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Enzymatic activity in disordered states of proteins

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Although disordered proteins are able to carry out a variety of different functions, particularly those involved in signalling and regulation, they have been observed to perform catalysis only in a small number of cases. The presence of structural disorder is indeed expected to be poorly compatible with enzymatic catalysis, which requires a well-organised environment in the active site of the enzyme in order to facilitate the formation of the transition state of the chemical reaction to be catalysed. Despite this stringent requirement, current evidence suggests that certain partially disordered proteins could be catalytically active by becoming structured in the regions of their active sites, even if their overall states retain a significant degree of conformational heterogeneity. This type of mechanism, however, does not appear to be not very common, perhaps because the time required to the conformational search within a disordered state to establish a catalytic environment in the presence of the substrate should not be longer than the overall turnover time required for optimal function. In addition, the catalytic environment should be maintained for long enough despite the structural fluctuations to enable the catalytic reaction to take place. As some partially unstructured proteins have been reported to be capable of overcoming these severe limitations and act as enzymes, their study can increase our general understanding of the mechanism of enzymatic catalysis, as well as extend our ability to control the range of functions that can be performed by disordered proteins.

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Introduction

Protein molecules in their natural environments are constantly undergoing conformational fluctuations that take place on timescales ranging from nanoseconds to milliseconds and beyond. The roles of these dynamics in enabling protein functions are being increasingly recognized, as they generate ensembles of conformations among which there are those capable of performing biochemical activities [1–7].

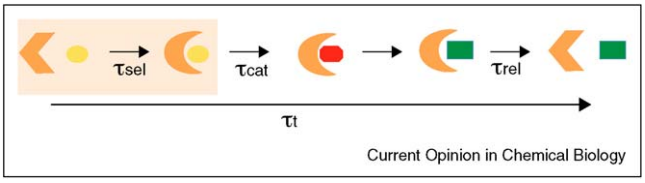
Although this concept was initially identified in the case of the well-defined native structures of globular proteins, it is becoming clear that it has a more general validity, as evidence accumulates about the activities of disordered and partially disordered proteins [8–11]. It is now well established that disordered proteins can perform effectively a range of different functions, in particular those that require molecular recognition such as transcriptional and translational regulation, and signalling. The key advantage that the structural flexibility associated with disorder can provide over the greater rigidity of fully folded native states is that it enables an effective search of the conformational space to be carried out. Disordered proteins can thus readily explore a range of possible structures to bind their partners, which, depending on their specific functions, can be other proteins, nucleic acids, lipids or smaller metabolites [11–22]. This 'conformational selection' mechanism has been exploited by evolution and we now know that a significant fraction of the proteins in complex organisms are either entirely or partially disordered in their native states [10,11].

Enzymatic catalysis, however, appears to be much less compatible with the presence of structural disorder, and this function is not normally considered among those that disordered proteins can perform. The catalytic step in an enzymatic process requires a protein to provide an environment in which the transition state of the chemical reaction can be reached more readily than in the absence of the enzyme [23–26]. As a very specific organisation of the interacting centres within the catalytic site of the enzyme appears generally to be required for this process to take place with optimal efficiency [27], disordered proteins are expected to be poorly suitable as enzymes — but recent evidence suggests that this needs not to be always the case.

Examples of enzymatic activity in disordered states

Although the catalytic process, as observed above, requires a highly accurate structural arrangement of the active site, there have been reports of enzymatic activity in disorder states [28,29•,30–36,37•,38]. These observations suggest that the conformational selection mechanism can be exploited effectively in disordered states to favour the initial association of enzymes and substrates (Figure 1). Indeed, a recent study has demonstrated that site-directed mutagenesis can be used to tune conformational fluctuations to maximize the rate of substrate binding, as well as that of product diffusion [39].

Figure 1



Scheme of the main steps in an enzymatic reaction. In the first step (orange shaded area) a conformational search takes place for a structure of the enzyme (orange crescent) capable of forming a complex with the substrate (yellow circle); this step takes on average a time τ_{sel}. In the second step the transition state of the reaction (red circle) is reached within the favourable environment provided by the catalytic site; this step takes a characteristic time τ_{cat}. In the third step the reaction is completed, and in the fourth step the product (green square) is released and the enzyme returns to its original state (orange chevron); this final step takes on average a time τ_{rel}. Overall, the enzymatic reaction takes a turnover time τ_t. All the characteristic times discussed in this work are the inverse of the corresponding rates, which are more commonly discussed in the literature.

One of the first examples of a protein capable of performing enzymatic activity in a non-native state has been that of ribonuclease T1 [28,30]. This protein contains four proline residues and populates various folding intermediates that reflect different proline isomerisation states and are generally characterized by the presence of extensive secondary structure elements and of native-like topologies. Some of these intermediates have been shown to exhibit a significant fraction (40–50%) of the ribonuclease activity of the native state [28,30]. Subsequently, other examples have been provided, including those of two circular permutants of dihydrofolate reductase [40] and of a double mutant of a staphylococcal nuclease [31], which were found to be enzymatically active *in vitro* in their molten globule states.

A particularly well-characterized case is represented by a monomeric variant of chorismate mutase, which is normally a homodimeric enzyme, obtained by inserting a hinge-loop sequence within the long N-terminal α-helix of the protein to disrupt the dimer interface [41]. The mutant protein was shown to be enzymatically active in its molten globule state, with efficiency comparable to the wild-type dimeric enzyme [33,35,37•,38]. It was also found that in the presence of transition state analogues the mutant enzyme adopts a native-like structure, while retaining an overall high flexibility. Structural fluctuations were suggested to be at play through conformational selection both during the initial enzyme-substrate recognition process and to facilitate the release of the products. In particular, this latter step was observed to take place faster than in the folded wild-type counterpart, suggesting that the presence of structural disorder can also be beneficial in the final step of an enzymatic reaction. These observations have recently been complemented by a computational study that demonstrated that in chorismate mutase a range of conformations distinct from the native state could also lead to significant catalytic activity [42•].

Another case that has been studied recently concerns the enzymatic activity of a folding intermediate of an acylphosphatase from *Sulfolobus solfataricus*, which was estimated to have about 80% of the enzymatic activity of the native state [29•]. Through a combination of protein engineering methods and molecular dynamics simulations [43], the intermediate state was characterized as comprising a well-structured region acting as a native-like scaffold to support a conformationally more heterogeneous region, which includes the catalytic site [29•]. Despite being relatively disordered, this intermediate state was found to contain conformations in which the catalytic residues are in native-like positions. It was suggested that this structural organisation creates an environment in the active site in the presence of the substrate resembling that required for efficient catalysis [29•].

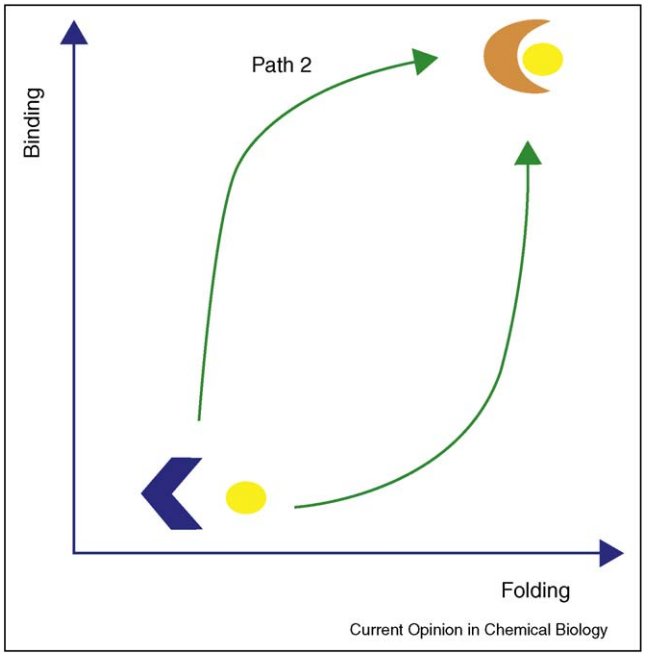
Equilibrium dynamics and enzymatic activity

The dynamics that we consider here concern the thermal fluctuations that enable conformational searches to be carried out within specific minima in the free energy landscape of proteins — native, intermediate or molten globule states. These dynamics are of essential importance for enzymatic reactions since they enable the initial molecular recognition events to take place, as proteins visit conformations capable of binding the substrates. The problem of carrying out this conformational search is usually quickly solved in native states (path 1, Figure 2), but it can severely limit the ability of enzymes to function efficiently in disordered states (path 2, Figure 2) because it can take a significant time. By contrast, these conformational fluctuations are unlikely to play a major role in the catalytic step in an enzymatic process (Figure 1), as such a step involves chemical reactions that are likely to be only weakly, if at all, coupled with the structural fluctuations by which the initial conformational search is performed [27,44].

A disordered-dependent conformational selection time

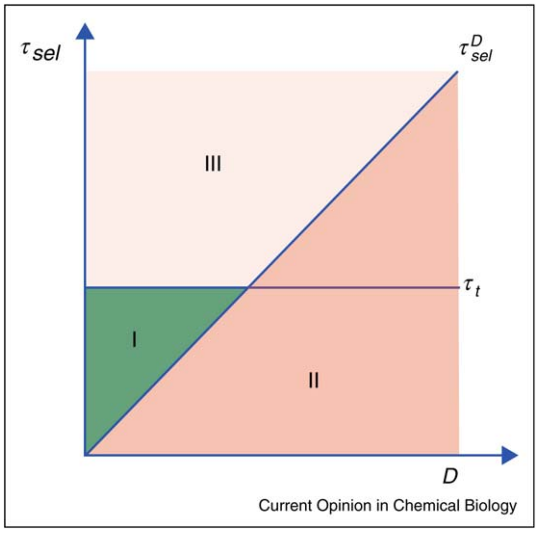
The mechanism of conformational selection is important in determining the functions of disordered proteins, in particular those that involve the molecular recognition events that are required for signalling and regulation. Although conformational selection could also help the molecular recognition process between disordered enzymes and their substrates, biologically relevant turnover times can be shorter than the time required by conformational selection within highly flexible states (Figure 3). In this sense, the conformational selection time τ_{sel} must not exceed the overall turnover time τ_t, which is required for optimal enzymatic activity. As the conformational selection time must increase with the

Figure 2



Schematic illustration of the coupling between the folding and binding processes in the molecular recognition events between enzymes and their substrates. In most cases, enzymes fold before binding their substrates (Path 1). In the case of disordered proteins, they fold upon or after binding (Path 2).

Figure 3



The disorder-dependent conformational selection time τ^D_{sel} is assumed to be proportional to the degree of structural heterogeneity D, which represents the size of the conformational space to be explored by the enzyme to find a structure capable of binding the substrate. For enzymes with well-formed native structures, D is small and correspondingly τ^D_{sel} is short, thus making the overall turnover time be determined by τ_{cat} or τ_{rel}. Even if D is relatively large there could be enzymatic activity, at least when the conformational selection time τ_{sel} is shorter than the overall turnover time τ_t (green-shaded region, zone I, $\tau^D_{sel} < \tau_{sel} < \tau_t$). When $\tau_{sel} < \tau^D_{sel}$ no enzymatic activity is possible (red-shaded region, zone II) since there is not enough time for the enzyme to establish a catalytic environment. Also when $\tau_t < \tau_{sel}$, no enzymatic activity is possible (light red-shaded region, zone III) since the enzyme is too inefficient to be viable in living organisms.

degree of structural heterogeneity D, we can expect an enzyme to require a time of the order of $\tau^D_{sel} \propto D$ to complete the conformational search (Figure 3). Conformational searches longer than τ^D_{sel} are not compatible with turnover times in living cells, which are normally in the range of milliseconds. In the case of acylphosphatase discussed above, the enzymatic reaction takes about 10 ms in the native state, whereas the folding process from the catalytically active folding intermediate to the native state requires a time about 20-fold longer [29•]. By taking this latter time as an estimate of τ_{sel}, as both times are associated with a conformational search within the intermediate state, one should conclude that the folding intermediate would not constitute by itself a viable enzyme, as it falls in the forbidden region in Figure 3. Of course, this is not much of a problem for *S. solfataricus*, as most of the enzymatic activity of this acylphosphatase is actually carried out in the native state [29•].

Maintenance of the organisation of the catalytic site

In addition to the condition, which was described in the previous section, on the duration of the conformational search imposed by the optimal turnover time, disordered enzymes should also be able to maintain a productive catalytic environment for at least a time τ_{cat}. Such a condition may be difficult to meet in partially structured states, even when the substrate is bound, since the overall conformation of the enzyme is experiencing significant fluctuations. The catalytic activity may therefore take place from within more structured substates, which would appear as local free energy minima within broader partially structured states. Although this type of substates are likely to be visible through standard methods of structural biology only in favourable cases, as for example recently shown for the molten globule of the nuclear coactivator binding domain (NCBD) of CREB binding protein [45], there may be opportunities to determine their structures by exploiting the recent advances that have been made in the characterization of 'invisible' states by nuclear magnetic resonance spectroscopy methods [46].

The view that enzymes fluctuate within an ensemble of substates also plays a key role in our current understanding of the mechanism of action of promiscuous enzymes, which are enzymes that catalyze functions that are different from those that they have evolved to promote [47]. Also in the case of promiscuous enzymes the selection of a

particular substate results in the acquisition of a catalytically competent conformation [7,48,49]. The considerable understanding that we have of promiscuous enzymes [7,48–50] may lead to further insight into the way in which disordered enzymes work.

Perspectives

Although disordered enzymes have been rather uncommonly observed, it could be possible to establish strategies for creating them in a more controlled manner, and thus extend the range of functions performed by disordered proteins for applications in biotechnology and synthetic biology. In order not to exceed the disordered-dependent conformational selection time even in partially structured states, the formation of the catalytic environment should be speeded up, for example by identifying or designing substrates capable of nucleating the structuring process of the catalytic site. In this sense, the enzymatic activity in disordered states can be achieved through a mutual action in which the substrates promote the folding of catalytic sites, which in turn become capable of catalyzing chemical reactions within the substrates themselves.

Conclusions

We have discussed the possibility of enzymatic activity of disordered states and described specific examples for which such an activity has been reported. We have suggested that this phenomenon is not observed very often because the time required to establishing the well-organised catalytic environment necessary for catalysis should not exceed the overall turnover time required for optimal function in living organisms. This condition is difficult to achieve in disordered states where the conformational space to be searched can be quite extensive. In addition, a steady catalytic environment should be maintained for long enough to enable the catalytic process to be completed, which may be particularly arduous in the absence of an overall well-defined native structure. Despite these stringent conditions, enzymes active in disordered states have been observed, which can be as catalytically efficient as in their native states, suggesting that through further research it might become possible to extend in a controllable manner to enzymatic catalysis the range of functions performed by disordered proteins.

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