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Biophysical Chemistry 129 (2007) 289–297

Biophysical Chemistry

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

Kinetics of binding the mRNA cap analogues to the translation initiation factor eIF4E under second-order reaction conditions

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Received 10 May 2007; received in revised form 20 June 2007; accepted 25 June 2007
Available online 3 July 2007

Abstract

The kinetics of binding of five analogues of the 5′-mRNA cap, differing in size and electric charge, to the eukaryotic initiation factor eIF4E, at 20 °C, pH 7.2, and ionic strength of 150 mM, were measured, after mixing solutions of comparable concentrations of the reagents, in a stopped-flow spectrofluorimeter.

The registered stopped-flow signals were fitted using an efficient software package, called Dyna Fit, based on a numerical solution of the kinetic rate equations for assumed reaction mechanisms. One-, two-, and three-step binding models were considered. The quality of fits for these models were compared using two statistical criteria: Akaike's Information Criterion and Bayesian Information Criterion. Based on resulting probabilities of the models, it was concluded that for all investigated ligands a one-step binding model has essentially no support in the experimental observations.

Our conclusions were also analysed from the perspective of kinetic transients obtained for cap–eIF4E systems under the so called pseudo-first order reaction condition, which result in the linear correlation of the observed association rate constant with ligand concentration. The existence of such a linear correlation is usually considered as proof of a one-step binding mechanism. The kinetic and optical parameters, derived from fitting a two-step cap-binding model with the DynaFit, were used to simulate kinetic transients under pseudo-first order reaction conditions. It appeared that the observed association rate constants derived from these simulated transients are also linearly correlated with the ligand concentration. This indicated that these linear dependencies are not sufficient to conclude a one-step binding.

Keywords: eIF4E; 5′-mRNA cap; Binding; Kinetics; DynaFit

1. Introduction

Eukaryotic mRNAs possess a cap structure consisting of a 5′-5′-tri-phosphate bridge of a general form m^7GpppN^1, where m^7G is the 7-methyl-guanosine, and N can be any of the four nucleosides [1]. One of the essential steps in the initiation of eukaryotic mRNA translation is the recognition of this cap structure by a eukaryotic initiation factor 4E (eIF4E) [2]. The protein eIF4E is a part of a trimeric complex, termed eIF4F, which also contains an adaptor protein eIF4G and RNA helicase eIF4A [3]. The crucial element of the cap recognition consists of sandwiching of the alkylated base between the side chains of two conserved tryptophans (Trp-56 and Trp-102 in the murine eIF4E). The interaction can be explained in terms of enhancement of π–π stacking enthalpy, because of charge transfer between the electron-deficient 7-methylguanine (which carries a delocalised positive charge secondary to methylation) and the electron-rich indole groups. Moreover there is a number of hydrogen bonds stabilising the complex, N1 and N2 make hydrogen bonds with the carboxylate oxygen atoms of Glu103, and Arg157 and Lys162 make such contacts with oxygen atoms of the phosphate groups. Many of these hydrogen bonding interactions include contributions due to direct electrostatic interactions because the interacting groups are ionised. The interaction energies also include contributions due to shifts in the appropriate pK_a values upon binding. These insights into structural characteristics of cap–eIF4E complexes were obtained from three-dimensional structures by X-ray crystallography

Abbreviations: G, guanosine; m^7G, 7-methyl-guanosine; ppp, 5′-5′-tri-phosphate bridge; GMP (GDP, GTP), guanosine 5′-mono-(di-tri-)-phosphate; eIF4E, eukaryotic initiation factor 4E.

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doi:10.1016/j.bpc.2007.06.009

[4–6] and multi-dimensional NMR study [7], as well as from spectroscopic investigations in vitro, both equilibrium [5,8,9], and kinetic [10–14].

The reported kinetic studies were based on transient fluorescence measured in stopped-flow spectrometers. Kinetic methods allow direct measurement of rate constants governing individual steps of molecular processes. One of the issues considered in the kinetic studies was the consideration of the most appropriate model for the mechanism of cap binding by the protein initiation factor.

The simplest receptor–ligand binding model is a one-step mechanism written as

$$P + L \underset{k_D}{\overset{k_R}{\rightleftharpoons}} K. \tag{1}$$

In principle, however, when a protein binds a single ligand molecule, some rearrangement of the protein–ligand complex following the initial collision can be expected, which leads to a two-step binding kinetics [15], described by

$$P + L \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} K. \tag{2}$$

It can also happen that the association process is best described by a distribution of many relaxations, in such case one can expect that a three-step or even a multi-step model is the best way to describe protein–ligand association. Here we will consider three-step association as the most complex model:

$$P + L \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} D \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} K. \tag{3}$$

In the above equations, k_i are the appropriate rate constants, C and D represent intermediate forms and K represents the final form of the complex of protein P and ligand L.

One of the important aspects of investigating the receptor–ligand association reactions by stopped-flow spectrometry is that the initial state of an investigated system, at the moment of the reagent mixing, can be arbitrary far from the thermodynamic equilibrium achieved during the course of the initiated reaction. Therefore, data analysis is more complicated than in the case of the so called relaxation methods. In the past, this led to restrictions to simplified reaction schemes and/or experiments designs. A classical example in this respect is performing stopped-flow experiments under the so called pseudo-first order reaction conditions, where the concentration of one component is much higher than the concentration of the other reagent. Under these conditions, the rate equations can be integrated to yield mono-exponential time dependence of the concentration of reactants [15]. The observed F signal is also a mono-exponential function of time

$$F(t) = F_\infty + (F_o - F_\infty) \cdot \exp(-k_{\text{obs}} t) \tag{4}$$

where k_{obs} is the observed first-order rate constant, and F_o and F_∞ is the initial and final signal, respectively, registered for the solution. Analysis of k_{obs} versus total ligand concentration can be used to distinguish between the one-step and two-step association mechanism for ligand binding [15–19].

For a one-step mechanism k_{obs} is a linear function of the total ligand concentration $[L]$

$$k_{\text{obs}} = k_R[L] + k_D. \tag{5}$$

For the above two-step binding mechanism, and ligand concentrations much larger than the protein concentration, the steady-state assumption and the condition that $[C]=[K]=0$ at time zero, the observed rate constant reads [16–18]

$$k_{\text{obs}} = \frac{k_{+1}[L](k_{+2} + k_{-2}) + k_{-1}k_{-2}}{k_{+1}[L] + k_{-1} + k_{+2}}. \tag{6}$$

Assuming that $k_{-1} \gg k_{+2}$, the last equation can be simplified to a hyperbolic dependence

$$k_{\text{obs}} - k_{-2} = \frac{k_{+1}k_{+2}[L]}{k_{+1}[L] + k_{-1}} \tag{7}$$

which, by neglecting k_{-2}, can be also presented as a linear relation between the inverse of the observed rate constant and the inverse of the ligand concentration. The hyperbolic saturation phenomenon of k_{obs} as a function of the total ligand concentration $[L]$ is commonly considered as an indication of more than one step in the protein–ligand binding mechanism [15–18]. According to Eq. (7), at some high concentration of the ligand, the observed rate is limited by first-order isomerisation of the protein–ligand complex, i.e. $k_{+2}+k_{-2}$ [15].

Advances in computational methods to analyse reaction time courses by numerical integration [19–21] removed the necessity to restrict data analysis to simplified reaction schemes and/or particular design of the experiments. Particularly useful for numerical analysis of transient kinetic signals registered in stopped-flow spectrometers is the DynaFit program developed by Petr Kuzmic [21]. With this program one can fit a postulated theoretical model to given experimental data or one can simulate pseudo-experimental data.

A considered reaction model can be fit simultaneously to several sets of experimental data, e.g. several kinetic transients registered for different initial concentrations of the reagents. When several transients are analysed simultaneously within restrictions imposed by a particular reaction mechanism, a larger number of model parameters might be reliably determined from data fitting. The program allows for a discrimination analysis of various models which results in a statistically justified choice of the most appropriate reaction scheme for a given system.

Stopped-flow investigations of the binding of m^7GpppG to eIF4E protein, under pseudo-first order reaction conditions, were performed by Goss and coworkers [10,13] and by Slepenkov and coworkers [14]. The first group concluded that the binding is a two-step process and according to the second group a one-step reaction is sufficient to describe experimental data. Our group investigated the association of eIF4E protein with cap analogues using stopped-flow spectrofluorimetry under secondary-reaction conditions [11,12]. In the earlier study we have used mono- and bi-exponential functions in experimental data fitting and the obtained rate constants were interpreted in terms of plausible one-