Heuristic Considerations Suggesting a Role for Electron Pairing in Thermodynamically Consistent Pathways of Enzyme Catalysis

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Z. Naturforsch. **33a**, 1206—1209 (1978); received July 26, 1978

Nuclear motions accompanying enzyme catalysis are described in terms of a hypothetical reaction mechanism in which instabilities of electronic structure arise from short-lived pairing resonances of parallel spin electrons. A pairing interaction is proposed which has its basis in confinement constraints imposed by complex formation.

A fundamental requirement for any satisfactory model of enzyme catalysis is that it allow for high specificity and speed in a way which is compatible with an energy balanced regeneration of the enzyme. The purpose of this paper is to point out that this requirement is fulfilled by a class of reaction schemes in which instabilities of electronic structure arise from short-lived resonances of parallel spin electrons. Such resonances are possible under conditions of high confinement and downward fluctuations in kinetic energy. The confinement itself has its origin in shape complementarity, hence in the same weak interactions which contribute to the binding energy of the complex and (in the case of the r^{-6} van der Waal's interactions) to its specificity. The direct contribution of the orbital instability resulting from this confinement is to the macromolecular shape changes which accompany catalysis, including shape changes which facilitate decomposition of the enzyme-substrate complex.

Pairing of large numbers of antiparallel spin electrons (due to an interaction arising from lattice vibrations) is known to be the basis of superconductivity [1]. A number of early investigators also considered the possibility of permanent antiparallel pairing in a chemical context, although again in connection with a condensation process [2, 3, 4, 5]. In the reaction scheme described here (see Fig. 1) the spins are parallel, only one or a few electron pairs are involved, and the pairing is unstable and short-lived [6]. The underlying idea is that the behavior of electrons which are pushed together is indistinguishable from the behavior of electrons which are attracted to one another (e.g. by an exchange of virtual phonons). The pushing (or confinement constraint) is possible if the cumulative van der Waal's and other weak interactions which hold the complex together are sufficiently strong to compress the equilibrium distance between nuclei orbited by nonbonding, parallel spin electrons. These electrons are thus pushed together by complex formation and at the same time pushed apart by exclusion interference (and to a lesser extent by a

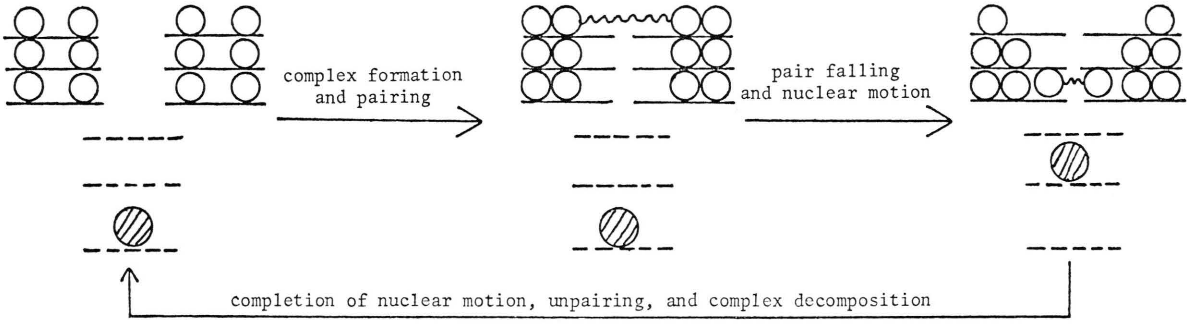

Fig. 1. *Schematic illustration of the role of electron pairing in enzyme catalysis.* Small circles represent electrons, pairs of small circles connected by a wavy line represent parallel spin pairs, solid energy level lines are electronic energy levels, cross-hatched circles represent nuclei, and dashed energy level lines represent energy levels of the nuclear configuration. As explained in the text, the scheme allows a temporary exchange of potential energy between electronic and nuclear configurations due to a short-lived, complex-induced electron pairing. Note that the delocalization of the pair in the normally forbidden energy levels is not represented.

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screened Coulomb repulsion). The resulting spatial confinement energetically favors a phase-correlated motion, thereby potentially establishing a configuration which is indistinguishable from a bound pair (except for its inherent instability). The appearance of the pair means the creation of new quantum numbers for the electrons, distinguishing them from other electrons in the system and (because of the parallelism of spin) from each other. Thus once the pair forms, there is nothing to prevent it from immediately falling through the energy floor normally maintained by the Pauli exclusion principle. However, its instability prevents it from remaining below this floor and hence any instability in electronic structure arising from pair formation will be self-annihilating, either requiring re-absorption of the energy released by falling or leading to an electronic structure of lower energy than the original structure. In the former (cyclic) case the temporary destabilization potentially provides an energy loan which is capable of supporting an energy balanced nuclear motion. This will happen if the energy of falling is temporarily channeled into an increase in the potential energy of the nuclear configuration before being channeled back into the unpairing process. *The advantage is that the nuclear motion can dynamically open up a low activation energy reaction route for complex decomposition, therefore allowing for the binding energy of the complex (hence the specificity) to increase without decreasing the speed of complex decomposition.*

Points to note are:

(i) The interaction between electrons can be thought of in terms of an induced attractive interaction since the correlation in phase means that the electrons will be influenced in a coherent way by the nuclear charge densities. From a field theoretic point of view the resulting oscillatory motions of the nuclei lead to an exchange of virtual particles between the electrons.

(ii) In general the coupling interaction would be too small relative to kT to effect coupling. However, only a single, short-lived pairing event is necessary and thus a coupling interaction smaller than kT is sufficient, provided that the waiting time for a sufficiently large downward fluctuation in the kinetic energy is short. Taking the waiting time, $\bar{\tau}$, for the first pair as proportional to the size of the ensemble of confined systems which on the average would be required to find one pair as soon as an equilibrium distribution is reached, an estimate can be obtained by considering the reaction $\mathrm{e} + \mathrm{e} \leftrightharpoons \mathrm{e}_2$. The ratio of pairs to single electrons is

$$n_{\mathrm{p}}/n_{\mathrm{u}}^2 = \exp(\Delta F/N_0\, kT)\,,$$

implying that the waiting time is given by

$$\bar{\tau} = K[\exp(-\Delta F/2N_0\, kT) + 2]/2\,,$$

where K^{-1} is the rate of equilibration, ΔF is the difference in Gibbs free energy between the unpaired and paired configurations, and k is Boltzmann's constant. If there are more than two pairable electrons in the system, the waiting time will be shorter. Note that for zero or positive ΔF the waiting time depends for all practical purposes on the rate of equilibration and not on the pairing energy, which must in any case be positive ($E_{\text{paired}} < E_{\text{unpaired}}$). If the free energy change is negative (i.e. if the entropy decrease associated with pairing dominates the energy decrease) the dependence on ΔF becomes important, but pairing is still possible and the waiting time can be short if K is small.

(iii) Confinement means a decrease in σx and therefore an increase in the uncertainty of the kinetic energy by $\hbar^2/2m(\sigma x)^2$. This increases the probability of pair formation since the increased fluctuation leads to lower as well as higher values of the kinetic energy. Thus the same confinement constraint which gives rise to the pairing interaction also increases the probability of downward fluctuations in kinetic energy which favor short-lived pairing. Note that the confinement argument does not work for antiparallel spin electrons.

(iv) Falling through the energy floor allows delocalization, thus decreasing uncertainty fluctuations in the kinetic energy, but also reducing or eliminating the basis for the phase correlation. The pair may persist for a short time, however, either as a virtual pair or because unpairing requires energy. If it persists long enough to channel the energy of falling into an increase in the potential energy of the nuclear configuration, it may be trapped until the nuclear motion is complete. The instability of the pair increases the liklihood that its annihilation will mediate any possible decrease in potential energy of the system and therefore cyclic processes should be common. From the thermodynamic point of view the paired state is a low entropy state (because of the phase correlation) and therefore unpairing will be driven by entropy. If the process is not cyclic, the system cannot be an enzyme, but a role as a specific reactant in energy transfer is possible.

The pairing scheme illustrated in Fig. 1 has an important implication for the free energy changes accompanying enzymatic reactions. This is summarized in the two-pathway free energy diagram (called an energy loan diagram) illustrated in Figure 2. Upward thermal fluctuations bring enzyme and substrate into an activated state $\varepsilon s^{\ddagger}$, or alternatively bring the enzyme and product into the activated state $\varepsilon p^{\ddagger}$. These may either fall directly back into the uncomplexed states ($\varepsilon + s$ or $\varepsilon + p$) from which they originated or into the complexed state, εs, in which case they can jump into either of the uncomplexed states. In the usual, single-pathway diagram the complex sits on a minimum of relatively high free energy (described by the dashed line) and the barriers to complex formation and decomposition are the same, except traversed in different directions [7]. In the two-pathway diagram the complex sits in a minimum of lower free energy (described by the solid line) and the barriers to complex decomposition (lower pathway, with arrows) are different than the barriers to formation. The equilibrium of s and p are not affected as long as the activation energy advantages, F_1^- and F_2^-, are equal (since the only effect in this case is to speed the decomposition process by a factor $\exp(F_1^-)$ in both directions). This activation energy advantage is possible because the energy released by pair falling causes a nuclear motion which temporarily opens up the low activation barrier pathway to complex decomposition, thereby making possible a complex of lower free energy without decreasing the speed of decomposition (provided that the minimum associated with the complex does not fall below the highest noncomplexed minimum). Clearly this allows for greater specificity and also more precise orientations of enzyme and substrate.

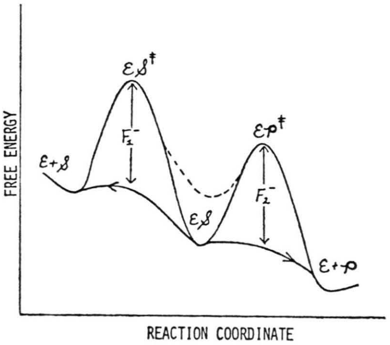

Fig. 2. *Energy loan diagram for* $\varepsilon + s \rightleftharpoons \varepsilon s \rightleftharpoons \varepsilon + p$ See text for explanation.

The arrows on the lower, decomposition pathway indicate that this pathway can only be entered from the complexed state, implying the existence of a constraint (such as the confinement constraint) associated with complex formation. If the decomposition pathway could be entered from the uncomplexed configurations, the barriers would be small for formation as well as decomposition and therefore the advantage for specificity of a deeper minimum lost. The minimum described by the dashed line also has an interpretation. Since the complex is unstable, its equilibrium concentration will be smaller and its rate of decomposition larger than would be the case for a stable minimum of the same depth as the minimum described by the solid line. Thus the shallow minimum described by the dashed line would correspond to the phenomenological free energy of the complex as determined by equilibrium or rate measurements, whereas the deeper, solid line represents the free energy (as determined, e.g. by heat measurements) of the real, but unstable minimum. The destabilization should not be thought of in terms of the ejection of the substrate (which would require work) but rather in terms of the use of an energy loan to temporarily remove activation barriers to complex breakup. Note that the activation energy advantage is not the same as the energy loan. The former is equal to the difference in height between the original barriers and the barriers associated with the pathway opened up by the shift in nuclear configuration. The latter is equal to the energy released by pair falling and is responsible for the shift in nuclear configuration.

The importance of downward fluctuations for pair formation leads to the prediction of an anomalous temperature dependence for the stage of catalysis in which pair formation takes place. Because of the parallelism of spin, this stage should be marked by the appearance of a spin one particle. The model also implies that the real and apparent free energies of the complex are not the same (as described above). The pairing scheme presented here is not intended as a theory of the bond modification process except insofar as this may be influenced by pair falling and the resulting nuclear motion. This nuclear motion should be distinguished from induced fit type shape changes concomitant to complex formation [8]. Instead it serves to destabilize the complex, thus facilitating its decomposition. Alternatively, it may facilitate allosteric switching or related forms of ordered nuclear motion in macromolecules.

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