ENZYME FUNCTION:
RANDOM EVENTS or COHERENT ACTION ?

by

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Abstract

This article compares the traditional statistical approach to our understanding of enzyme function with that of the opposing structuralist view. To introduce this investigation, attention is drawn to several points regarding the statistical approach: firstly, it is not possible to reconcile values of thermodynamic and kinetic parameters obtained in solution studies with protein stability and enzymic activity as known in the cell; secondly, the sequence of reactions in metabolic pathways would resemble a collection of average events, in which each substrate molecule entering the initial step of the pathway has a defined probability of becoming the final product at the end, whereas the cell requires certainty; thirdly, the basic assumption that the role of water is that of a random background solvent does not fit the biologists' picture of the cytoplasm, which can adopt any of several active physical states such as the extending, contracting, streaming, gelling; and fourthly, a random background solvent would necessarily play a destructive rather than a constructive role in the cell's mechanical processes.

In contrast, the structuralist approach is based on the cluster model in which water and protein are equal partners in cell function. Enzyme complexes are large protein assemblies which are stabilized by internal tensile forces. These forces arise from structural, as opposed to thermal energy, and simple work cycles establish that this energy can be converted into work by machines exerting tension. This result is used to develop a speculative model of enzyme activity. In the model, catalysis and product translocation steps are synchronized by a pressure-tension switch operating at the water-protein interface and powered by osmotic energy available in the solvent.

Introduction

The study of the energetics of living processes can be approached from the standpoint of either one of two different views. Firstly, we can take the classical or traditional view, that the reactions of the cell happen as the result of random thermal motion, which is an incessant, inevitable and unavoidable phenomenon expressed at the molecular level. This reductionist approach has a firm basis in the field of physics called statistical mechanics. Or secondly, we could adopt the structuralist view, that sees subcellular events as being co-ordinated through mechanisms operating at the cellular level. In this holistic approach, causation can be effected in the top-down direction via long-range structures extending through the cytoplasm which can transmit information. It is not the purpose of this article to compare the basic tenets of these opposing philosophies, but rather to illustrate how the knowledge about living matter we already have in the year 2000 supports the structuralist view.

According to classical precepts, the reaction of the cell to its environment is determined by events at the molecular level. In the final analysis, the cell does not respond as though it senses that external conditions contain "meaning" or "message": it responds chemically to collisions with molecules. Similarly, there is no purpose in its response to internal conditions. A metabolic pathway is no more than a sequence of independent reactions occurring as they would if all the reagents were present in a solution. The cell itself is a complicated, but nonetheless statistical, collection of chemicals and its behavior at any moment is an average behavior.

In the structuralist view on the other hand, the chemical reactions do not occur independently in isolation, but belong to a strict sequence. Thus, collisions with an external chemical agent initiates, or not, a set of ordered internal reactions, which can spread across the length and breadth of the subcellular space. In this way, the cell responds to stimuli which have "meaning" to it. Today, we are familiar with the concept of DNA as the information molecule: the data-input tape carrying "meaning" that is read by the gene processing machinery. This picture of a coherently functioning machine is extended to all the other enzyme complexes, including the metabolic and motor complexes in which it is energy, rather than information, that is being processed. A consequence of this view is that the cell's energy sources are not spent in a random way, but stored and manipulated in co-ordination with large-scale function, just as information is. It is a view of the cell as a unit, in which the internal processes are under its control, in contrast to one of a chaotic collection of chemical reactions.

Let us look a little deeper into the subcellular world down to the level of the protein molecules – the building blocks that make up the enzyme complexes responsible for the biochemical reactions. It has been known since the early work of Kauzmann (1959) and Tanford (1968) on the thermodynamics and kinetics of protein denaturation, that proteins are not very stable. In thermodynamic terms, the stabilities lie in the range 20 – 60 kJ/mol. However, H-bond energies are quoted also in the range 12 – 38 kJ/mol (Fersht, 1999). Comparing these figures, one is struck by their apparent incongruity – they mean that protein stability relies on a few H-bonds. It is even conceivable that some H-bonds are more stable than small proteins. For example, Finney (1982) gives the stability of lysozyme and ribonuclease as equivalent to 4 H-bonds each. One naturally asks, how can a molecule containing thousands of atoms be held together by a few H-bonds? I am not saying that these figures are wrong, but rather that another energetic mechanism for protein stability has to be found – one that has not been detected by classical methods.

One is reminded here of the problem of protein folding. As I have pointed out elsewhere (Watterson, 1997), that problem also arises from applying classical theories, since they predict an average, not a unique fold. That these questions remain unsolved still today after 50 years of intense research effort, highlights a two-fold failing of statistical methods: firstly, they did not predict the existence of a stable folded state, and secondly, once given as an experimental fact, they cannot explain it.

The elucidation of catalytic mechanisms has made rapid progress over the last decade, especially as a result of the use of genetic engineering to modify active sites. The results of kinetic studies are usually interpreted in terms of the Michaelis-Menton theory, which is itself based on classical chemical kinetics. Here too, the approach gives rise to puzzling conclusions. For example, the central tenet of the theory holds that enzymes use the energy of substrate binding to lower the activation energy barrier to the transition state, where the configuration of the substrate has a high affinity for the active site. The energy (Free Energy of Binding) released on binding a substrate with a K_M of 1 μ M (a moderate affinity) to the active site is about 30 kJ/mol. Again we see that this corresponds to the formation of a few H-bonds.

Like many readers, I find these values confusing, not enlightening. Most biochemists imagine the binding of substrate to form the enzyme-substrate complex as a stabilizing step. They picture these partners locking together. But if the enzyme retains the energy made available on binding, it must become less stable – in fact, as we have seen above, 30 kJ/mol is enough to denature some proteins! Again, I am not proposing that these values are wrong. They are the result of many corroborated investigations, which have yielded a great wealth of data on enzyme kinetics. However, I wish to emphasise that these data have not yet been linked to protein structure, which is a necessary step in improving our understanding of biological function. Speaking realistically, it is very doubtful that nature evolved catalytic machines which are on the verge of breakdown each time they begin to function. Surely biological function relies on machines that operate consistently without failing, and indeed biologists believe this to be the case in enzyme activity. Clearly then, new energetic factors have yet to be identified to account for the reliability of cellular function.

Even bigger problems arise when we come to consider mechanical steps that enzymes must carry out in effecting their work cycles. Most enzymes are protein complexes composed of several, in some cases many, protein domains associated together. Since many steps involve large conformational changes, made visible by domain rotations and displacements, such domains can be viewed as moving parts in an integrated machine. In proposed working models, these movements are visualized as taking place in empty space. But the presence of the surrounding solvent cannot be ignored, because it possesses the same density and thermal energy as the protein solute. As explained elsewhere, these properties mean

that the surroundings must have a disruptive influence on enzyme action and hinder functioning (Watterson, 1997). For a 25 kDa domain to displace its own volume, about 1500 water molecules must be mobilised. As a consequence, thousands of H-bonds must be broken – an event requiring energy hundreds of times greater than that of the thermal stabilities of domains. We must conclude that cellular processes involve energies far in excess of those supplied by metabolic steps like substrate binding.

These problems are never mentioned in the classical approach, because chemical events are pictured in the following way: – the surrounding solvent plays the role of a heat bath for the reactants. Chaotic energy is always available, and in fact, is necessary, since collisions between reactants and solvent lift the reactions over their activation energy barriers. In other words, it is an integral part of solution chemistry, underlying all reactions. It is therefore taken for granted, and so reaction sequences can be considered as being played out in empty space. Although this approach is valid for solution chemistry, I do not think it is suitable for biological systems. Rather than being involved in random collisions, protein and water must act in concert, implying that water is an active reaction partner and not just a background solvent. The cytoplasm can be pushing, pulling, extending, contracting, flowing or gelling. These states are not possible without the direct involvement of the aqueous medium, and to understand them we need to find a new energy source which operates above the level of thermal collisions and which can override their disruptive effects. In the next section we will see how closely interrelated water and protein are according to the cluster model of liquid structure.

The Cluster – Domain Model

Liquids are an intriguing state of matter because they possess the properties of both gases and solids. In the familiar situation, liquids are under pressure, e.g., a solution in a beaker open to the atmosphere. Yet liquids adopt a condensed shape, because like solids, their molecules are linked together giving an overall macroscopic form. This means that they exert both pressure and tension simultaneously, and thus lie between gases and solids in their properties. Their ability to exist in this seemingly contradictory state is due to the dynamic nature of their molecular interactions. The opposing forces of pressure and tension do not cancel one another, but co-exist on separate levels: pressure on the macrolevel of the container and tension on the microlevel of the molecules.

In water, bonded aggregates of molecules are constantly forming and decaying, so that they travel as a wave through the liquid medium. These clusters take on a definite size, which represents a balance between the co-operative bonds holding the molecules together and collisions breaking them apart: between tension inside and pressure outside. I have called this critical size the “pressure pixel”, since the term “pixel”, borrowed from the information sciences, aptly describes the concept of a hierarchical divide between information and noise (Watterson, 1996). The internal state of liquids also presents us with a hierarchical divide: as we go down in scale, we reach the size of a cluster. Pressure has no meaning in smaller volumes. A cluster can be seen as a giant flexible 3D molecule, pulled into shape by temporary internal bonds.

This is just the flexibility we need when considering the state of cell water. The cytoplasm displays truly dynamic behavior, manipulating forces as it switches between flowing and stationary states. In the cluster model, this behavior is the outcome of lower level organization. We can see this in a schematic way by comparing the mechanical steps of a reaction sequence proceeding under conditions of classical solution chemistry on the one hand, and in a biological system on the other. In Fig 1, we see that each step happens at random locations in the solution, and according to statistical analysis, such a sequence can be regarded as an average sequence. On the other hand, in the subcellular world, the reaction series is carried out by a catalytic complex, such that each step occurs in the right place at the right time. In every metabolic pathway, the initial reactant is converted into the final product with a surety that leaves no room for chance. The reaction steps do not proceed back and forth in any order at random, they proceed from beginning to end in a predetermined sequence. This behavior does not resemble an average outcome in any way. On the contrary, it resembles a programmed outcome. Biological pathways do not obey the laws of statistical mechanics.

To achieve the correct spatial and temporal sequence, the enzyme complex must function as an integrated whole. The structural units of this sequence are shown as equally sized boxes in Fig. 1. They represent the globular domains of the enzymes involved in the pathway. We know that large proteins are subdivided into smaller domains. Surprisingly, these smaller units are roughly the same size independent of their function. I believe, that this property of uniformity is indicative of some underlying principle of biological organization at this hierarchical level. In 1988 (Watterson, 1988), I suggested that domains of

larger size were not possible. As far as I am aware, none have been reported in the intervening years. In fact, the 3D structure of chaparonin reveals an opening of diameter about 4 nm into its folding chamber. If, as workers presently propose (Sigler et.al., 1998), this chamber is the space for the folding of globular domains, its opening limits the dimensions of folded proteins. We may conclude, that the cell requires protein domains of a certain predetermined size, and no larger.

The concept of the pressure pixel opens up a new avenue of attack on the unsolved thermodynamic problem of the structural stability of proteins. The classical approach cannot explain the phenomenon, because the essential step in the statistical analysis of a system is the calculation of its partition function, which describes its average state. The partition function is used in turn as the basis for calculating all the thermodynamic properties of the system. Applying these principles to proteins shows that they too must adopt an average conformation in solution. However, they do not. They adopt a unique fold. As every biochemist knows, each and every enzyme must be in its native conformation in order to function. In the cluster-domain model, the size of protein domains fits the space defined by the pressure pixel. They resemble water clusters, but because they are held together by covalent peptide bonds, they are permanent. Conversely, water clusters resemble protein domains, but because they are held together by flexible H-bonds only, they are transient (for a fuller picture see Watterson, 1991).

The cluster-domain model depicts the cytoplasmic space as a densely packed world: not as a fluid solution, and not as protein molecules in empty space. Although the building blocks give solidity to the construction, it is not a rigid edifice. The building blocks are macroscopic, or better mesoscopic, entities, which occupy the hierarchical level above water molecules. However, being themselves held together by flexible bonds they have a dynamic internal structure oscillating with the frequency of the structure wave. Rather than solid blocks, we can view them as large quantized particles with definite modes of vibration, and so they are able to receive, store and transmit energy.

The hydration force illustrates the flexibility of cluster-domain interactions. This force is a tension exerted laterally in the layer of water next to hydrophilic surfaces, both biological and mineral. It was extensively studied in the 1970s and 80s, and was found to be so strong that pressures up to 1 000 atmospheres are needed to remove it from between surfaces that approach closer than 3 nm to one another (Rand and Parsegian, 1989). The cluster model predicts that such a layer would form at flat extended surfaces as a result of aligned clusters aggregating together. In this way, clusters can grow laterally to macroscopic proportions, covering an area as large as the interface itself (Watterson,1991).

However, we do not need sophisticated measurements to recognize the existence of this force. Biochemists know that spinning a protein solution at 300 000 x g does not produce a protein concentration greater than 50 mg/mL. Sedimented pellets retain more than 90 % water content even in the presence of such enormous centrifugal forces. With this everyday experience, the hydration force demonstrates its power to bond water and protein together. The effect with DNA, lipids and glycans is even more dramatic. The fact that large amounts of water are under tension in these biological systems, explains the gelled state of the pellets. This familiar phenomenon shows us that we cannot ignore the role of the hydration force in any realistic picture of protein function.

Mechanical Events

Every enzyme has to carry out mechanical steps. The activities of many rely on large conformational changes which open and close a groove between domains containing the active site. But even in cases where there is no relative movement of domains, substrates and products have to be moved. As explained above, the classical approach regards these steps to be the result of thermal fluctuations and the energies involved are ignored.

Because enzymes are links in a sequence, they rely on the correct and efficient function of the other members: those downstream need the products of their neighbors upstream. They are interdependent, and together form a coherent whole. Diffusion has no part to play in this organisation. If the classical picture of the subcellular world were correct, enzymes would be soluble entities without fixed locations, and a multitude of metabolic reactions would be proceeding simultaneously. In addition, the concentration of metabolites, [S], would have to be sufficiently high to ensure efficient reaction rates, since the concentration of the catalytic complex, [ES], is proportional to reactant concentrations. However, this requirement that the cell contains high concentrations of free metabolites is not supported by experiment. On the contrary, with the exception of

special molecules like ATP, metabolite concentrations are usually lower than the concentrations of their corresponding enzymic binding sites.

The structuralist view of subcellular organisation sees the components of metabolism arranged in a way similar to an assembly line (Fig 1). Elementary metabolites are not let loose into the environment, but passed from enzyme to enzyme until the particular pathway is completed. Concentrations of metabolites do not have to be high, because enzymes do not rely on diffusion. Their substrates are supplied by the stepwise operation of the assembly line, where the individual moving parts must execute back-and-forth mechanical steps ensuring continuous flow through the pathway. If the individual enzymes do not leave their locations, then the flow must occur in the adjacent aqueous phase. The lateral force in the adjacent hydration layer is an obvious candidate to play the role of a "conveyor belt", that shuttles metabolites through the pathway.

In many cases, enzymes have to be moved as well as substrates. The restriction enzymes that process DNA, the complexes that read and translate genes, the motor molecules that move along cytoskeletal filaments, chaparones, ATPases that rotate, reporter proteins, activators like G-proteins and calmodulin, all make step-wise translocations or shuttle to-and-fro in performing their cyclic functions. In large assemblies like the polymerases, smaller domains are moving parts in the overall machine. For the required outcome to be successful, these elements must act in co-operation, since the cytoplasm is densely packed without holes of empty space where moving parts can freely enter and exit. Clusters and domains must swap locations in a reciprocal way, otherwise their motion would be always obstructed by the environment.

The mechanical events carried out by the cell are examples of work performed by machines at the molecular level. We have seen that, from a realistic point of view, heat supplied by disruptive collisions cannot be their energy source, and further, that the energy released in metabolic reactions is minuscule in comparison to that required for the totality of movement involved, including rearrangement of the solvent. We must therefore find a source of energy which is immediately at hand and large enough to fuel these subcellular tasks. The osmotic energy of water fulfils these requirements: it is directly available and in plentiful supply. In the next section we will see how it can be harnessed to do work through the action of tensile forces in an elementary way.

An Osmotic Machine

In analogy with Carnot's machine, the osmotic machine is a cylinder fitted with a piston, containing a solution (the working solution), and immersed in an external solution (the reservoir). The walls of the cylinder allow the passage of solvent (e.g. water) but not solute (e.g. salt), i.e they function as semipermeable membranes. In the usual situation, the reservoir is pure water at atmospheric pressure, P_0, and the working solution contains solute, and hence the pressure inside is higher than outside by an amount called the osmotic pressure, Π,

$$P = P_0 + \Pi \qquad (1)$$

This is illustrated by the machine A in Fig. 2. The pressure difference between inside and outside is positive, as in the familiar case, as long as the concentration of solute, Z/V, is higher inside than outside. But if the concentrations are reversed, the pressure inside is lower than outside and then the piston has to be pulled as opposed to pushed. The technique of osmotic shock is an example of this reversed situation. When cells are placed in a hypertonic solution, water is drawn out of the cell and the cytoplasm comes under tension (machine C and D in Fig. 2). This pulling force can be quite high, e.g. sea water, an important osmotic agent, produces a pressure of –24 atm in pure water, thus, assuming the working solution is pure water and the reservoir is sea water at atmospheric pressure, then the water inside is under a tension of 24 atm and the osmotic pressure is –25 atm. In other words, the piston rod must be pulled with a force that will oppose the tension on the inside (24 atm) and the pressure on the outside (1 atm) to maintain equilibrium. This means that the water molecules inside are pulling on the piston, on the walls and on one another. There is tension throughout the whole of the space occupied by the working solution. Under these conditions, it has become a single macroscopic cluster. Extending this picture we see that, if the reservoir solution is itself wholly enclosed in a vessel with rigid walls so that it also can be held under tension, then the machine can be under any combination of conditions, with pressure or tension, inside or outside.

Let us follow the machine around the work cycle in Fig. 3. At A, it starts with the low volume V_1, so that the corresponding solute concentration inside is high, giving a high osmotic pressure. As the machine expands

down the curve A to B, the concentration falls to Z/V_2, and hence the osmotic pressure falls also. However, it performs work during the step, because the piston moves in a direction against the external pushing force acting on it. At B, the machine is immersed in a hypertonic reservoir solution, so now solvent tends to leave the machine and the pressure inside drops below zero. At this point C, the piston must be pulled to maintain equilibrium. However, no work is done in the step B to C because the piston does not move. Next, the volume decreases back to V_1, so the concentration inside, and correspondingly the osmotic pressure, increases. During this step C to D the machine performs work, this time again because it displaces the piston in the direction against the external pulling force exerted on it. From D to A the machine is placed back in the first reservoir solution, and the pressure is returned to the original pressure without doing further work. The total work done during the cycle is just the area enclosed by the figure ABCD.

If the machine had expanded further down the curve AB, more and more solvent would enter, continually diluting the solution inside until it resembled the pure solvent outside. Since the reservoir is open to the atmosphere, the solution inside would eventually end up at atmospheric pressure also. Thus the curve AB is asymptotic to the pressure P_0 as V increases to large values. If the inside volume expanded down the curve DC on the other hand, the pressure approaches P_1 asymptotically, which, in this set-up, is a negative pressure, because the hypertonic solution is open to the atmosphere while the pure solvent is enclosed inside the machine (just as in the osmotic shock experiment described above). Thus the two curves are parallel with the difference between them being the difference between the asymptotes, $(P_0 - P_1)$. Hence the area of the cycle is given by

$$W = (P_0 - P_1) (V_2 - V_1) \qquad (2)$$

Just as with Carnot's heat machine, the cycling of this osmotic machine performs work. However, there is no suggestion that machines of this type exist inside the cell: – of course they do not. Carnot's method is used because it is a clear, unambiguous line of reasoning to demonstrate that there is an energy source in liquids which can be tapped for useful purposes. Furthermore, because the machine is not driven by a temperature difference, this source is not thermal. As explained fully elsewhere (Watterson, 1995), osmotic phenomena are accompanied by an increase the Free Energy. The fact that they happen spontaneously contradicts the thermodynamic principle requiring that a spontaneous process must be driven by a fall in Free Energy. Osmotic energy is therefore distinct from random energy, on which thermodynamics is based, and is structural in its nature. This conclusion has special significance for biological systems, since it means that this new type of energy is not supplied by random collisions, yet it is very effective at exerting force via molecular action. There can be no doubt that the molecular properties that give rise to osmosis exist, and are in operation in every solution, whether these solutions are behind a semipermeable membrane or not.

Let us consider what happens to the solvent molecules inside the machine during the step B to C. When the machine is immersed in the second reservoir, they will tend to pass outwards because the solute concentration is higher outside. But since the volume is held constant, only an insignificant number can leave and the pressure inside falls sharply as a consequence. When the pressure drops below atmospheric, the piston has to be pulled, rather than pushed, to keep the volume constant. Then as the pressure continues to fall below zero, the piston must be pulled even harder, because the inside solution is now under tension, and the pulling force on the piston must balance this opposing internal tension plus the atmospheric pressure in the reservoir pushing on the piston from outside. Thus we see why Π in Equ. 1 is a bigger negative number (say –25 atm) than the internal pressure P (say –24 atm).

The operation of the machine at negative pressures raises intriguing questions. How are the molecules in the contact region within the pores of the membrane wall maintaining the equilibrium? We need to answer specifically, what are the molecules in the contact region doing to prevent them from all moving in the common direction of the two forces? At the point C in the cycle, the molecules in the pores are being pulled inwards by the solution inside and pushed inwards by the solution outside. Since gases cannot exert tension, there is no gas analogue of this circumstance. Also, in the case of two solid bodies in contact, one pushing and one pulling, an equilibrium is impossible (Fig. 4). As far as I am aware, this important question is not dealt with in texts on liquid physics, although the editors of many journals claim that it is known. For example, the editors of the journal *Science* say they "sense" it is well known. So if indeed it is known, then what is the answer? I have not found one, and I feel sure that readers have not found one either. The lack of information on this basic issue highlights the urgent need for increased research effort in applying our understanding of liquid physics to biological systems.

It is vital for our understanding of cellular mechanisms, that tension is recognised and investigated. The predominantly gelled state of the cytoplasm supports the conclusion, that tension is the underlying cause of cytoplasmic cohesion. In the structuralist view, clusters and domains are connected together like links in a chain, so that when the connections strengthen, the chain tightens. Thus tension is indeed an effective mechanism for transferring energy over a long range, since tension makes a chain go rigid, not pressure. However in the classical view, enzyme machinery operates by collisions between and within its moving parts. For example, in popular models of how force transducing molecules work, motion is produced by the forceful rotation of rigid levers (but see Block, 1996, for an insightful review).

Pressure is an entropic mechanism. Its force spreads and gets lost unless it is contained by strong rigid walls. It does not suit the subcellular world, which is constructed out of soft dense material. The cytoplasmic environment is not like the inside of a car motor. Therefore, as the analogy of a chain suggests, tension is a superior mechanism to effect long-range vectorial action. On the other hand, pressure is used by the cell to power expansions on the larger scale. Cells develop regions under pressure, e.g., when budding, exocytosis and extending growth processes are in progress. In addition, many cell types are surrounded by stiff cell walls, so that they can develop and maintain turgor generated by their vacuoles. These examples of the multiple roles adopted by the cytoplasm serve to illustrate the inadequacy of the concept depicting cell water as an inert background solvent. In the next section we will examine how the dynamic properties of the cytoplasm may be under the control of a pressure-tension switch operating at the protein-water interface.

Energetic Events

The Free Energy of ATP hydrolysis is about 30 kJ/mol. This thermodynamic quantity is derived from solution chemistry and gives us the energy released as a result of molecular collisions between ATP and its surroundings as it moves back and forth in a random way along its reaction sequence ("stochastically" (Simmons and Hill, 1976)), converting one mole of reactant into one mole of product. The motor enzyme complexes use this energy to fuel subcellular movement, for example, the kinesin motors are known to move along microtubules at speeds in the range 0.05 – 0.5 μm/s. An elementary "processive" step in this movement is thought to extend over one α/β tubulin dimer (Vale and Fletterick, 1997). Since this distance is about 10 nm, the complex as a whole moves along the filament taking a step at every 0.1 s on average. However according to rigorous statistical mechanics, this step takes place along with a large number of thermal fluctuations, which are happening continuously to this molecule, to ATP and to the surroundings. Assuming a radius of 10 nm for this large duplex motor molecule gives a diffusion coefficient of $2.5\ 10^{-11}$ m^2/s, from which the Einstein formula, $\bar{x}^2 = 2Dt$, predicts that thermal collisions would propel it through this distance in just 2 μs !

We see immediately that these values are entirely incompatible. The thermal displacements are 5 orders of magnitude faster than the biological ones. In addition, we would expect the values to be reversed in magnitude, because it is reasonable to assume that the biological process ought to be faster than the thermal, to ensure that the physiological response is not nullified by disruptive collisions. In the language of information science, these values mean that the signal would be lost in the noise.

In the structuralist view, random collisions play no part in these events in vivo. Protein domains and water clusters move together in a co-ordinated way. The thermal motions of proteins are prevented by the action of the hydration force, which locks proteins into position, so that their physical state resembles that in a crystal rather than in a solution. Not only are the enzyme complexes assembled in an ordered architecture, but the catalytic reactions are themselves also ordered. In this picture, the chemical and mechanical events are orchestrated, and therefore, so too are the energies that fuel them. We can now reconsider the sequence of reaction steps in Fig. 1 from a dynamic standpoint (Fig. 5). For the complex to function in an ordered way, the catalytic steps and the transport of their products have to be synchronized. In an oversimplified picture, we can imagine that the individual enzyme units catalyze simultaneously, the products are released into the hydration layer simultaneously, these are then displaced one unit along the sequence simultaneously and rebound to the next enzyme site simultaneously. This picture presents the metabolic products being displaced together as a block along the physical pathway. The lateral force within the hydration layer itself is ideally suited to effect this step. As described in the above section on the cluster-domain model, the existence of the layer is due to the tensile force, which holds it in position like a taut chain. Everyday experience tells us that when a link in a chain weakens or breaks, then the remaining intact portion pulls suddenly away from the break. Hence, a local disruption in the water structure causing a weakening in the hydration force at the beginning of the sequence, could result in the whole layer moving physically along the sequence.

However, the analogy with a solid chain should not be developed too far. As we have seen in the osmotic machine, solid bodies have limitations in comparison to liquids in the way they transmit force. Since both pressure and tension can be exerted at the same place simultaneously in liquids, it is naturally of interest to speculate how biological systems could utilize this flexibility. We know that even small changes in protein conformation can dramatically affect the hydration force. For example, the binding of metabolites causes subunits to associate in some cases, or complexes to dissociate in others. These switching mechanisms which are essential for function, are effected through subtle modulation of the hydrophilic-hydrophobic character of the protein surface. A hydrophilic surface binds water preferentially and builds a firm hydration layer, while a hydrophobic one repels water and induces direct protein-protein contacts. Such switches were earlier considered to be the result of particular allosteric changes controlled by the binding of special effector molecules (GTP, cyclic AMP, Ca etc). However, today they are recognized as being so wide-spread in protein behavior in general, that they are accepted as a fundamental part of protein function.

The precise and efficient movement of solvent is essential for the smooth operation of enzymes. This point is well illustrated by the motor complexes, which must displace water as they move along filaments. For spatial co-ordination we need reciprocating displacement of solutes and solvent. For example, for motors that track along microtubules, water flow in the opposite direction inside the tubule could be an effective mechanism for compensating the space required by these large protein solutes. A hydrophilic-hydrophobic switch associated with microtubules has recently been suggested to control antibody migration towards the nucleus in fluorescent labelling experiments (Mentre and Debey, 1999).

Switching mechanisms of this kind are predicted by the cluster-domain model. Fig. 6 (derived from Fig. 7 of Watterson, 1991), depicts an enzyme site located between two domains. Binding of substrate (effector, metabolite, messenger) builds interconnections from one domain to the other resulting in the high affinity enzyme-substrate complex. Upon catalysis however, the tight interactions across the interface are broken, so the cleft reopens releasing the products. In terms of the cluster-domain model, the switch is an harmonic transition in the structure wave. A node at the domain interface occurs when the surfaces are repulsive and so exert pressure on one another. Concomitant with the transition to an antinode, the surfaces become attractive and tension is exerted throughout the space of both domains. The bonding and antibonding orbitals of electrons in simple molecules are an illustrative parallel to these states of the structure wave. They emphasize the quantum principle underlying the model and introduce the idea that we are dealing with energetic states of biological matter, and not just short-range forces between amino acid side groups localized at the interface. Thus the model is not an alternative to the present-day theory of protein-protein and protein-ligand interactions, it is an extension of it. X-ray crystallographic data have located hundreds of short-range interactions across protein interfaces with high precision. In the model presented here, these bonds belong to the larger network which extends throughout the whole space of the bonded domains, so that all are contributing together to the interaction.

We can now extend the model in Fig. 5 a little further and assume that the release of product from the enzyme binding site exposes a hydrophobic surface. This step in turn disrupts the hydration layer, giving rise to local pressure. Rather than the crude physical break of a rigid body as we had in the chain analogy, we still have here a stable structure, since pressure and tension can co-exist in liquids. In other words, the overall organization is not destroyed, as we would expect from a sudden fracture; it is still intact. But now the driving energies are redirected. Indeed, the emergence of local pressure would promote the displacement of the hydration layer away from this point, transporting the metabolic products along the protein-water interface to the next binding site. That is we have a pressure-tension switch which is linked to a step in the catalytic cycle, controlling the movement of the conveyor belt. In this scenario, the hydration layer slides along the surface of the protein aggregate in quantized, “start-stop” displacements.

Although this description of enzyme action is both speculative and oversimplified, it illustrates the high degree of co-ordination needed for successful functioning. As soon as one step fails to happen, or happens out of rhythm, the overall machine begins to falter, random energies take over and, as a result, the “living” quality fades away. The energy carried in the structure wave, i.e. in the oscillating network of liquid water, must be maintained as the energy source for all the individual, but interdependent, movements of each component to avoid thermalization and death. Heat does not drive the osmotic machine, and nor does it drive living machines. That is why the subunits of enzyme complexes are not associated together in a random jumble, but are positioned relative to one another in a predetermined way. This spatial geometry is necessary for the structure wave to resonate throughout the integrated whole and form a single, large-scale living energy.

Conclusion

We have seen how the traditional view cannot explain the mechanical and energetic events of the cell. The high level of organization required for the events to proceed without failure runs counter to statistical mechanical principles. The contrasting organizing principle governs the way, not only in which biological matter is assembled but also how energy is managed. Energy is associated with quantized steps which move material along predetermined catalytic sequences. We also saw how the multitude of such events require an energy source far in excess of that supplied by what we know of metabolism.

The protein-water network is an energized gel whose units, protein domains and water clusters, manipulate pressure and tension to perform mechanical steps. The interplay between these opposing forces is controlled by conformational changes switching between the hydrophilic and hydrophobic character of the protein-water interface. Thus proteins operate a pressure-tension switch, which may be responsible for the cytoplasmic transition between the gelled and fluid states. The cell can be seen, from a somewhat extreme structuralist point of view, as organized water. There is an incipient order in liquid water, which is given long-range coherence and permanence by the protein framework. In the words of A. Szent-Gyorgyi, "Life is water dancing to the tune of solids".

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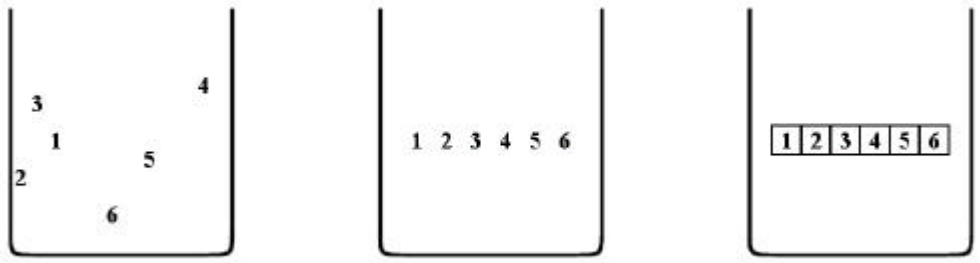

Fig. 1. Random and ordered reaction sequence.

The first panel is a representation of the locations of sequential steps of a hypothetical reaction occurring in solution. Each of the positions labelled 2 to 6 were obtained by throwing dice to determine the moves 1 – 6 places left or 1 – 6 places right and 1 – 6 places up or 1 – 6 places down. There are thus 144 possible outcomes for the next location on the grid for each of the 5 steps, giving a total 62 billion sequences.

The second panel shows the ordered linear sequence. The chance of this sequence of random events happening is thus one in 62 billion.

In the third panel, the steps are shown in separate boxes to illustrate the pixel concept of subcellular organization. The space in which the biological reaction occurs is subdivided into volumes of definite size occupied by the enzymes which catalyze the steps. Thus each step occurs in its own box and the product is moved into the next one along the sequence. An example of such a sequence is the citric acid cycle, which consists of 8 basic steps. Since the metabolites which enter the pathway at step 1 all pass through to be released as products after step 6, the chance of this sequence occurring equals one. Although this model is artificial, it demonstrates dramatically the difference between the two approaches.

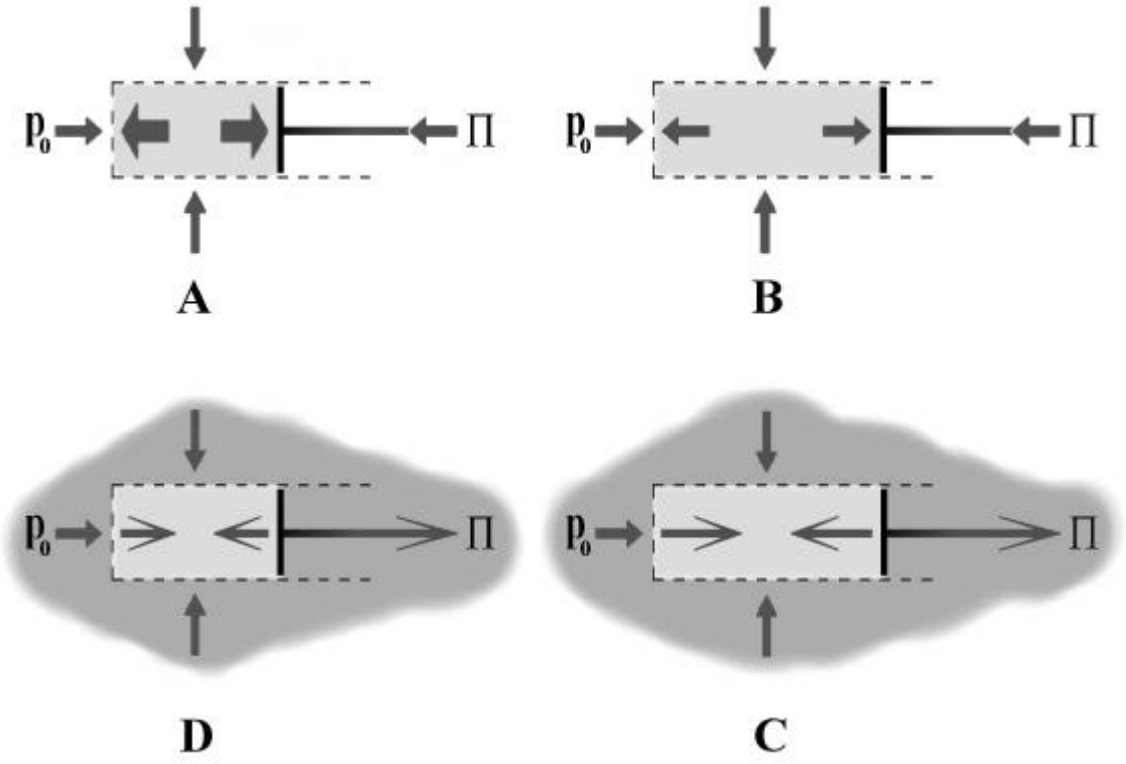

Fig. 2. The osmotic machine.

Schematic representation of the forces exerted on and by the osmotic machine in 4 different configurations corresponding to the work cycle in Fig. 3. The machine contains a solution of Z molecules of solute (e.g. salt), which cannot pass through the walls, in a solvent (e.g. water) which permeates through the walls from inside to outside and vice-versa. The pressure inside is highest in the configuration labelled A, because the volume V_1 is small so the solute concentration Z/V_1 inside the machine is high (light shading) and there is pure solvent outside. When the piston expands to volume V_2 at B, the solute concentration and correspondingly the internal pressure falls. At C and D the machine is immersed in a hypertonic solution, so the solute concentration inside is lower (light shading) than outside (dark shading). Now the solution inside is under tension (negative pressure) and so it is pulling on the walls and the piston. The osmotic pressure, $\Pi = (P-P_0)$, equals the difference between the pressure inside and outside. It is the pressure exerted on the rod connected to the piston and maintains the equilibrium. Thick arrows represent pressure and thin arrows represent tension.

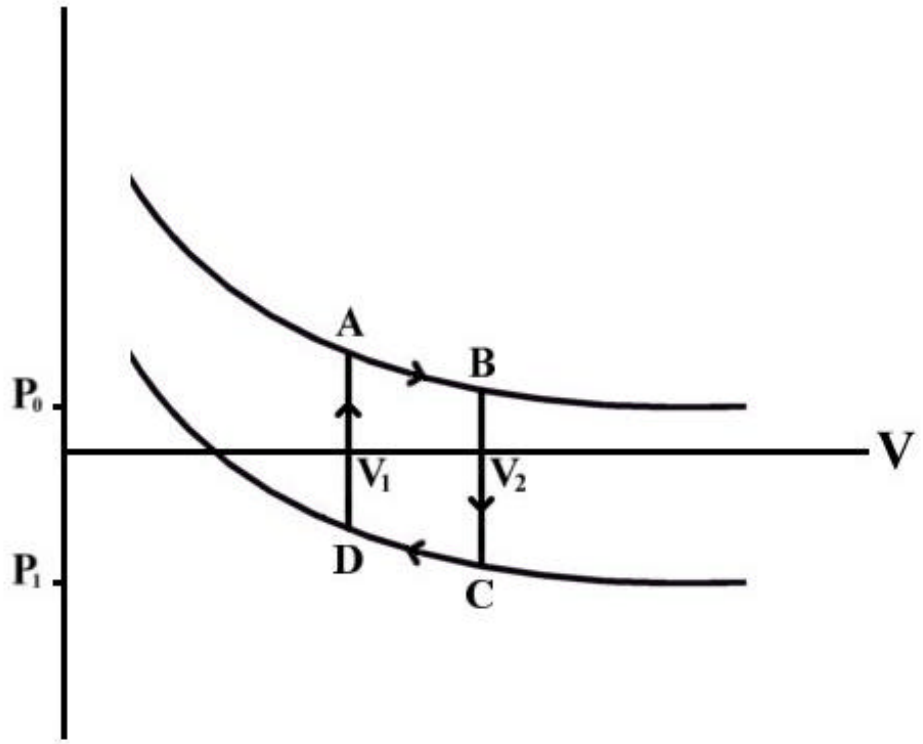

Fig. 3. The osmotic work cycle.

Graphical representation of the work cycle on a P,V diagram in analogy to the Carnot Cycle in which the pressure inside the machine is plotted against its volume. The cycle starts at A with the machine immersed in the pure solvent which is open to the atmosphere. The solution inside is thus at a higher pressure and equilibrium is maintained by the osmotic pressure, $\Pi = (P\text{-}P_0)$, pushing on the rod connected to the piston. This arrangement thus corresponds to the usual experimental set-up for measuring Π. As the volume expands, solvent enters the machine diluting the solution inside and the osmotic pressure decreases down the hyperbolic curve according to van't Hoff's equation, $\Pi = (Z/V)kT = cRT$. During this step the piston does work analogous to that done in the Carnot Cycle. The return stroke C to D is performed while the machine is immersed in a hypertonic solution, so that the solution inside is under tension and the piston rod must now be pulled (see Fig. 2). For purposes of discussion, the expansion A to B is curried out with the internal solution under positive pressure and the return stroke C to D under tension, however, the cycle may be carried out wholly under pressure, i.e., above the V-axis, or wholly under tension, i.e., below the V-axis.

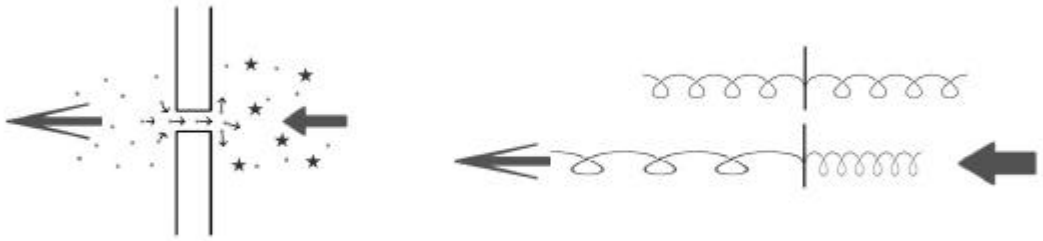

Fig. 4. Molecular mechanisms involving tension.

Left panel: Pure solvent on the left is separated from the solution on the right by a semipermeable membrane wall. Solvent molecules (dots) can pass through the pore, while the solutes (stars) cannot. Solvent tends to pass from left to right to establish osmotic equilibrium (little arrows). In the familiar experimental set-up, the solvent (left side) is open to the atmosphere while the solution (right side) is enclosed where the pressure in excess of 1 atm builds up. If, as depicted here on the other hand, the solvent is enclosed while the solution is open to the atmosphere, then tension is generated within the solvent (left side). At the point of contact, solvent molecules flow spontaneously to the right against both these forces, tension (thin arrow) on the left and pressure (thick arrow) on the right, and establish equilibrium.

Right panel: For solid bodies in contact this equilibrium is impossible. When two springs joined end-to-end are at their rest lengths (or both stretched or both compressed) they are in equilibrium, i.e., there is no resultant force exerted at their point of contact. However, when one is stretched and the other compressed, there is a resultant force, and mass on both sides of the contact point is accelerated to the left.

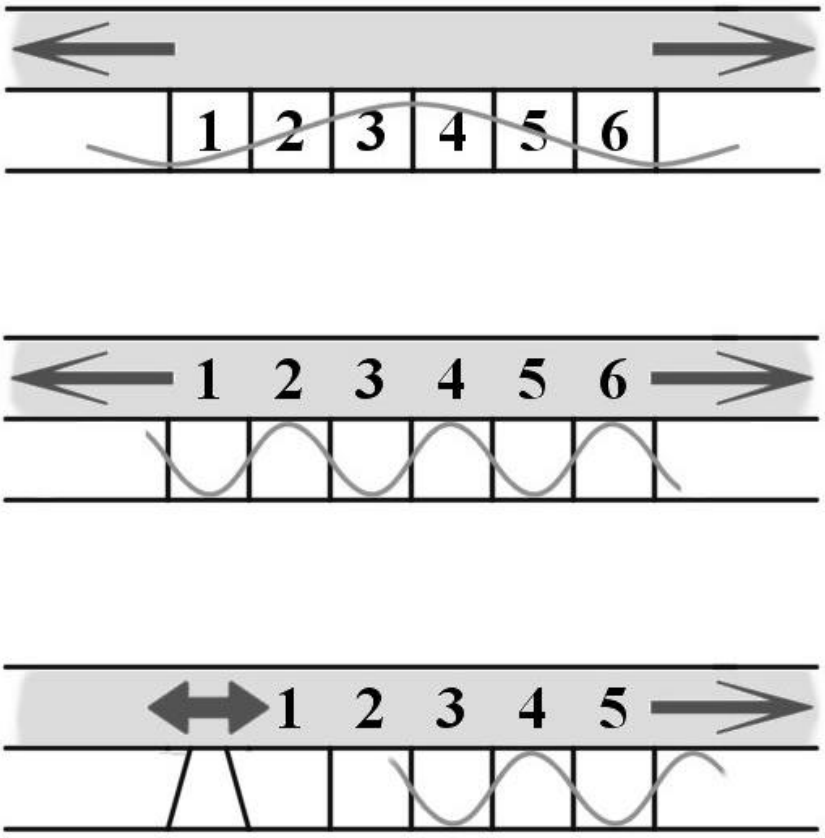

Fig. 5. Synchronization of enzymic function.

The layer of water of hydration is shown situated on top of the line of protein domains making up the enzyme complex described in Fig. 1. It is not a random liquid without form but a gel held in place by strong lateral tension and bonding to the protein surface, i.e., it is a single water cluster. When tension also extends through the whole complex as shown by the long wavelength of the structure wave (see Fig. 6.), the substrates react simultaneously. After catalysis, the domains become disconnected as shown by the short wavelength with nodes at the domain interfaces. Pressure is now exerted at the active sites between the domains and the products are pulled into the adjacent hydration layer. A conformational change in the protein subunits at the start of the sequence exposes a hydrophobic surface to the hydration layer. The lateral force in the water now switches from tension to pressure changing the balance of mechanical forces at this location, and the layer is moved as a unit to the right.

Fig. 6. Bonding and antibonding protein states.

The substrate molecule (zig-zag line) binds in an open groove between two domains of an enzyme complex, introducing attractive interactions across the interface. Initially, these two domains are oscillating as two separate clusters with a node between them located at the binding site. The tight enzyme-substrate complex which forms is denoted by the full wave. At this point the two clusters have fused to form a single cluster with an antinode at the binding site. After catalysis, the domain interface is again repulsive opening the groove and releasing the products (v shapes). In terms of the structure wave, there is an harmonic transition as the two clusters fuse doubling the wavelength, followed by the reverse transition halving the wavelength back to the initial value. Thus the sequence illustrates an antibonding-bonding-antibonding transition between the domains.