Journal of Theoretical Biology 411 (2016) 16–26

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Equilibria of oligomeric proteins under high pressure – A theoretical description

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ARTICLE INFO

Keywords:
Oligomeric protein
High pressure
Theory
Equilibrium
Inflection point

ABSTRACT

High pressure methods have become a useful tool for studying protein structure and stability. Using them, various physico-chemical processes including protein unfolding, aggregation, oligomer dissociation or enzyme-activity decrease were studied on many different proteins. Oligomeric protein dissociation is a process that can perfectly utilize the potential of high-pressure techniques, as the high pressure shifts the equilibria to higher concentrations making them better observable by spectroscopic methods. This can be especially useful when the oligomeric form is highly stable at atmospheric pressure. These applications may be, however, hindered by less intensive experimental response as well as interference of the oligomerization equilibria with unfolding or aggregation of the subunits, but also by more complex theoretical description. In this study we develop mathematical models describing different kinds of oligomerization equilibria, both closed (equilibrium of monomer and the highest possible oligomer without any intermediates) and consecutive. Closed homooligomer equilibria are discussed for any oligomerization degree, while the more complex heterooligomer equilibria and the consecutive equilibria in both homo- and heterooligomers are taken into account only for dimers and trimers. In all the cases, fractions of all the relevant forms are evaluated as functions of pressure and concentration. Significant points (inflection points and extremes) of the resulting transition curves, that can be determined experimentally, are evaluated as functions of pressure and/or concentration. These functions can be further used in order to evaluate the thermodynamic parameters of the system, i.e. atmospheric-pressure equilibrium constants and volume changes of the individual steps of the oligomer-dissociation processes.

1. Introduction

High-pressure methods became a common tool of investigation of structure and function of proteins during the last two decades (Gross and Jaenicke, 1994; Royer, 1995; Mozhaev et al., 1996; Silva et al., 2001; Marchal et al., 2005; Rivalain et al., 2010; Silva et al., 2014). In some cases they are used to study the properties of proteins from marine organisms living deeply under the sea level (Shrestha et al., 2015), but vast majority of these studies is aimed at elucidation of the structure-function relationships of proteins from common organisms extrapolating their high-pressure behavior to the atmospheric pressure. High-pressure methods are used to investigate protein denaturation, unfolding, conformational changes, enzyme kinetics, etc., but they also have valuable application in studying quaternary structure and equilibria of oligomeric proteins. Many oligomeric proteins have been investigated by high-pressure methods, including those of low number of subunits, mainly dimers (Paladini and Weber, 1981; Silva et al., 1986; Ruan and Weber, 1988; Erijman et al., 1993; Kornblatt et al., 2004; Marchal et al., 2012; Ingr et al., 2015) and tetramers (Jaenicke and Koberstein, 1971; Royer et al., 1986; Ruan and Weber, 1989; Pin et al., 1990; Devillebonne and Else, 1991; Ruan and Weber, 1993; Girard et al., 2010), hexamers (Foguel and Weber, 1995), higher oligomers and viral capsids (Silva and Weber, 1988; Silva et al., 1989, 1992; Da Poian et al., 1993; Silva et al., 1996; Weber et al., 1996) or prion oligomers (Torrent et al., 2015), protein aggregates with less organized structure like casein micelles (Gebhardt et al., 2005, 2006, 2011) and even polymeric structures, e.g. TMV-virus (Bonafe et al., 1998) or microtubules and microfilaments (Messier and Seguin, 1978; Kobori et al., 1996; Nishiyama et al., 2010). Structural changes of oligomeric proteins prior to subunit dissociation were studied, too (Cioni and Strambini, 1996). These studies were concerned with different structural and functional features and in some cases the key thermodynamic parameters, especially the volume change of the oligomer dissociation ΔV and the atmospheric pressure equilibrium

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http://dx.doi.org/10.1016/j.jtbi.2016.10.001
Received 16 May 2016; Received in revised form 14 September 2016; Accepted 3 October 2016
Available online 04 October 2016

constant K_{atm}, were determined (Silva et al., 1986; Ruan and Weber, 1988, 1989; Pin et al., 1990; Da Poian et al., 1993; Erijman et al., 1993; Ruan and Weber, 1993; Foguel and Weber, 1995; Kornblatt et al., 2004; Ingr et al., 2015). These studies exploit the fact that the high pressure favors a process accompanied with a negative change of the total volume of the system. They show that the oligomeric form is destabilized by high pressure, i.e. the total volume of the monomers is lower than that of the oligomer, which is considered as a general rule for the oligomer dissociation processes supported by whole the experimental evidence. This fact allows us to study the dissociation equilibrium even for oligomers highly stable at atmospheric pressure and dissociating only at very low concentration where the signal of the detection methods is insufficient (Royer, 1995). Application of high pressure is most often coupled with different spectroscopic and fluorometric detection techniques, but methods of light (Meier and Kriegs, 2008) or neutron (Shrestha et al., 2015) scattering, as well as optical microscopy (Nishiyama et al., 2006, 2010) or gel electrophoresis (Paladini et al., 1987, 1994), can be used, too. In addition, properties of the monomeric forms of highly stable oligomers as well as intermediate structures of protein unfolding can be studied by high-pressure X-ray crystallography and NMR (Collins et al., 2011).

Application of high pressure can induce various structural changes from oligomer dissociation via reversible unfolding to an irreversible aggregation, sometimes observable at a single protein (Dumay et al., 1994; Seefeldt et al., 2005; Ingr et al., 2015). It is, therefore, necessary to be able to distinguish among these processes, especially unfolding and oligomer dissociation. The processes can be identified according to the concentration dependence of their transition curves. As was shown in numerous experimental studies (Lange et al., 1996; Mozhaev et al., 1996; Ruan et al., 2001; Royer, 2002; Rouget et al., 2010, 2011; Cioni et al., 2014), the transition curve of a reversible folding-unfolding equilibrium, i.e. the dependence of the fraction of one of the forms on pressure, is a concentration independent sigmoid with the inflection point pressure

$$p_{\mathrm{inf}}=\frac{RT}{\Delta V}\ln K_{atm} \tag{1}$$

and the fraction of the unfolded form α_u is ½ (for the proof follow the derivation given by Eqs. (3)–(14) below for n=1). The slope of the transition curve in the inflection point is

$$\frac{d\alpha_u}{dp}=-\frac{\Delta V}{4RT}. \tag{2}$$

These two quantities allow us to determine the thermodynamic characteristics ΔV and K_{atm} of the process. On the contrary, the transition curve of the oligomer-monomer equilibrium moves towards higher pressures when the concentration grows. This shift can be used to determine the volume change of the process ΔV and the equilibrium constant K for any pressure, including the atmospheric pressure equilibrium constant K_{atm}, as was previously shown for several oligomeric proteins (Ruan and Weber, 1988, 1989, 1993; Foguel and Weber, 1995; Kornblatt et al., 2004; Ingr et al., 2015). In addition, processes with negative ΔV in the direction of association of monomeric subunits are also known. They are usually aggregations with high and not precisely defined number of monomeric units, as was demonstrated on the case of myoglobin (Gebhardt et al., 2003).

Besides many experimental studies, some theoretical works dealing with the thermodynamics of oligomeric-proteins dissociation under high pressure were published as well (Weber, 1986, 1993). In this paper we provide a contribution to the theoretical analysis of some of these equilibria with the stress on the detailed description of the transition curves, especially their significant points, i.e. inflection points and extremes, which may be used as a versatile tool for evaluation of eventual future experiments with oligomeric proteins.

2. Results and discussion

2.1. General assumptions

In this work we describe closed equilibria (i.e. equilibria between the highest oligomer and the monomer without any intermediate states) of homooligomers of any degree, heterodimer and heterotrimers, and consecutive equilibria (containing intermediate oligomers of lower degree than the highest one) of homo- and heterotrimer. In all the cases only pressure-independent negative volume changes of oligomer dissociations will be considered as it seems to be a good approximation supported by the overall experimental evidence as well as our recent theoretical simulation (Kutalkova et al., 2014).

The parameters that should be determined using the proposed theoretical background are especially the volume changes ΔV accompanying the individual oligomer-dissociation steps and the atmospheric-pressure equilibrium constants of these processes K_{atm}. Their determination is based on the analysis of the transition curves – i.e. responses of the experimental device to the system under changing pressure at a given concentration or, in a reciprocal approach, changing concentration at a given pressure.

For simplicity, the concentrations of individual chemical entities are denoted by simple capital letters with intuitive meaning (M – monomer, A – subunit A, D – dimer, etc.) equal with those used in the respective chemical equations. All the concentrations are considered as dimensionless relative quantities related to the standard concentration of 1 mol dm^{-3}. Accordingly, the equilibrium constants are dimensionless, too. As many of the mathematical derivations are rather lengthy, they are presented in Electronic Supplementary Information, hereafter referred to as ESI.

2.2. Closed equilibria systems

2.2.1. Homooligomers

Consider the equilibrium between a homooligomeric protein M_n, consisting of n monomeric subunits, and its subunits M described by a chemical equation

$$M_n \quad \rightleftarrows \quad n\,M. \tag{3}$$

The equilibrium constant of this process for a given pressure p is

$$K(p)=\frac{(M)^n}{M_n}. \tag{4}$$

Denoting the total protein concentration related to the monomeric form M_0 and considering the balance equation

$$M_0 = M + n\,M_n, \tag{5}$$

the relation between M_0 and the monomer fraction $\alpha = M/M_0$ is given by the equation

$$n\ \alpha^n + \frac{K(p)}{M_0^{n-1}}\alpha - \frac{K(p)}{M_0^{n-1}} = 0. \tag{6}$$

The reaction change of the Gibbs energy is

$$\Delta G = \Delta G_{atm} + \Delta p \Delta V \tag{7}$$

where ΔG_{atm} is the same quantity at atmospheric pressure and $\Delta p = p - p_{atm}$ is the difference between the current and atmospheric pressures. For simplicity, we approximate Δp by p because p_{atm} is negligible in comparison with p, which is in the order of tens to hundreds MPa in all relevant experiments. As

$$K(p)=\exp\left(-\frac{\Delta G}{RT}\right)=\exp\left(-\frac{\Delta G_{atm}+p\Delta V}{RT}\right)=K_{atm}\exp\left(-\frac{p\Delta V}{RT}\right) \tag{8}$$

where R is the molar gas constant and T is the thermodynamic temperature, Eq. (6) becomes