ELECTRON PAIRING AS A SOURCE OF CYCLIC INSTABILITIES IN ENZYME CATALYSIS

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Received 3 July 1978

Enzymatic reactions can be interpreted in terms of a thermodynamically consistent potential surface with different pathways for complex formation and decomposition. The construction suggests that unstable pairing of parallel spin electrons provides the basis for pathway switching.

The specificity of enzymes for particular substrates is generally attributed to shape complementarity and cumulative van der Waal's interactions (since the r^{-6} dependence of the latter allows for a significant interaction only in the event of a close fit). If the complex is to form rapidly, it should be possible for the enzyme and substrate to fall into this specific fit from a large variety of initial contacts. However, this means a lower free energy for the complex and therefore slower decomposition. The purpose of this note is to show that it is possible to re-interpret the free energy changes accompanying enzymatic reactions in such a way that within a limit determined by the highest uncomplexed free energy this conflict is eliminated. This re-interpretation is based on the construction of a thermodynamically consistent free energy diagram with different pathways for complex formation and decomposition. The diagram suggests that the instability of the complex has its basis in a short-lived, unstable pairing of parallel spin electrons deriving from confinement constraints imposed by complex formation [1]. The temporary destabilization of electronic structure arising from such pairing in effect provides an energy loan which finances the conformation change associated with the switch to the low activation energy pathway.

The construction is illustrated in fig. 1. $\mathcal{E}$ is the enzyme, $\mathcal{S}_1$ and $\mathcal{S}_2$ the substrates, and $\mathcal{E}\mathcal{S}$ the enzyme–substrate complex. $\mathcal{E}\mathcal{S}_1^{\ddagger}$ and $\mathcal{E}\mathcal{S}_2^{\ddagger}$ are activated states of enzyme and substrate. The curve *abcfgh* (including the dotted line) runs through the free energy of the complex, $\hat{F}_{\mathcal{E}\mathcal{S}}$, as determined by either kinetic measurements or measurements of equilibrium concentration (using the formula, $[\mathcal{E}][\mathcal{S}]/[\mathcal{E}\mathcal{S}] = \exp[\hat{F}_{\mathcal{E}\mathcal{S}} - F_{(\mathcal{E}+\mathcal{S})}]/N_0 kT$). The lines *acd* and *hfe* (to be called 1 and 2) represent formation pathways and *dba* and *egh* (to be called 3 and 4) decomposition pathways. All points along these pathways represent real free energies, as might be determined by heat measurements or in principle calculation. The formation and decomposition pathways may both be traversed reversibly, except that the decomposition pathway can only be entered from the complexed state. This is indicated by the arrowheads on lines *bd* and *eg*. F_1^- and F_2^- are the activation energy advantages of the decomposition over the formation pathways for the left and right peaks respectively.

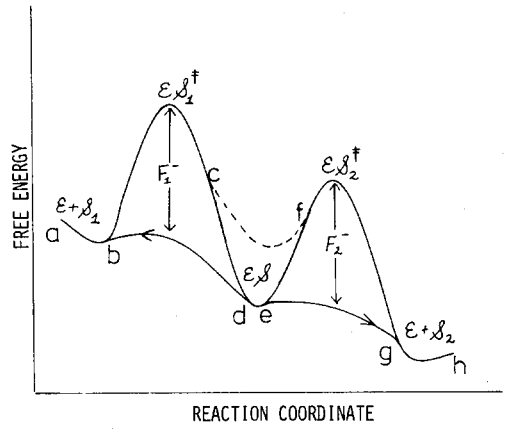

Fig. 1. Two pathway (energy loan) diagram for $\mathcal{E} + \mathcal{S}_1 \rightleftharpoons \mathcal{E}\mathcal{S} \rightleftharpoons \mathcal{E} + \mathcal{S}_2$.

Upward thermal fluctuations bring the uncomplexed systems ($\mathcal{E} + \mathcal{S}_1$ or $\mathcal{E} + \mathcal{S}_2$) into the activated states ($\mathcal{E}\mathcal{S}_1^{\ddagger}$ or $\mathcal{E}\mathcal{S}_2^{\ddagger}$). These may either fall back into the uncomplexed states or into the complexed configuration associated with the minimum between d and e. Falling into this minimum is followed by the switch to the decomposition pathway, implying that the complexed configuration is unstable and therefore that its concentration will be smaller than it would be for a stable minimum of the same free energy. The advantage is that this low real free energy is compatible with a drawing in of the substrate (possibly corresponding to the notion of induced fit [2]), yet does not interfere with the rate of complex decomposition. It may be decreased to allow for greater speed and specificity of formation without any decrease in the rate of decomposition provided that it does not fall below the free energy of the highest noncomplexed minimum.

The switch from the formation to the decomposition pathway does not affect the equilibrium of $\mathcal{S}_1$ and $\mathcal{S}_2$ provided that $F_1^- = F_2^- = F^-$ and thus does not lead to any disagreement with thermodynamics. This can be verified by taking the rate constants as given by the classical Arrhenius formula, $k_i = A_i \exp(-\Delta F_i^{\ddagger}/N_0 kT)$, where $\Delta F_i^{\ddagger}$ is the activation energy along pathway i. Since F^- is the difference between the maxima of both pathways, decomposition is faster by a factor $\exp(F^-)$ along the decomposition than along the formation pathways and speeded up equitably in both directions from the minimum. This assumes that $A_1/A_2 = A_3/A_4$. This (and also the symmetry of F_1^- and F_2^-) is reasonable on physical grounds since the weak bonding between enzyme and reactant is the same whether or not a covalent bond has been formed or broken, implying that barriers 1 and 3 describe the same physical situations as 2 and 4, respectively, or at most appear different if the mechanism of decomposition is different in the two directions. However, it can also be assumed that the major mode of motion of each of the activated states is translational motion and therefore (following the theory of absolute reaction rates [3]) that each of the A_i can be taken as kT/h.

The energy loan construction implies that the decomposition pathway is dynamically opened up in the course of the reaction (otherwise its existence would obviate the barriers to complex formation) and in a way which allows an energy-balanced regeneration of the enzyme. The following interpretation is consistent with this requirement. The switch to the decomposition pathway occurs when downward uncertainty and thermal fluctuations make possible a short-lived potential energy dominance which allows parallel spin electrons to pair and thus frees them to drop below the energy floor normally maintained by the Pauli exclusion principle. Such pairing is possible if the cumulative van der Waal's and other weak interactions which hold the complex together are sufficiently strong for complex formation to push nuclei together which ordinarily repel because of nonbonding, parallel spin electrons. At the same time these electrons repel because of the exclusion principle and to a much lesser extent because of a (screened) Coulomb repulsion. The net result is a spatial confinement which increases uncertainty fluctuations in their kinetic energy and weakly favors a phase correlation in their motion, thereby opening the possibility of establishing a short-lived composite system. The paired configuration is highly unstable and energy released by pair falling is either immediately recaptured to reestablish a normal orbital structure, or if the pair persists long enough to produce a nuclear motion, recaptured at the end of this motion. This nuclear motion is the conformation change which provides the mechanism of switching in the energy loan construction. The release of energy can be thought of as an energy loan since it gives rise to the activation energy advantage F^- without compromising an energy-balanced regeneration of the enzyme. Such an active barrier reduction makes it possible to support a recognition process which is active in the sense that it leads to a complex of low real free energy, thereby increasing the speed of complex formation without decreasing the speed of decomposition. The increase in the allowed strength of the complex and the nuclear motion are also capable of facilitating conventional forms of bond modification, or in an alternative process paired electrons may resurface in a way which favors bond formation or breakage for other electrons.

A single, transient pairing event can initiate the process. Thus a coupling interaction smaller than kT is sufficient provided that the waiting time to which it gives rise is short. An indicative expected waiting time for a small pairing energy $\Delta E < N_0 kT$ can be obtained from the equilibrium formula for the ratio of the two configurations at constant temperature and pressure and is given by $\bar{\tau} = K\langle N\rangle = K[\exp(-\Delta F/2N_0 kT) + 2]/m$,

where ΔF, taken as the difference in the Gibbs free energy between the unpaired and paired configurations, is pictured as an average on a single system taken over a large number of time periods, each just long enough for an ensemble to reach equilibrium, N is the number of such periods, m is the number of pairable electrons (with $mN = 2n_p + n_u$), and K is a proportionality constant which depends inversely on the rate of equilibration. This rate should be fast and thus the waiting time for a single pairing event could be short even though pairing is accompanied by an entropy decrease.

The proposed coupling interaction (allowed to be weak) can be thought of in terms of a compression of the average distance between constrained nuclei, with the consequence that the electrons repel because of exclusion interference and are therefore influenced in a coherent way by the nuclear charge densities. Alternatively, since the phases are not random the nuclei oscillate and the electrons can be thought of as exchanging virtual particles. Other sources of oscillation may be present and alternative mechanisms of weak coupling are possible. [4] However, the confinement constraint has the additional property that it reduces σx and therefore leads to an increase in the uncertainty of the kinetic energy of the order $\hbar^2/(2m_e)(\sigma x)^2$. This prevents permanent pairing, but also favors pairing during short time intervals of downward fluctuation. Since paired electrons differ from other electrons in the system (and from each other) in some quantum number other than spin, nothing prevents them from falling as soon as they are formed and any fluctuations favoring pairing may thus serve to trigger a critical sequence of events.

Falling and delocalization reduce the Coulomb repulsion and momentum fluctuations, but also eliminate the original coupling interaction. To break up, however, the pair must re-absorb energy sufficient to re-establish a normal orbital structure (since unpairing at lower energy levels would conflict with the exclusion principle). This may happen immediately, but if the pair persists long enough the destabilization of the electronic structure may release sufficient energy to produce the nuclear motion, causing the pair to be trapped until the nuclei return to their low energy configuration. However, the pair is so unstable an addition to the normally allowed orbital structure that the requirement for its annihilation in effect dominates the dynamics of the system. Alternatively, unpairing can be thought of in terms of an entropy driven increase in the energy of the electronic configuration. This is why some or all of the energy released by pairing must be released on loan and therefore why the pairing mechanism makes feasible an energy-balanced regeneration of the enzyme.

The mechanistic model is to some extent prescribed by the energy loan construction. The construction requires a constraint which makes the decomposition pathway inaccessible except from the complexed configuration, implying that the instability of the complex ultimately derives from the same shape complementarity responsible for specificity. The energy-balanced switching from the formation to the decomposition pathways requires an energy loan. This suggests that the constraint pairs electrons, since pairing makes possible an instability of the normal electronic structure which is self-annihilating. Downward uncertainty and thermal fluctuations assume a role since any coupling interaction between electrons is likely to be weak and because only one or a few pairing events are necessary. This suggests that the constraint is a confinement constraint on parallel spin electrons, since this would increase the uncertainty fluctuations in their momentum, therefore increasing the chance of a short-lived pairing event which triggers the process. Such a confinement constraint also provides a weak coupling interaction and is the natural type of constraint to associate with complex formation. The non-reactivity required for confinement and the importance of instability in the process also suggests parallel spin pairing. For antiparallel spins the confinement argument does not work and covalent bond formation, if possible, would be favored.

The model has a number of implications. These include the difference between the real and apparent free energy of the complex, the persistence of a magnetic moment for the duration of the nuclear motion, and a (non-Arrhenius) temperature dependence during the pair formation phase of the enzymatic process (since pair formation is more likely at a lower temperature). Variations of the energy loan diagram can be made which correspond to a number of processes involving macromolecular motions, including sequential processes in catalysis, allosteric control, self-assembly, putative diffusive macromolecular motions in membranes, channeled transport, protection against inhibition, and processes (such as energy transfers) in which the loan is not fully repaid.

References

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