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Review

A novel type of allosteric regulation: Functional cooperativity in monomeric proteins

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ABSTRACT

Cooperative functional properties and allosteric regulation in cytochromes P450 play an important role in xenobiotic metabolism and define one of the main mechanisms of drug–drug interactions. Recent experimental results suggest that ability to bind simultaneously two or more small organic molecules can be the essential feature of cytochrome P450 fold, and often results in rich and complex pattern of allosteric behavior. Manifestations of non-Michaelis kinetics include homotropic and heterotropic activation and inhibition effects depending on the stoichiometric ratios of substrate and effector, changes in the regio- and stereospecificity of catalytic transformations, and often give rise to the clinically important drug–drug interactions. In addition, functional response of P450 systems is modulated by the presence of specific and non-specific effector molecules, metal ions, membrane incorporation, formation of homo- and hetero-oligomers, and interactions with the protein redox partners. In this article we briefly overview the main factors contributing to the allosteric effects in cytochromes P450 with the main focus on the sources of cooperative behavior in xenobiotic metabolizing monomeric heme enzymes with their conformational flexibility and extremely broad substrate specificity. The novel mechanism of functional cooperativity in P450 enzymes does not require substantial binding cooperativity, rather it implies the presence of one or more binding sites with higher affinity than the single catalytically active site in the vicinity of the heme iron.

Epigraph

S.J.G.: Let's talk about the ideas that were gradually forming for the concept of allosterism.
J.W.: They had really begun earlier. The paper with David Allen was published in 1951. But it was during my first trip to Japan that I really had a clear vision of conformational changes as giving rise to an entire array of functional properties.
S.J.G.: And this idea just came to you?
J.W.: It came to me as I was walking in Kyoto in a Zen garden

Conversations with Jeffries Wyman. Interview by Stanley J. Gill [1]

Introduction

Most of the key biological processes, such as formation of native biomolecules and their functional assemblies, as well as cellular signaling and regulation, are highly cooperative [2–4]. Cooperativity and allostery are observed as a result of a mutual perturbation of functional properties of a biological macromolecule interacting with two or more ligands [2]. This feature is commonly used in living systems to improve sensitivity to external chemical perturbation and to amplify the response of receptors, transport molecules [5–7], or xenobiotic metabolizing systems, including cytochromes P450 [8–11]. Homotropic and heterotropic cooperative effects in cytochromes P450 are commonly known as one of the main sources of drug–drug interactions [12–18]. We recently reviewed cooperativity concepts applied to cytochromes P450 [19]. However, many recent results suggest an update and refinement of the earlier conclusions on the molecular interactions giving rise to the cooperative functional response of cytochromes P450.

In biochemistry and biophysics, cooperativity is typically defined as the mutual perturbation of interactions of the ligand with a macromolecule (protein or DNA) at different binding sites [20]. Various aspects of cooperative effects in macromolecular binding have been thoroughly described in several excellent books [2,21–25]. Positive cooperativity is defined as the increase of the binding affinity at one site when other site is also occupied, and negative cooperativity is manifested if the second binding event

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is disfavored. Cooperative enzymes typically display a sigmoid plot of the reaction rate against substrate concentration, and an experimentally observed sigmoid dependence of activity on the substrate concentration is often interpreted as indication of cooperative binding, but this is not always true. Deviations from the simple Langmuir or Michaelis–Menten hyperbolic dependence of the enzyme properties on the substrate concentration can suggest the presence of more than one binding site and simultaneous interactions with several substrates and/or effectors. Importantly, sigmoidal steady-state kinetics is also possible in monomeric enzymes which bind only one substrate molecule at the single catalytic site [26]. An example of such system is represented by the human glucokinase, where slow conformational rearrangements between the states with low activity and high activity upon substrate binding explains significant functional cooperativity [27], as reviewed in this issue by Larion and Miller [28].

The word 'allosteric' was introduced by Monod and Changeux 50 years ago, as described in [29], while the idea of thermodynamic coupling between binding and conformational changes in macromolecules "...which gives rise to the entire array of functional properties..." [1] have been put forward by Jeffries Wyman as early as in 1951 [30]. His analysis of the cooperative binding of oxygen and carbon monoxide to the tetrameric human hemoglobin, and of the pH dependence of cooperativity known as Bohr effect, successfully attributed all observed effects to the large scale pH-dependent conformational equilibrium of hemoglobin tetramer between two well defined states with substantially different affinities. This work was the first seminal study which suggested the concept of the thermodynamic linkage between functional properties of macromolecules and their conformational and dissociative equilibria, which was developed further in 60s and 70s as reviewed [20,31–36].

In the simplest case, the overall binding of the small ligand to the macromolecule with N identical and non-interacting binding sites is described by the Langmuir isotherm (Eq. (1)), which does not depend on N:

$$Y = \frac{[ES]}{[E_0]+[ES]} = \frac{[S]}{K+[S]} = \frac{\frac{[S]}{K}}{1+\frac{[S]}{K}} \tag{1}$$

As shown on the Fig. 1, the shape of the binding isotherm plotted as the full line, is the same for $N = 1,2,3$. However, concentrations of the binding intermediates (dashed lines) are not identical. As seen from Eqs. (2 and 3), the binding isotherms can be represented as $Y = y_1/2 + y_2$ for two-site macromolecule, and $Y = y_1/3 + 2\cdot y_2/3 + y_3$ for three sites, where y_1, y_2, and y_3 are fractions of intermediates with one, two or three ligands bound.

$$Y = \frac{\frac{[S]}{K_1}+\frac{2[S]^2}{K_1K_2}}{2\left(1+\frac{[S]}{K_1}+\frac{[S]^2}{K_1K_2}\right)} = \frac{1}{2}y_1 + y_2 \tag{2}$$

$$Y = \frac{\frac{[S]}{K_1}+\frac{2[S]^2}{K_1K_2}+\frac{3[S]^3}{K_1K_2K_3}}{3\left(1+\frac{[S]}{K_1}+\frac{[S]^2}{K_1K_2}+\frac{[S]^3}{K_1K_2K_3}\right)} = \frac{1}{3}y_1 + \frac{2}{3}y_2 + y_3 \tag{3}$$

The multipliers 1/2 and 1 for two-site binding (Eq. (2)), and 1/3, 2/3 and 1 for three-site binding (Eq. (3)) yield the fractional contribution of corresponding binding intermediates to the overall binding isotherm, as they represent the fractions of occupied sites for each macromolecule. For non-cooperative binding to the macromolecule with N identical sites, the population of jth intermediate reaches it is maximum value at the same time when the average saturation reaches j/N [37].

In case of highly cooperative binding, the population of all binding intermediates except the last is low because of the large increase of the binding affinity when macromolecule approaches saturation. This results in the change of the shape of binding isotherm from convex (hyperbolic, non-cooperative Langmuir isotherm, Fig. 1) to concave, or "sigmoidal", as shown in Fig. 2 for dimeric hemoglobin HbI from *Scapharca inaequivalvis* [38] and tetrameric human hemoglobin [39].

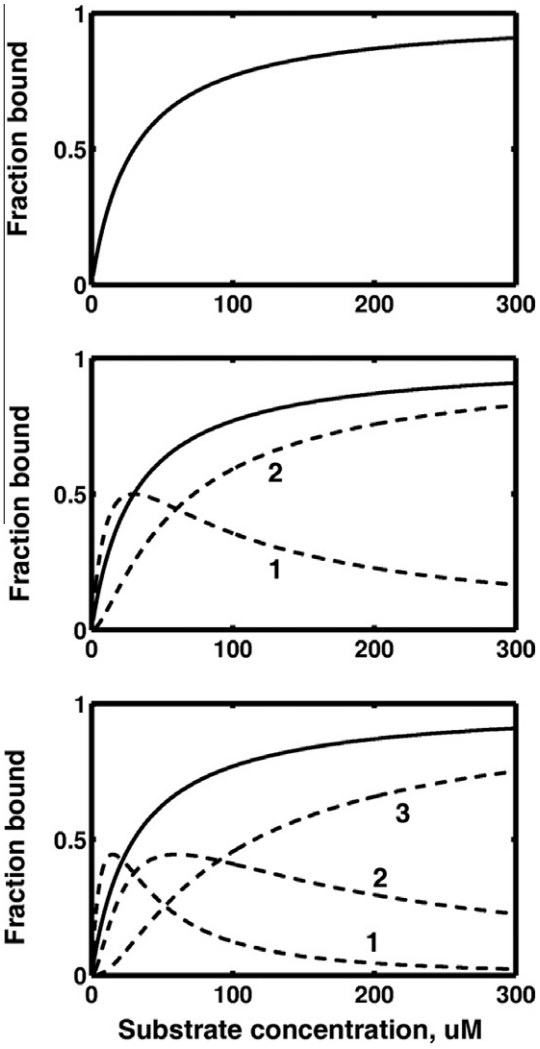

Fig. 1. Non-cooperative binding isotherms for one (top), two (middle), or three (bottom) sites without cooperativity are shown. The resulting isotherms are identical, however the difference in distributions of binding intermediates is shown as dashed curves.

Importantly, the same result can be observed for the functional response of macromolecule even in the case of non-cooperative binding, if the fractional contributions of the binding intermediates into the overall observed macromolecular function are different because of the different activity at the different levels of saturation. For the molecule with three binding sites the overall response can be expressed as the linear combination of contributions from each binding intermediates as shown in Eq. (3a):

$$Y_a = \frac{a_0 + a_1\frac{[S]}{K_1} + a_2\frac{[S]^2}{K_1K_2} + a_3\frac{[S]^3}{K_1K_2K_3}}{1+\frac{[S]}{K_1}+\frac{[S]^2}{K_1K_2}+\frac{[S]^3}{K_1K_2K_3}} = a_0y_0 + a_1y_1 + a_2y_2 + a_3y_3 \tag{3a}$$

Here the shape of the overall observed response Y_a (typically measured as a spectral signal or activity) as a function of the substrate concentration, depends not only on the stepwise dissociation constants K_i, ($i = 1,2,3$), but also on the fractional amplitudes a_i.