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A distinct intermediate of RNase A is induced by sodium dodecyl sulfate at its pK_a

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Abstract

The chemical denaturation of RNase A was found to be mediated by sodium dodecyl sulfate (SDS) at various pH. The characterization of the unfolding pathway was investigated by spectrophotometry and differential scanning calorimetry (DSC), and was analyzed by multivariate curve resolution (MCR) as a chemometric method. The spectrophotometric titration curve of RNase A upon interaction with SDS indicated a distinct complex intermediate in glycine buffer at pH 3.3. This was accompanied with the catalytic activation of the enzyme and was concurrent with maximum population of the intermediate, determined by MCR. This was confirmed by the DSC profile of RNase A in the presence of SDS, indicated by two transitions in thermal unfolding. The kinetic data on the unfolding process of RNase A upon addition of SDS showed a two-phase pathway under the same conditions. The intermediate appeared at low pH especially at the pK_a of SDS (pH 3.3). These results provide strong evidence of the influence of low pH (around the pK_a of SDS) on the existence of an intermediate upon interaction of RNase A with SDS.

Keywords: RNase A; Sodium dodecyl sulfate; Chemometry; Intermediate; Denaturation; Differential scanning calorimetry

1. Introduction

Bovine pancreatic ribonuclease A (RNase A) has played a crucial role as a model system in the studies of protein structure, folding and unfolding pathways and enzyme catalysis [1,2]. In most of the reported studies, the unfolded state of the protein was achieved using changes in temperature, pH, urea and guanidine hydrochloride as well as the use of chemical denaturants such as detergents [3–7]. A cooperative, two-state reversible unfolding transition has been observed by thermal, urea and guanidine hydrochloride induced unfolding [8]. In contrast, other investigators demonstrate a stable intermediate during the thermal denaturation [9–12]. Later studies suggested that RNase A folds and unfolds through multiple pathways determined by transition intermediates [13,14].

Anionic detergents, such as sodium dodecyl sulfate (SDS), can denature proteins at low concentrations of the order of millimolar. SDS binds to most proteins with a high affinity via interactions between the sulfate head group and the positively charged amino acid chains of the protein, on the one hand, and between the surfactant alkyl chain and the protein's hydrophobic side chains, on the other [15]. Recently,

Abbreviations: DSC, differential scanning calorimetry; SDS, sodium dodecyl sulfate; cCMP, cytidine 2′,3′-cyclic phosphate; MCR, multivariate curve resolution; FA, factor analysis

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it has been demonstrated that the binding of SDS results in a conformational change in the protein to form a partially denatured state, before the occurrence of the major unfolding transition [16] or the induction of a molten globule state at very low concentration of SDS [17].

Differential scanning calorimetry (DSC) has been extensively used to study the protein thermal denaturation [8]. Analysis of DSC transitions can provide a direct measurement of small structural transitions and lead to the characterization of their thermodynamic aspects [18–20].

On the other hand, spectroscopic methods are in general simple, highly sensitive and very suitable for the study of chemical reactions in solutions. When the components involved in the chemical reaction have distinct spectral responses, their concentration can be monitored directly. However, in many cases, the spectral responses of two and sometimes even more components overlap considerably and the analysis is no longer straightforward. Nowadays, by using chemometric methods, one can analyze whole spectra, thereby utilizing all spectral information [21]. This enables chemists to monitor the complex chemical reactions by spectrophotometric techniques even if the component spectra are highly overlapped. Spectral curve deconvolution or multivariate curve resolution methods are chemometrics techniques for extraction of the pure spectra of components involved and their corresponding concentration profiles from evolutionary processes [22]. Self-modeling methods extract the concentration profiles without having any information about the shape of the spectra. Several self-modeling approaches have been developed since the pioneering work by Lawton and Sylvestre in 1971 [23]. Among these are the factor analysis-based methods such as automated spectral isolation (ASI), iterative target transformation factor analysis (ITTFA), evolving factor analysis; (EFA), iterative key set factor analysis (IKS-FA) [22], windows factor analysis; (WFA) and alternative least squares (ALS) [21–24].

Here, we provide further information regarding the intermediate state produced upon interaction of RNase A with SDS utilizing spectroscopic titration via chemometrics and calorimetric indications. This was accomplished through multivariate curve resolution as well as a kinetic model in order to know more about the mechanisms for intermediate pathway during the unfolding state.

2. Theories

2.1. Kinetics of denaturation

The theoretical analysis of the linear three-state model for protein unfolding is as follows:

$$\mathrm{N} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{I} \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \mathrm{D} \tag{1}$$

where N, I, D are native, intermediate and denatured states, k_1 and k_2 are the forward rate constants, k_{-1} and k_{-2} are the reverse rate constants. The concentration of a particular specie at each time ($c_{i(t)}$), could be followed as a function of time on the basis of relaxation time method [25] as below:

$$c_{i(t)} = \sum a_i \exp\left(\frac{-t}{\tau_i}\right) \tag{2}$$

where i is the number of kinetic phases and a, t, and τ are amplitude, time and relaxation time, respectively. Amplitudes included the initial concentrations of the denaturant and the enzyme and the microscopic rate constants at final conditions. A physical property that has a linear dependency on concentration of specie i of protein (e.g., absorbance) could be used for monitoring the process [26] as:

$$A_t = A_\infty + \sum a_i \exp\left(\frac{-t}{\tau_i}\right) \tag{3}$$

where A_t is absorbance of phase i at time t and A_∞ is absorbance at $t = \infty$ corresponding to the end of the reaction. In the exact treatment of the three-state model, Eq. (4) for two kinetic phases can be used:

$$A_t - A_\infty = a_1 \exp\left(\frac{-t_1}{\tau_1}\right) + a_2 \exp\left(\frac{-t_2}{\tau_2}\right) \tag{4}$$

where terms 1 and 2 denote the kinetic phases 1 and 2, respectively.

Details of the theory of kinetics of protein denaturation and the expression of τ_1 and τ_2 have been previously reported [26]. In summary, the correlation between observed relaxation times and microscopic rate constants depends on the unfolding and refolding mechanisms. In a three-state model including a single intermediate, the expressions for τ_1 and τ_2 and microscopic rate constants according to Eq. (1) are given as follows [25,27]:

$$\frac{1}{\tau_1} = k_1 + k_{-1}\left[\frac{k_2}{k_2 + k_{-2}}\right] \tag{5}$$

$$\frac{1}{\tau_2} = k_2 + k_{-2} \tag{6}$$

or

$$\frac{1}{\tau_1} = (k_1 + k_{-1})\left[\frac{K_{\mathrm{ID}}}{K_{\mathrm{ID}} + 1}\right] \tag{7}$$

where

$$K_{\mathrm{ID}} = \frac{k_2}{k_{-2}} = \frac{[\mathrm{D}]}{[\mathrm{I}]} \tag{8}$$

under limiting conditions, in a complete unfolding experiment, since $k_2 \gg k_{-2}$ and $k_1 \gg k_{-1}$ therefore, $1/\tau_1 = k_1$ and $1/\tau_2 = k_2$.

2.2. MCR analysis

In this method, the spectral data, recorded at each reaction step, were collected in a data matrix (D) with $m \times n$ dimension, m being the number of spectra collected and n being the