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Review

Essential role of conformational selection in ligand binding

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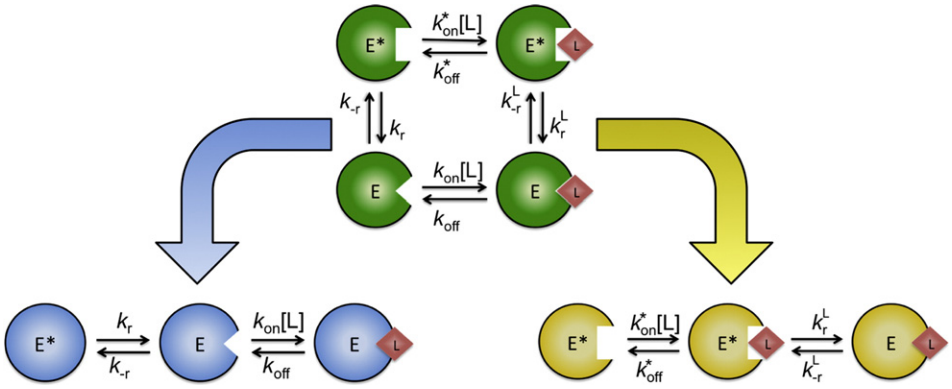
HIGHLIGHTS

- Conformational selection is always sufficient and often necessary to account for the relaxation kinetics of ligand binding.
- Induced fit is never necessary and only sufficient in a few cases.
- The long assumed importance and preponderance of induced fit as a mechanism of ligand binding should be reconsidered.
- Conformational selection is an essential component of any mechanism of ligand binding.

GRAPHICAL ABSTRACT

Linkage scheme containing four species is depicted in green. Conformational selection and induced fit, two special cases of the linkage scheme, are shown in blue and yellow.



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ABSTRACT

Two competing and mutually exclusive mechanisms of ligand recognition – conformational selection and induced fit – have dominated our interpretation of ligand binding in biological macromolecules for almost six decades. Conformational selection posits the pre-existence of multiple conformations of the macromolecule from which the ligand selects the optimal one. Induced fit, on the other hand, postulates the existence of conformational rearrangements of the original conformation into an optimal one that are induced by binding of the ligand. In the former case, conformational transitions precede the binding event; in the latter, conformational changes follow the binding step. Kineticists have used a facile criterion to distinguish between the two mechanisms based on the dependence of the rate of relaxation to equilibrium, k_{obs}, on the ligand concentration, [L]. A value of k_{obs} decreasing hyperbolically with [L] has been seen as diagnostic of conformational selection, while a value of k_{obs} increasing hyperbolically with [L] has been considered diagnostic of induced fit. However, this simple conclusion is only valid under the rather unrealistic assumption of conformational transitions being much slower than binding and dissociation events. In general, induced fit only produces values of k_{obs} that increase with [L] but conformational selection is more versatile and is associated with values of k_{obs} that increase with, decrease with or are independent of [L]. The richer repertoire of kinetic properties of conformational selection applies to kinetic mechanisms with single or multiple saturable relaxations and explains the behavior of nearly all experimental systems reported in the literature thus far. Conformational selection is always sufficient and often necessary to account for the relaxation kinetics of ligand binding to a biological macromolecule and is therefore an

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essential component of any binding mechanism. On the other hand, induced fit is never necessary and only sufficient in a few cases. Therefore, the long assumed importance and preponderance of induced fit as a mechanism of ligand binding should be reconsidered.

Contents

1. Introduction

The interaction between a ligand, L, and its macromolecular target, E, forms the basis of function and regulation in all biological systems. Understanding how ligands recognize their targets in terms of structure, energetics and kinetics remains a central issue to biochemistry and biophysics and a prerequisite to rationally design efficient enzymes, effective drugs, and new therapeutics [1]. The interaction involves two components: specific binding of L to E, and linked conformational changes that may precede and/or follow the binding step. The combination of binding steps and conformational transitions in any given mechanism of recognition generates the repertoire of kinetic behaviors accessible to experimental measurements. The challenge facing the experimentalist is to decipher the nature of conformational transitions involved in the recognition process from analysis of the transient behavior of the system relaxing to equilibrium [2,3]. A system of N independent species gives rise to N independent relaxations, some of which may be too fast to measure by conventional stopped-flow techniques or may be spectroscopically silent. Analysis of a ligand binding interaction must therefore rely only on the relaxations accessible experimentally and interpretation of the underlying mechanism should be based on the simplest kinetic scheme consistent with the observations.

2. Simplest binding mechanism: the lock-and-key model

Over the years, our interpretation of binding interactions has evolved alongside the emerging view of the plasticity of biological macromolecules buttressed by the successes of x-ray crystallography and NMR spectroscopy [4]. Energy landscapes portraying the conformations available to the macromolecule have replaced the static view of a single "species" converting from free to bound upon ligand binding (Fig. 1), as envisioned by the classic lock-and-key model introduced by Fischer [5]. In this model, binding of L to E is cast in terms of the single step reaction scheme

$$\mathrm{E} \underset{k_{on}[\mathrm{L}]}{\overset{k_{off}}{\rightleftharpoons}} \mathrm{EL},$$

where k_{on} ($M^{-1}\,s^{-1}$) is the second-order rate constant for ligand binding and k_{off} (s^{-1}) is the first-order rate of dissociation of the EL complex into the parent species E and L. The strength of the interaction is quantified by the equilibrium dissociation constant K_d (M) defined as k_{off}/k_{on}. The lock-and-key model predicts a relaxation of the system to equilibrium that is linear in [L], i.e.,

$$k_{obs} = k_{off} + k_{on}[\mathrm{L}]. \quad (1)$$

Measurements of k_{obs} as a function of [L] yield both k_{off} and k_{on} as the intercept and slope of the plot, respectively. Hence, for a simple binding interaction, the value of k_{obs} equals the rate of ligand dissociation for [L] = 0 and grows without limits as [L] increases.

It has been argued for a long time that the lock-and-key model is too crude an interpretation of ligand binding because it envisions a rigid body collision between the ligand and its target, thereby neglecting the conformational flexibility of the macromolecule [2,3,6] recently supported by x-ray crystallography [7], NMR spectroscopy [8,9] and single-molecule fluorescence detection [10]. Although this argument has important merits, it is not completely rigorous. The existence of multiple conformations is not necessarily incompatible with the lock-and-key model. An ensemble of conformations that interconvert on a time scale faster than binding and dissociation events is indistinguishable from a single species as far as the equilibrium and kinetic properties of the system are concerned. The partition function of a system capable of binding L at a single site, as in the lock-and-key model, is linear in [L] regardless of the number of conformations accessible to the macromolecule [11,12]. Likewise, the transient properties of the system depend only on the slowest kinetic steps in the mechanism and replacing E or EL in the lock-and-key model with an ensemble of rapidly interconverting conformations does not change the observed relaxation kinetics [6]. Therefore, the lock-and-key model describes the behavior of a macromolecule that exists in a single conformation but also applies to an ensemble of rapidly interconverting conformations, in which case k_{on} and k_{off} represents ensemble averages of rate constants over the entire population. In general, any individual species in a kinetic scheme can be replaced by an ensemble of rapidly interconverting species without changing the kinetic properties of the system. Therefore, the dynamic nature of proteins and the existence of an ensemble of conformations will not produce a departure from the simple properties predicted by the lock-and-key model, unless the conformational transitions take place over time scales that eventually affect events of functional significance.

3. Binding coupled to conformational transitions: conformational selection and induced fit

Consider next the relevant case of the macromolecule existing in multiple conformations interconverting on the ms–μs time scale, which is directly relevant to binding/dissociation reactions [10]. Two limiting cases should be considered, depending on whether the conformational transitions precede or follow the binding step. In both cases, relaxation to equilibrium no longer follows a straight line as in the lock-and-key model because the macromolecule assumes alternative