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Review

Allostery in pharmacology: Thermodynamics, evolution and design

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ABSTRACT

This review focuses on basic models of allostery, the ambiguous application of the allosteric term in pharmacology illustrated by receptors, the role of thermodynamics in allosteric mechanisms, evolution and design of allostery. The initial step of ligand activation is closure of the agonist-binding cavity. Large entropy increases accompany the agonist-elicited conformational changes of pentameric ligand-gated ion channels due to cavity closure and rearrangement of transmembrane helices. The effects of point mutations on thermodynamic parameters of binding and function can reveal energetic coupling of neighbouring (and distant) amino acid residues in activation. High-order double-mutant cycle analysis and rate-equilibrium linear free-energy relationships can identify the trajectory and conformational spread of activation.

Protein assembly and allostery can be deduced from colocalization and physicochemical principles. Molecular evolution has led from homooligomerization of protomers to heterotropic cooperativity and to allosteric regulation. Examples are discussed such as similar paths of protein (dis)assembly and evolution, irreversible evolution, statistical analysis of sequence homology revealing coevolution, different impacts of adaptation and evolution on hemoglobin, and the flagellar motor switch of bacteria. The driving force of dynamic allostery is associated with funnel-like free energy landscapes of protein binding and shifts in conformational fluctuations upon binding. Allostery can be designed based on our increasing knowledge of natural allosteric mechanisms and evolution. The allosteric principle has been applied for various bio/macro/molecular and signal transduction systems as well as in cognitive sciences.

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1. Introduction

Fifty years ago Max Perutz et al. (1960) revealed the structure of hemoglobin via X-ray crystallography and opened the way to reveal allostery and cooperativity within protein oligomers. The importance of Perutz' achievements was soon acknowledged by a Nobel Prize in Chemistry (1962). The allosteric term was subsequently introduced for enzyme regulation to distinguish regulatory and

Abbreviations: $GABA_A$, *A*-type γ-aminobutyric acid; KNF, Koshland, Némethy and Filmer; MWC, Monod, Wyman and Changeux; pLGIC, pentameric ligand-gated ion channels; TM, transmembrane.

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catalytic sites (Monod et al., 1963). The term of allostery has Greek origin: *allos* means other, *stereos* is steric (object, effect). One of the definitions of allostery (Fenton, 2008) says that allostery is a chain of interactions when binding of a ligand changes the affinities of distant binding sites of a biopolymer, cooperativity of its subunits, or efficacy of its function (e.g. signal transduction and catalysis). The cooperative term has similar meaning but it has been mostly used for interactions of whole subunits while allostery for those of distant (binding) sites.

Nowadays a search for "alloster*" in data bases yields tens of thousands of publications. Half of the hits still fit in the field of biochemistry where the term has been introduced first. Consequently, protein allostery has been the topic of several excellent reviews (e.g. Fenton, 2008; Goodsell and Olson, 2000; Jardetzky, 1996; Kern and Zuiderweg, 2003; Luque et al., 2002; Mandell and Kortemme, 2009; Oh et al., 2009; Ostermeier, 2005). Another half of the hits refers to further (life) sciences with pharmacology in leading position. This can be attributed to the increasing number of known oligomeric structures and to the importance of pharmacological receptors. This is a review of the recent progress related to pharmacological allostery, thermodynamics and evolution.

2. Allosteric models

Monod et al. (1965) introduced a concerted, symmetry or MWC model of allostery to interpret regulatory mechanisms of oligomeric enzymes. According to the original MWC model, ligand-free homooligomers exist in equilibrium of different states or conformations. It is assumed that 1) oligomeric structures are symmetric; 2) the conformation of protomers changes in a concerted way (all-or-nothing); and 3) upon ligand binding, the symmetric arrangements of protomers are maintained while the equilibrium of pre-existing oligomeric conformations is shifted. In contrast to the MWC model, Koshland et al. (1966) introduced a sequential or KNF model of allostery. According to the KNF model, conformational changes of protomers are consecutive and asymmetric. Ligands bind sequentially with increasing affinity when the structure of a ligand and its binding site are accommodated to each other via induced fit.

Advanced methods such as nuclear magnetic resonance (NMR) relaxation can reveal very rapid (ps–μs) dynamics of conformational ensembles of proteins. The role of NMR in protein dynamics has been reviewed thoroughly elsewhere (Boehr et al., 2006; Jardetzky, 1996; Kern and Zuiderweg, 2003). The corresponding dynamic model and the driving force of allostery will be discussed in detail in the thermodynamic chapter 4. Briefly, ligands select from preexisting conformations of proteins, binding to the most suitable one shifts the equilibrium of conformations, according to the MWC model (Boehr et al., 2009; Smock and Gierasch, 2009; Tsai et al., 1999). But the side chains of the oligomer are accommodated to ligand binding, according to the KNF model. Although the effects of induced fit are not necessarily propagated in transduction, both conformational selection and induced fit play important roles in molecular recognition. Thus, we progress from the antithesis toward the synthesis of MWC and KNF models of allostery (Boehr et al., 2009; Csermely et al., 2010). Fig. 1 shows a cyclic combination of conformational selection and induced fit. Here, the binding-competent conformations of the protein and ligand (lower forms) both pre-exist in minority, yet induced fit results in mutual conformational changes and a shift toward the accumulation of the ligand-bound species (lower right corner).

The "morpheein" model of allostery has been introduced for the dual quaternary structures of porphobilinogen synthase (Jaffe, 2005). This model requires equilibrium of protomer conformations which dictate different quaternary structures of finite

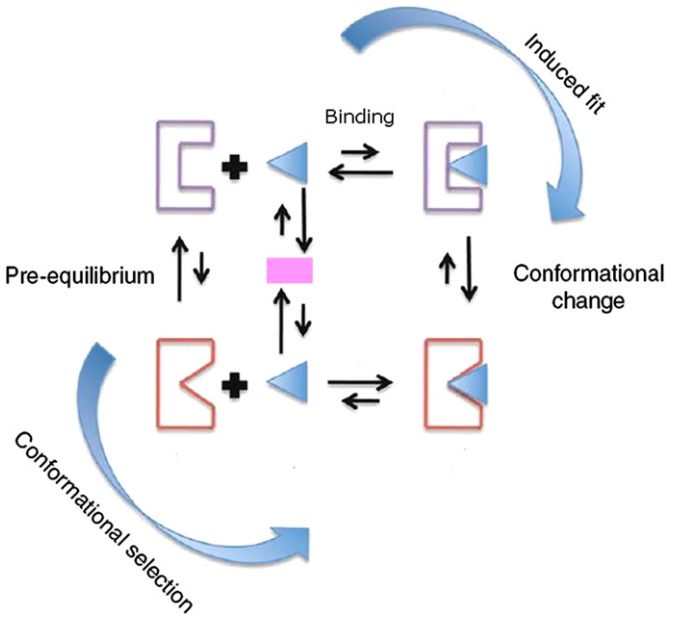

Fig. 1. Unified scheme of conformational selection and induced fit based on the MWC (left side) and KNF models (right side) of allostery. Left side: both receptor and ligand exist in pre-equilibrium of conformations. Note the ratio of arrows indicating a preferential direction. Modified from Boehr et al. (2009).

multiplicity and different functionality. Magnesium binding to this enzyme facilitates transition from hexamer to octamer by stabilizing a subunit interface that is present in the octamer but not in the hexamer. The binding of the alloster requires oligomeric dissociation, conformational change and reassembly into a functionally distinct oligomer with different stoichiometry (Jaffe, 2005). This model promises a functionally selective, new way of allosteric regulation.

3. Pharmacological allostery and receptor activation

In biochemistry, the allosteric term was introduced for interactions between protein subunits. However, it has been raised that allostery is an intrinsic property of all dynamic proteins based on the redistribution of conformational ensembles upon binding (del Sol et al., 2007; Gunasekaran et al., 2004). In pharmacology, the allosteric term has been frequently applied for interactions of binding sites within one subunit. Further, the term "orthosteric" has been subsequently introduced in order to distinguish a prominent (orthosteric) binding site of agonists (and antagonists) from the sites of allosteric agents. Pharmacology has imported the term allosteric in a developmental phase when receptors were faceless substances without structural information. Therefore the distinction of orthosteric and allosteric sites has been obscure. Moreover, according to the original biochemical meaning of allostery, both orthosteric and allosteric agents elicit remote conformational, that is, allosteric effects during signal transduction.

Changeux extended the MWC model to chemical signal transduction while Karlin was the first to use the nicotinic acetylcholine receptor as an experimental model of allostery (Changeux et al., 1984; Changeux and Edelstein, 2005; Karlin, 1967). However, allostery has key roles in several other processes such as enzyme-catalyzed metabolism, protein folding, receptor trafficking, gene regulation and apoptosis as well. Nevertheless, signal transduction has proved to be an ideal target because the investigation of chemical neurotransmission has been extremely successful in the last decades. First, it is not ideal that agonists and competitive