Biophysical Chemistry 189 (2014) 33–39

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Distinguishing induced fit from conformational selection

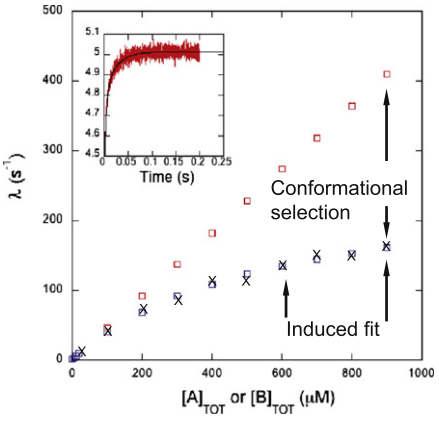
Stefano Gianni [a,b,*], Jakob Dogan [a], Per Jemth [c,*]

[a] Istituto Pasteur Fondazione Cenci-Bolognetti, Dipartimento di Scienze Biochimiche "A. Rossi Fanelli" and Istituto di Biologia e Patologia Molecolari del CNR, Sapienza Università di Roma, P.le A. Moro 5, 00185, Rome, Italy
[b] Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom
[c] Department of Medical Biochemistry and Microbiology, Uppsala University, BMC Box 582, SE-75123 Uppsala, Sweden

HIGHLIGHTS

- Protein interactions may involve conformational changes.
- The conformational change can occur before or after the initial encounter.
- Both mechanisms may give complex kinetics.
- Induced fit and conformational selection can be distinguished by varying the reactants' concentrations.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 January 2014
Received in revised form 13 March 2014
Accepted 13 March 2014
Available online 1 April 2014

Keywords:
Induced fit
Conformational selection
Kinetics
Protein–protein interactions

ABSTRACT

The interactions between proteins and ligands often involve a conformational change in the protein. This conformational change can occur before (conformational selection) or after (induced fit) the association with ligand. It is often very difficult to distinguish induced fit from conformational selection when hyperbolic binding kinetics are observed. In light of a recent paper in this journal (Vogt et al., *Biophys. Chem.*, 186, 2014, 13-21) and the current interest in binding mechanisms emerging from observed sampling of distinct conformations in protein domains, as well as from the field of intrinsically disordered proteins, we here describe a kinetic method that, at least in some cases, unequivocally distinguishes induced fit from conformational selection. The method relies on measuring the observed rate constant λ for binding and varying both the protein and the ligand in separate experiments. Whereas induced fit always yields a hyperbolic dependence of increasing λ values, the conformational selection mechanism gives rise to distinct kinetics when the ligand and protein (displaying the conformational change) concentration is varied in separate experiments. We provide examples from the literature and discuss the limitations of the approach.

1. Introduction

Mechanisms of protein–ligand interactions have been studied since the dawn of modern structure-based biochemistry. Strikingly, it is almost 60 years ago that Daniel Koshland Jr. introduced a theory to explain enzyme specificity, based on the concept that ligands may induce changes in protein structures upon binding [1]. This pioneering work, together with the subsequent studies of the group of Monod [2], lead to the establishment of what are still considered the two "standard mechanisms" in protein–ligand recognition, i.e. the concerted (nowadays more frequently called conformational selection) [2] and induced fit [3]

* Corresponding authors.
E-mail addresses: Stefano.Gianni@uniroma1.it (S. Gianni), Per.Jemth@imbim.uu.se (P. Jemth).

http://dx.doi.org/10.1016/j.bpc.2014.03.003
0301-4622/

scenarios. The field experienced a revival with the development of sophisticated NMR and molecular dynamics methods that identified high energy states in proteins that resemble ligand bound states [4–6], and allostery based on protein dynamics [7,8] rather than conformational changes, but the underlying questions remain the same [9]. Thus, it appears that proteins can sample high energy states, which are proposed to bind the ligand, while the most stable ground state does not bind the ligand. The rates of interconversion between these high and low energy states are usually fast and it is therefore hard to prove what comes first, the conformational change or the binding. This question is also of center stage in the organically growing field of intrinsically disordered proteins (IDPs) [10], where the definition of the order of events in the mechanism of binding induced folding is critical to investigate the advantages (if any) of disorder [11]. Based on different experimental and computational techniques the general consensus in the field is that the induced fit mechanism is the most common [12] but conclusive evidence are scarce.

Recently, Di Cera et al. published three papers in which the two mechanisms, induced fit and conformational selection, were subjected to a critical appraisal [13–15]. They showed that, whilst an increase of the observed rate constant as a function of reactant concentration might be consistent with both models, a decrease in the observed rate constant is a characteristic signature of conformational selection. Their analyses suggest that, in general, conformational selection is a more versatile model to describe kinetic data and the papers also offer a valuable method to distinguish between the two different scenarios.

Indeed, the first example where kinetics was used to distinguish induced fit from conformational selection clearly showed that the latter model applies to the binding of nicotinamide-adenine dinucleotide (NAD^+) to yeast glyceraldehyde-3-phosphate dehydrogenase [16]. While conformational selection is now gaining popularity, there are examples from the literature of well-established induced fit mechanisms, such as for an exonuclease-deficient mutant of T7 DNA polymerase studied by Johnson et al. [17–19]. This DNA polymerase represents an interesting example, in which a complex physiological mechanism is regulated kinetically by a binding step followed by a conformational change to achieve specificity towards the correct nucleotide. Binding of an incorrect nucleotide, resulting in a mismatched DNA, is characterized by a slow forward conformational change and fast reverse dissociation step, thereby allowing sufficient time for the release of the mismatched nucleotide from the polymerase active site. On the other hand, the slow release of the correct substrate and faster forward step allow the conformational change to occur and commit the enzyme towards incorporation of the nucleotide to the growing chain.

In this review we wish to complement Vogt and Di Cera's analyses [13–15] on these two mechanisms with a kinetic method that indeed can distinguish induced fit from conformational selection, even when the observed rate constant increases hyperbolically. It is our hope that the present paper will spur interest in this kinetic method which, while known since many years [20–26], has been rarely used, has never been explicitly analyzed in a dedicated paper and discussed together with its advantages and limitations.

2. How to distinguish induced fit from conformational selection

The binding kinetics of protein–ligand interactions have been reviewed previously [27,28] and Di Cera et al. have recently provided a comprehensive description of the kinetics for induced fit and conformational selection [13–15] (Fig. 1). In this section we complement these studies by describing a straightforward approach, which was previously used by for example Halford [20,29], Olson et al. [21], Galletto and Bujalowski [22–24] and ourselves [25,26], and that may be employed to distinguish between these two alternative scenarios.

A common method to study the time dependence of second order reactions is to carry out experiments in the presence of a very high concentration of one of the reactants. Under such conditions, commonly known as pseudo-first-order, the reaction rate (defined as the derivative of the observed signal as a function of time) will depend on the concentration of the reactant present at low concentration, with an apparent rate constant k_i' equal to the microscopic rate constant k_i multiplied by the concentration of the species at high concentration. For example, by considering a simple reaction

$$A + B \underset{k_2}{\overset{k_1}{\rightleftarrows}} AB^*$$

If [A] > > [B], the reaction rate will depend only on the concentration of B and the system will approach a first order scenario such that

$$B \underset{k_2}{\overset{k_1[A]}{\rightleftarrows}} AB^*$$

We will now describe how some simple considerations regarding the pseudo-first-order assumption may be employed to distinguish between induced fit and conformational selection.

The induced fit mechanism can be described by the scheme in Fig. 1. Importantly, under pseudo-first-order conditions where [B] > > [A], this scheme will simplify to

$$A \underset{k_2}{\overset{k_1[B]}{\rightleftarrows}} AB \underset{k_4}{\overset{k_3}{\rightleftarrows}} AB^*$$

or, if [A] > > [B]

$$B \underset{k_2}{\overset{k_1[A]}{\rightleftarrows}} AB \underset{k_4}{\overset{k_3}{\rightleftarrows}} AB^*$$

The analytical solutions of these two scenarios correspond to the following eigenvalues (denoted λ_i), which are the observed rate constants λ_1 and λ_2.

$$\lambda_{1,2} = \frac{(k_1[A] + k_2 + k_3 + k_4) \pm \sqrt{(k_1[A] + k_2 + k_3 + k_4)^2 - 4k_1[A]k_2 - 4k_1[A]k_4 - 4k_2k_4}}{2}$$

And

$$\lambda_{1,2} = \frac{(k_1[B] + k_2 + k_3 + k_4) \pm \sqrt{(k_1[B] + k_2 + k_3 + k_4)^2 - 4k_1[B]k_2 - 4k_1[B]k_4 - 4k_2k_4}}{2}$$

It is evident from inspection of these equations that, due to their symmetry with respect to the reactants, in the case of induced fit, the solutions of the respective kinetic system are identical when pseudo-first-order experiments are performed with respect to either A or B. Thus, in both cases, the slow phase λ_2 displays a hyperbolic dependence on A or B.

On the other hand, by following the conformational selection mechanism (Fig. 1), it is postulated that the protein may explore alternative conformations in the absence of the ligand and that the different conformations are selected depending on their relative affinities for the ligand.

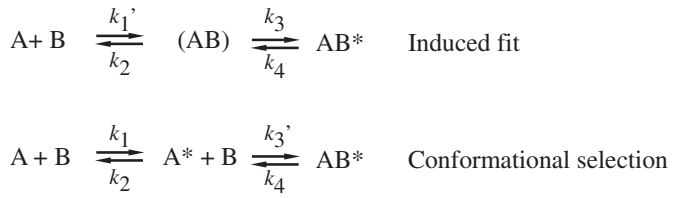


Fig. 1. Reaction schemes depicting induced fit and conformational selection. The prime indicates a pseudo-first order rate constant.