential energy e.g. optical isomers.

Finally, torsional angles are determined by the state of three successive rigid units. Therefore, the expression for V that is given in Equation (1) does not include their contribution explicitly, due to assumptions (a) and (c). Nevertheless, torsional strains appear implicitly in Equation (1) through the geometrical constraints, which correlate in principle the states of distant rigid units.

2. Conformations – Number and Flexibility

For given "working elements" the following questions are raised. What is the form of the elastic potential energy V of the active part [Eq. (1)] when the geometrical constraints, imposed by the supporting part, are taken into account? What are the number, the spatial forms and the flexibility of the active part at the states represent-

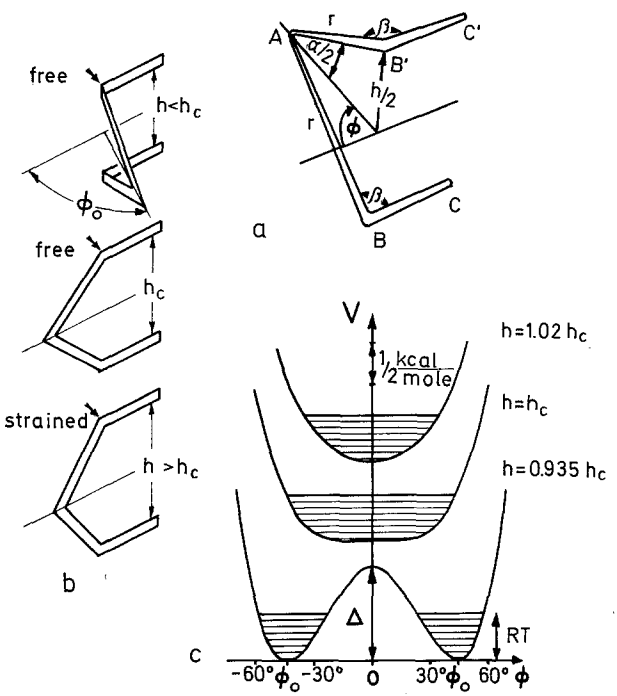

Fig. 2. A solved example of a system having two rigid units. **a)** Geometrical data. **b)** A schematic drawing of the possible types of conformations for given states of the supporting part. **c)** Potential energy curves for the situation in (b) given by Equation (A-2) for $\bar{\theta} = 110°$ $C_{\bar{\beta}} = 60$ kcal/mole. The shaded areas are related to the regions of ϕ in which thermal structural fluctuations dominate the dynamic state of the active part

ing the minima of V? How the results depend on the state of the ends of the supporting part? The answers to these questions for this system in its general form require a separate work. The simplest example of this kind is discussed here as follows:

Geometry (Fig. 2a): BC, $B'C'$ – the ends of the supporting part. AB, AB' – rigid units (narrow cylinders). A,B,B' – elastic units. $AB = AB' = r$, $BC \| B'C'$, BC and $B'C' \perp BB' = h$, $\hat{A} = \alpha$, $\hat{B} = \hat{B}' = \beta$, ϕ is the angle between the planes BAB' and $BCB'C'$. h determines uniquely the state of the supporting part, and ϕ- the state of the active part.

A detailed mathematical solution of the problem is given in the appendix. Its main features may be found by using simple arguments as follows:

Conformations: since $\alpha = \alpha(h)$ and $\beta = \beta(\phi)$ the state of the active part is determined by the state of the elastic units B,B' for a given h. Let us define by h_c the value of h for which $CBABC'$ is planar and $\beta = \bar{\beta}$ (Fig. 2b) i.e. B,B' are free at $\phi = 0$. For $h > h_c$ $\beta < \bar{\beta}$. $|\beta - \bar{\beta}|$ reaches a minimum at $\phi = 0$. For $h \leq h_c$ $\beta \geq \bar{\beta}$, B,B' are free at $\phi = \pm \phi_0$. They are strained for any other value of ϕ. Therefore, for $h < h_c$ the active part possesses two conformations (Fig. 2b). As h increases from $h < h_c$ to $h > h_c$ $\phi_0 \to 0$ as $h \to h_c$ (Fig. 3) and the two conformations are degenerated into a single one. The values of ϕ_0 are extremely sensitive to the values of h near h_c as can be shown by simple geometrical arguments (it vanishes as $\sqrt{h_c - h}$).

The above analysis is represented by V having the form shown in Figure 2c. The energy barrier Δ vanishes as $h \to h_c$. The dependences of Δ and ϕ_0 on h are given by Equation (A-4) and (A-3), respectively and are plotted in Figure 3.

Flexibility: the following elastic force constant may be assigned to the active part:

$$C_{\phi_0} = \partial^2 V / \partial \phi^2 \Big| \geq \phi_0 \,. \tag{3}$$

For $h \geq h_c$ ϕ_0 is identically zero. Since V has a minimum for $h > h_c$ and a maximum for $h > h_c$ at $\phi_0 = 0$, it turns out that $C_{\phi_0} = 0$ at $h = h_c$. Therefore as h passes through h_c the active part becomes elastically "soft" and extremely flexible. It should be noted that the above arguments involve only geometry, symmetry and the assumption of a single "free" state for the elastic units B,B'. Most of the quantitative results are sensitive only to the behaviour of ΔV_n near its "free" state and thus characterized by C_{θ_n}.

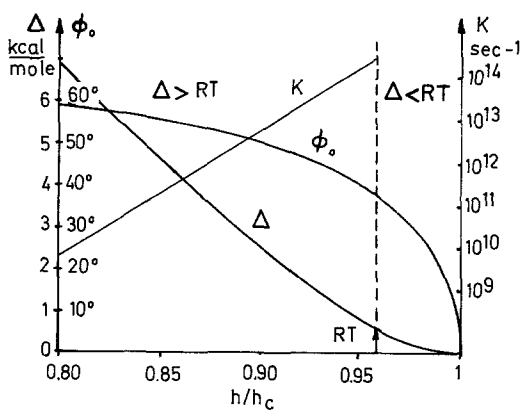

Fig. 3. The dependence on h (for $h \leq h_c$) of the height of the energy barrier – Δ, the minimum energy position of the active part ϕ_0 and the rate k of transitions between the conformations at $\pm \phi_0$ (see Fig. 2c). The analytical expressions are given in the appendix. $\bar{\beta} = 110°$, $C_{\bar{\beta}} = 60$ kcal/mole, $r = 3.6$ Å, $\varrho = 5 \times 10^{-15}$ gm/cm, $\eta = 0.01$ poise. The dashed line is the value of h for which $\Delta = RT$ and above which k is meaningless

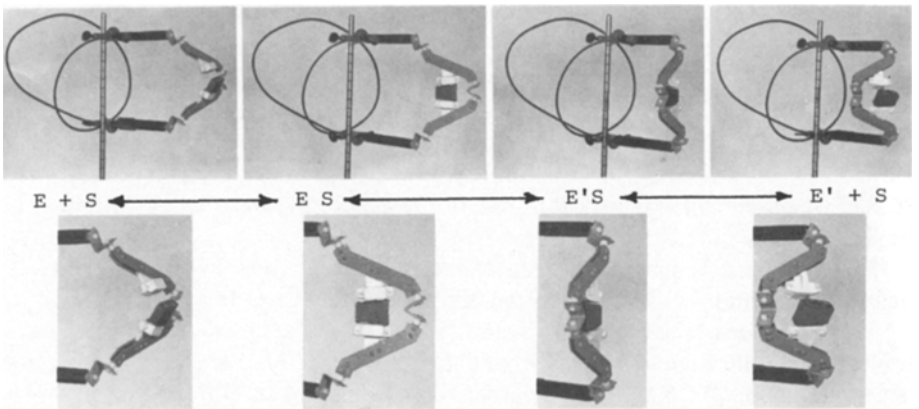

Fig. 4. A working mechanical model which was built by the author as a representation of the possible operations of the molecular machine as described by this model. The machine includes the "working elements" of Figure 1. A stiff coiled wire terminated by two parallel rigid objects represents the supporting part. Two wiggle-shaped rigid objects represent the rigid units. Three V – shaped plastic springs represent the elastic units. The springs are connected to the rigid units by low friction axes. The two white objects are magnets which represent the active site. The rectangular object represents the substrate. The entire structure is clamped to a calibrated stand in its upper side only in order to allow strain in the supporting part. The bottom series of photographs includes a magnification of the top series, and both correspond to discrete steps in the operation. In this case S forms a strained complex with E (or E'), ES (or $E'S$). The transition $E \leftrightarrow E'$ involves a large amplitude swing of the active part by which S is transported in space. In the presence of a separating membrane the machine achieves the function of passive transport

The behaviour of more complicated structure can be represented by using working mechanical models (Fig. 4). Such models show features similar to the above examples i.e. conformations whose number, spatial structure and flexibility are highly sensitive to the state of the ends of the supporting part.

3. Structural Fluctuation and Transition Between Conformations

The dynamical state of molecular structure which is embedded in a solvent at thermal equilibrium is discussed here in connection with the following questions: What is the mechanism which enables the active part to overcome an energy barrier? How does the rate of transitions between conformations depend on structural parameters and properties of the solvent?

A molecular structure in a solvent interacts with a great number of solvent molecules through random collisions. This interaction is conveniently split into two components [17, 18]:

1) a systematic part, which can be identified with friction forces operating on parts of the structure that are moving relatively to the solvent, according to a Stokes'-like law; 2) a random part, which is the fluctuation of total force acting on the structure due to the individual collisions with the molecules. This force has a highly irregular nature. The two parts must be related since they have a common

origin [17]. Part (1) acts to attenuate the motions of the structure by viscous damping. Since the net flux of energy into and out of the structure is zero at thermal equilibrium, on the average the net result of the action of the *random part* is to *energize* the structure. The simultaneous occurance of (1) and (2) leads to *structural fluctuations* around the minimal potential energy state, and may be identified with *fluctuating strains*. The randomness of the process gives the structure a finite probability to reach *any degree of strain.*

A molecular structure having a single conformation and only one degree of freedom will fluctuate around its equilibrium state and will possess a thermal energy $RT \sim 0.6$ kcal/mole (Fig. 2c). If the structure has two conformations E_1 and E_2 which are separated by an energy barrier, transitions between them will occur according to the above principles; Let $k_{1\to 2}$ be the rate of the process $E_1 \to E_2$ and similarly $k_{2\to 1}$ for $E_2 \to E_1$. These rates are the first order kinetic constants of the reaction $E_1 \rightleftarrows E_2$. Kramers [19] derived an expression for $k_{1\to 2}$ (or $k_{2\to 1}$) for a system whose potential energy depends on one variable, and obtained

$$k_{1\to 2} = (A/\eta) \exp(-\Delta/RT), \tag{4}$$

where Δ is the height of the potential energy barrier in the direction $1 \to 2$, η is the local viscosity of the solvent in the vicinity of the active part and A depends on structural parameters (see appendix). Equation (4) is applicable only to $\Delta > RT$ so that $\exp(-\Delta/RT) \ll 1$, otherwise E_1 and E_2 are indistinguishable due to the large amplitude thermal motions of the active part (Fig. 2c, as h approaches to h_c). Since A and Δ depend on the state of the supporting part, the latter governs $k_{1\to 2}$. In the example shown in section 2 the potential energy is a function of a single structural variable ϕ (Fig. 2) for a constant h. In addition: $k_{1\to 2} = k_{2\to 1} = k$, due to symmetry considerations and $A = 0(1)C_{\beta}/r^3$ (see appendix). The decrease of h below h_c causes the increase of Δ and thus the decrease of k. The plot of $k(h)$ (Fig. 3) extends up to values of h for which $\Delta = RT$.

The derivation of Equation (4) justifies the identification of a structural variable with the well known concept of "reaction coordinate" [20].

Equation (4) can be generalized straightforward [19] to include the case of transition states between E_1 and E_2.

It must, however, be emphasized that this way of overcoming a potential energy barrier is based on classical mechanics, and has got nothing to do with quantum effects (tunnelling). The latter can be shown to be negligible for an active part, whose mass is much greater than that of hydrogen atom [20].

4. Discussion

Biochemical Relevance of the Model. A protein which is biologically active is a kind of "black box" whose input and output are related to the biological function of the protein. This model suggests principles of action for a molecular machine which may represent the contents of such a "black box". The machine consists of a molecular structure having certain elastic properties and is subjected to geometrical constraints (sec. 1, Fig. 1). Its structure may be found in a few conformations (sec. 2, Fig. 2). The energy exchange between the structure and the solution is of fluctuating nature,

thus inducing random transitions between the conformations (sec. 3). The results of the possible operations of this machine have common features with specific biological functions. For this purpose its stands to reason to represent the protein by the machine.

Let us choose two out of the possible conformations [21] of the machine and name them E and E'. These two fit molecules S and P to form the complexes ES and $E'P$ respectively. ES and $E'P$ are taken to be the initial and the final states of

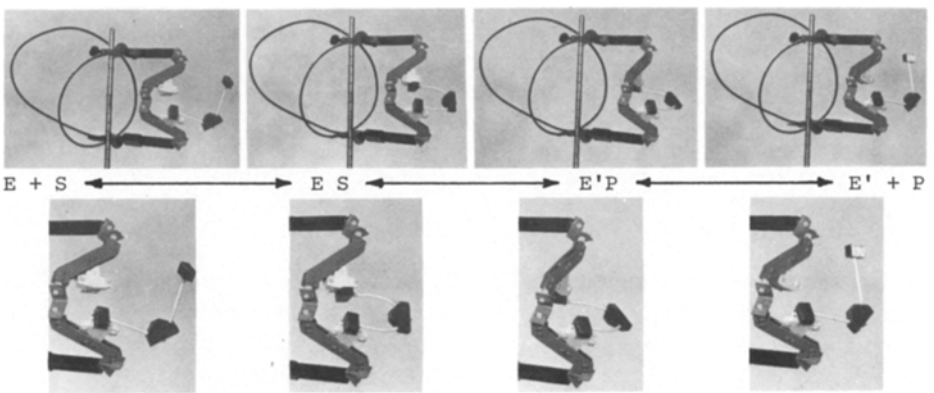

Fig. 5. An operation which involves a transition between conformations. During this operation one of the "groups" of S is rotated to give P. This rotation is shown by the white colour of these "groups" on their contact surface with the active site. In this case the function of enzymatic catalysis is achieved by the machine. The asymmetric conformations shown here are due to the wiggle shape of the rigid units

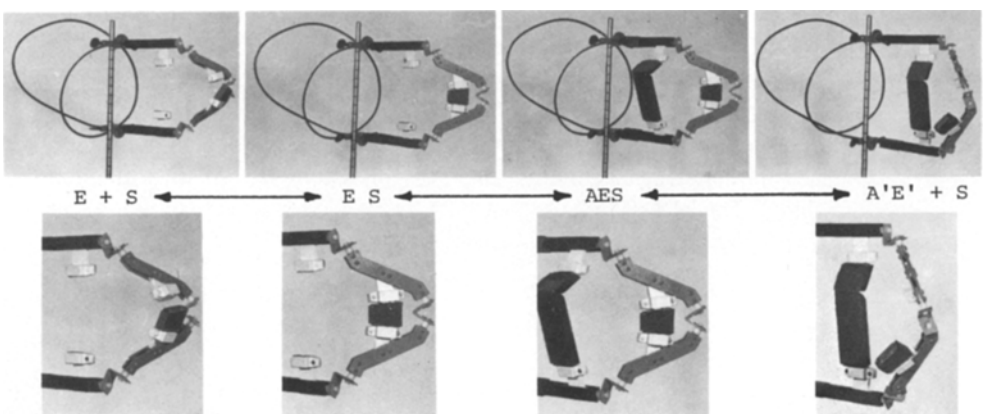

Fig. 6. An induced conformational change which is caused by an external factor. The supporting part binds molecule A after the formation of ES. The reaction $A \rightarrow A'$ results in a change in the state of the supporting part. This causes a drastic change in the active part, which is accompanied by the transport of S in space and loss of affinity for the active site. In the presence of a separating membrane the machine achieves the function of active transport. The formation of $AE'S$ is conditioned by the straining of E during its binding to S

operation, respectively. It is assumed that the process $ES \rightleftharpoons E'P$ is essentially the same as $E \rightleftharpoons E'$, but with possible modifications of the structural parameters. Straining of E and S or E' and P during the binding process is possible. We shall assume for the sake of simplicity that the "working elements" of the machine remain unchanged during the process and that there are no intermediates between ES and $E'P$. The generalization of the last assumption is straightforward. Discrete steps in the operation of the machine are shown in Figures 4–6, in which the initial and the final steps are states with partial binding of S and P.

The main features of the machine are as follows:

a) The possibility of defining conformation E and E' that fit S and P respectively and may be transformed into each other. This is due to the flexibility of the active part which permits large amplitude structural rearrangements (see E and E' in Figs. 4 and 5).

b) The possibility of induction of *transient oriented strains* in S during the transition $ES \rightarrow E'P$, since S is forced to follow the change in the geometry of the active site (Fig. 5). The specificity of these strains depends on the resistance of S to such changes, which may contribute significantly to the height of the energy barrier. The same argument holds when reversing the direction of the transport of S between two regions in space (Fig. 4). When they are separated by a membrane the machine achieves, the function of passive transport. When $S \neq P$ the function of enzymatic catalysis is achieved (Fig. 5). The well known fact that the above processes go in both directions [2] is explained by the random nature of the transitions between the conformation (sec. 3).

c) The possible variation of the state of the supporting part by external factors which may produce drastic changes in the state of the active part (Fig. 6). These changes appear as functional variations in the affinities of the protein to S and P and in its specificity, according to (a) and (b). Such external factors are, inter alia, temperature changes which cause thermal expansion [22] of the protein; a molecule A that reacts with the supporting part and thus functions as an effector (Fig. 6); and protein – protein interactions [23]. The last factor leads to allosteric effects. In Figure 6, the reaction $A \rightarrow A'$ causes a transport of S and its release ($S = P$). This situation is similar to active transport in the presence of a separating membrane, and it suggests simple concepts for the study of conformational coupling in biological energy transduction [24].

In the above description the active part has been taken to be a part of the polypeptide chain, and it defines completely the state of the substrate at any given moment. It may also happen that only the substrate in its complexed state undergoes conformational changes. In this case the substrate constitutes the active part and the protein may be taken as the supporting part. Therefore, both cases are similar in conception after suitably changing the definitions of the "working elements". In consequence Figures 4–6 should be interpreted in a more general sense.

Up till now we discussed the possible operations of a molecular machine. Let us now consider how such a machine should be designed in order to achieve a specific biological function, as it has been already done in nature for proteins. This model suggests that the existence of a small number of elastic units in a geometrically constrained molecular structure is necessary for giving the potential energy a few-minima form, as a function of some structural variables. This may be done within

the limits of the validity of Hook's law for the individual elastic unit (sec. 2). Complicated trajectories in space can be "designed" for the residues of the active part by using only three elastic units (Figs. 5 and 6).

On the other hand, a few-minima form of the potential energy as a function of "reaction coordinates" is a well known description of the kinetics of a chemical reaction [20]. In this description the different minima correspond to the states of the reactants during the reaction i.e. substrates, products and intermediates [20, 25].

Identification of some structural variables with the "reaction coordinates" forces the structural fluctuations to evolve along a specific reaction pathway. Therefore, a chemical reaction such as bond-formation, or bond-breaking, can be *defined* by a molecular structure without violating its elastic properties! The stated identification further explains the capability of a protein to control the rate of chemical reaction by changing the above trajectories. This might be achieved by small variations in the structural parameters that are induced by external forces (sec. 2). Rough quantitative estimations (see next subsection) justify the above suggestions.

Our model suggests that the specific biological function of proteins are governed by *transient strains which are developed during the transitions between conformations*. Static strains [1, 2] seem to be essential when the biological function involves an ordered sequence of reactions. The dynamic state of the protein, as it is presented here, suggests to what extent, if at all, the second law of thermodynamics is applicable in a micro-environment like an enzyme [2]. This problem arises since the specificity of enzymes may involve the reaching of improbable states of the substrate in the complex, and the storage of energy by elastic strains. The present model is based on the theory of Brownian motion, dealing with the above problem. It has been maintained by Chandrasekhar [26] that "the second law of thermodynamics is valid only for those diffusion processes in which the equalization of molecular concentrations which take place are by amount appreciably greater than the root mean square relative fluctuation ... A process appears irreversible (or reversible) according as whether the initial state is characterized by a long (or short) average time or recurrence compared to the times during which the system is under observation."

Assuming the system under observation to include a single substrate molecule S, then during the transition $S \rightarrow P$ the relative change in the concentration of S is unity. Therefore, the second law of thermodynamic is not violated by the above transient strains since it is not applicable to such short times. On the other hand, after long enough times the average concentrations of S and P will tend to equalize, according to the second law.

Justification of Basic Assumptions and Estimations of Relevant Quantities. Globular proteins possess compressibilities similar to semi-hard metals like tin [13] and contain dense regions that may reach a closely-packed structure [9]. Nevertheless, they include cavities running deep into the inside [7, 8]. This suggests that structural rearrangements of the polypeptide chain are rather limited and highly co-operative, resulting in a small number of degrees of freedom for such changes [7, 8]. The above features are found in structures containing the "working elements" of the model (Fig. 1). The strong structural inhomogeneity of the polypeptide chain due to its first, second and tertiary structure is related to elastic inhomogeneity. It was show very

recently [6] that the assumption of flexibility of the backbone of a rigid protein like Basic Pancreatic Trypsin Inhibitor is essential in order to explain the observed X-ray and NMR data by energy calculations. Therefore, the smallest rigid units and elastic units are amino acid residues and corresponding effective structural angles. Therefore $r \sim 3.8$ Å (the $C_\alpha - C_\alpha$ distance) and $C_{\bar{\theta}}$ is a few tenths of kcal/mole. Strains of $\sim 10°$ in structural angles (corresponding to few kcal/mole) are found in proteins by using X-rays analysis [15, 16]. Such strains are quite significant in this model. Helical and non-helical parts of the chain may be taken as an example for larger rigid units and elastic units, respectively. This division is justified for many globular proteins [10].

The choice of "working elements" in a specific protein seems to involve correlations of the results of different experiments, in order to simulate the model experiment of sec. 1, which defines these elements. The following two examples justify the applicability of the concepts of this model.

Cytochrome c. Its X-ray structure in the oxidized and reduced states [14] indicates that the most dramatic change in conformation occurs in residues 77–83. Apart from residues 19–25 the conformation of the rest of the protein in the two states appears to be the same. The structural rearrangements in residues 77–83 were suggested to be closely related to the biological function of cytochrome *c* [27]. This is supported by the findings that the residues 70–80 are evolutionarily invariant [28]. It was suggested that flexibility of that part of the polypeptide chain is essential for the biological function of cytochrome *c* [14]. The above description leads naturally to choose the segment of the polypeptide chain, including the residues 77–82, as the active part, and the rest of the protein as the supporting part. Since the ends of this segment are fixed, reasonable structural variables are the angles by which the energy of strain can be defined.

Lysozyme. This enzyme hydrolyzes polysaccharids. Its X-ray structure reveals [29] that in the enzyme/substrate complex the substrate (inhibitor) is anchored to six residues, and one of the sugar rings is distorted from its ground state conformation. X-ray difference-electron-density may shown [30] that only slight changes in the enzyme's conformation occur after the formation of the complex. Energy calculations suggest [31, 32] that the main contribution to the observed conformation of the distorted sugar ring is due to electrostatic interactions. This distortion weakens the adjacent glycosidic bond, which is cleaved later. It was shown there [32] that a few number of structural variables are sufficient for specifying the geometry and the potential energy of the strained substrate. Correlated fluctuations of charge in this region had been suggested to be the mechanism of catalysis for this enzyme [4].Therefore it is natural to choose the strained part of the substrate as the active part, and the rest of the complex as the supporting part. The suggestion [32] that the above strains are of electrostatic origin and not of mechanical origin, has no influence in the application of this model (see sec. 1).

Let us estimate the induced changes in the geometry and in the elastic potential energy of a globular protein, due to temperature variations. Typical values for the thermal expansion coefficient (collected data in ref. 22) and the compressibility [13] of globular proteins are $\sim 10^{-3}$/deg and $\sim 10^{-6}$/atm ($= 10^{-12}$ cm^2/dyne), respective-

ly. Therefore, a change of 10–30° C causes 1–3% change in the size of the protein. This costs in 1–10 kcal/mole of elastic potential energy and in a change of size of ~ 0.2–0.5 Å, for a typical globular protein having a volume of ~ 10^4 Å^3. Such variations of size, temperatures and free energy are quite common in biochemistry [16, 33], and are found in the range of values for which this model is sensitive (see Figs. 2c and 3).

Finally, let us estimate to what extent the model is sensitive to the fact, that the supporting part is not absolutely rigid. The energy of stabilization is 10–20 kcal/mole for a typical globular protein. If the conformational changes which occur in the active part do not exceed more then a few kcal/mole, the supporting part is expected to behave rigidly. The rigidity of the supporting part affects the ability of the active part to develop strains, in the substrate, according to this theory. Therefore, in order to develop large enough and well oriented strains, the supporting part must be of a massive and highly compact structure, relative to the active part. These conclusions are compatible with the present knowledge on proteins. The finite elasticity of the supporting part has been included in the mechanical models of Figures 4–6. The reaction $E + S \leftrightarrow ES$ involves a small deformation of the wire that represent the supporting part. The latter results in an "induced fit" [1] (Fig. 4) or productive binding [2] (Fig. 5). Additional small deformation (not shown) occur during the transition between conformations. It lowers the energy barrier as the strains at the active part become too large. Therefore, it changes the situation quantitatively but not qualitatively. This is a dynamic generalization of the above concepts which are related to static strains. The finite elasticity of the supporting part seems to be essential in the case of Figure 6.

Applications of Kramers' Law:

$$k_{1\to 2} = (A/\eta)\, e^{-(\Delta/RT)}. \tag{4}$$

Kramers' above equation shows the relationship existing between: temperature (T) and the viscosity (η) of the solution; the rate of transitions $k_{1\to 2}$ (and $k_{2\to 1}$) between conformations; and function (A) of some structural parameters which characterize the form of the potential energy as a function of some structural variable (see appendix). On the basis of Equation (4) we maintain that the so called "Arrhenius plot", which is extensively used for measuring the activation energy Δ, is being misinterpretated. The latter is due to the fact that η is a function of T!

It is known that for many liquids, including water [34],

$$\eta = \eta_0 e^{(W/RT)}, \tag{5}$$

where η_0 is constant over a wide range of temperatures, and W is some kind of activation energy. For water $W \sim 3$ kcal/mole.

Inserting Equation (5) into Equation (4) yields

$$k_{1\to 2} = (A/\eta_0)\, e^{-(W+\Delta)RT}, \tag{6}$$

therefore, W must be substracted from the value of the measured activation energy in order to find Δ. It must be noted that η is the viscosity in the vicinity of the active part which might be different from the bulk viscosity. The $1/\eta$ dependence of $k_{1\to 2}$

was confirmed experimentally for polymers solutions [35]. No relevant works involving similar experiments with proteins, which were designed to prove such relation could be found by the author. Nevertheless, η dependence of the relaxation time in cytochrom c was reported [36].

The rate of transitions may be identified with the turn-over-number in catalysis only if the diffusion rates of the reactants is not the rate limiting step. This may be the case in many reactions since the lower bounds for the rate constants of the diffusion control enzyme/substrate recombination are about [37] 10^8–10^9 $M^{-1}s^{-1}$.

Let us estimate the factor A/η. Given a potential energy barrier similar to that of Figure 2c, we found in the appendix that for a potential barrier of the form of Figure 2c

$$A \sim 0(1)\varrho\omega_1^2\,, \tag{7}$$

where ϱ is a linear mass density and $\omega_1/2\pi$ is the natural frequency of oscilation (in 1/s) of the active part around its stable state (one of them). Raman spectroscopy [38] and NMR [39] show the existence of modes of proteins at frequency $\sim 10^{11}$ Hz. This is compatible with the natural frequency of the active part as obtained from its definition at the appendix, by taking an elastic force constant of a few tenths of kcal/mole and radius of gyration of a few angströms. Therefore, 10^{11}/s seems to be a typical value for $\omega_1/2\pi$. ϱ is estimated as 10^{-14} gm/cm since this is the right order of magnitude in the case of a polypeptide chain. Taking $\eta \sim 10^{-2}$ poise (aqueous solutions) we find $A/\eta \sim 10^{12}$/s. For $\Delta \sim 10$ kcal/mole $k_{1\to 2}$ 10^5/s, which is a reasonable value for the rate of catalysis. Although the problem of rate acceleration of catalysis by enzymes is not discussed here, it is interesting to note from Equation (7) that the elasticity of the active part is essential in determining the rate of transitions.

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Appendix

Potential Energy Calculations. Assume for B,B' a potential energy of the following form (Fig. 1b):

$$\Delta V_B = \Delta V_{B'} = (1/2)\,(C_{\bar\beta}/\sin^2\bar\beta)\,(\cos\beta - \cos\bar\beta)^2 \tag{A-1}$$

This functional form simplifies the calculations. It reduces to $(1/2)C_{\bar\beta}(\beta - \bar\beta)^2$ (Eq. 2) for $|\beta - \bar\beta| \ll 1$. Unit A does not contribute to V since $\alpha = \alpha(h) = \text{const.}$ Let us choose $\bar\beta > \pi/2$ for convenience. Defining $z = h/2r = \sin(\alpha/2)$ and using the geometrical relation $\cos\beta = -\cos(\alpha/2)\cos\phi$ we find

$$V(z,\phi) = (C_{\bar\beta}/\sin^2\bar\beta)\,(\cos\bar\beta + \cos\phi\,\sqrt{1-z^2})^2 \tag{A-2}$$

$V(z, \phi)$ is plotted at Figure 2c with respect to the following analysis. Minima: at $\phi = \pm \phi_0 \neq 0$ for $h < h_c = 2r \sin \bar{\beta}$, where

$$\cos \phi_0 = -\cos \bar{\beta} / \sqrt{1 - z^2} \tag{A-3}$$

since $V(z, \pm \phi_0) = 0$, B and B' are then free; at $\phi_0 \equiv 0$ for $h > h_c$ and since $V(z, 0) > 0$, B and B' are then strained. Using Equation (3), (A-2) and (A-3) we find that $C_{\phi_0} > 0$ for $h > h_c$ and $h < h_c$, but vanishes for $h = h_c$ which indicates a "softening" of the structure. The height Δ of the energy barrier is found to be

$$\Delta = V(z, 0) - V(z, \phi_0) = (C_{\bar{\beta}} / \sin^2 \bar{\beta}) (\cos \bar{\beta} + \sqrt{1 - z^2})^2 \tag{A-4}$$

Δ and ϕ_0 as functions of h are plotted at Figure 3.
For $h \to h_c$ $\phi_0 \alpha \sqrt{h_c - h}$ and $\Delta \alpha (h_c - h)^2$.
Calculations of $k_{1 \to 2}$. Given a potential $V(\phi)$ which has a maximum at $\phi = 0$ and minima at ϕ_1 and ϕ_2. Kramers obtained [19]

$$k_{1 \to 2} = 2\pi(\omega_0 \omega_1 / \lambda) \exp(-\Delta RT) \tag{A-5}$$

in this model $I\omega_1^2 = \partial^2 V / \partial \phi^2 \big|_{\phi_1}$, $I\omega_0^2 = \partial^2 V / \partial \phi^2 \big|_0$, $\Delta = V(0) - V(\phi_1)$ where I is the moment of inertia of the active part. $I\lambda$ is defined as the friction torque, exerting by the solvent on the moving active part, divided by the corresponding angular velocity. $\omega_1 / 2\pi$ is the natural frequency of the conformation at ϕ_1. For a long and narrow cylinder having a linear density $\varrho\lambda \sim \eta / \varrho$ [40]. For the above example $\omega_0^2 \sim \omega_1^2 \sim 2C_\beta / I$ (when $\Delta > RT$) and $I \sim \varrho r^3$. Substitution in Equation (A-5) yields $A = 0(1) C_\beta / r^3$. The plot of K(h) in Figure 3 involves the exact expressions for various quantities which are based on Equation (A-2) and (A-4). The following rough estimations are believed to be relevant for proteins in aqueous solutions. $\eta \sim 10^{-2}$ poise, $\varrho \sim 10^{-14}$ gm/cm, $r \sim 4$–10 Å and $C_\beta \sim 10$–100 kcal/mole $\sim 10^{-11}$ erg. We obtain $I \sim 10^{-35}$–10^{-37} gm-cm^2, $\lambda \sim 10^{12}$/s and $\omega \sim \omega_1 \sim 10^{12}$/s. Expression (A-4) is a good estimation for $k_{1 \to 2}$ only if $\omega_0, \omega_1 \ll \lambda$ [19]. For these data $\omega_0, \omega_1 \sim \lambda$ and Equation (4) may serve only for rough estimations. There are refinements to the Kramer's result [41] but they would not be discussed here. The above estimations suggest that for $\omega_0 \sim \omega_1$

$$k_{1 \to 2} = 0(1) (\varrho \omega_1^2 / \eta) \exp(-\Delta / RT) . \tag{A-6}$$

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