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Computational modeling of acrylodan-labeled cAMP dependent protein kinase catalytic subunit unfolding

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ABSTRACT

Structure of the cAMP-dependent protein kinase catalytic subunit, where the asparagine residue 326 was replaced with acrylodan-cystein conjugate to implement this fluorescence reporter group into the enzyme, was modeled by molecular dynamics (MD) method and the positioning of the dye molecule in protein structure was characterized at temperatures 300 K, 500 K and 700 K. It was found that the acrylodan moiety, which fluorescence is very sensitive to solvating properties of its microenvironment, was located on the surface of the native protein at 300 K that enabled its partial solvation with water. At high temperatures the protein structure significantly changed, as the secondary and tertiary structure elements were unfolded and these changes were sensitively reflected in positioning of the dye molecule. At 700 K complete unfolding of the protein occurred and the reporter group was entirely expelled into water. However, at 500 K an intermediate of the protein unfolding process was formed, where the fluorescence reporter group was directed towards the protein interior and buried in the core of the formed molten globule state. This different positioning of the reporter group was in agreement with the two different shifts of emission spectrum of the covalently bound acrylodan, observed in the unfolding process of the protein.

1. Introduction

Acrylodan (6-acryloyl-2-dimethylaminonaphthalene), when covalently bound to a cystein residue of a native or mutated protein, is a useful fluorescent probe for investigation into the protein structural dynamics, as the emission spectrum of this dye is highly sensitive to changes of its environment (Prendergast et al., 1983). In water the emission spectrum of the acrylodan adduct with model thiol compounds was characterized with the λ_{max} value 540 nm (Prendergast et al., 1983), and significant blue-shift was observed if this dye was transferred into water-free polar organic solvents acetone (λ_{max} = 465 nm), dimethyl formamide (λ_{max} = 470 nm) and acetonitrile (λ_{max} = 468 nm) (Kawski et al., 2002). The fluorescence spectrum of acrylodan, which was covalently attached to cAMP-dependent protein kinase catalytic subunit (PKAc), was characterized by the λ_{max} value 496 nm (Lew et al., 1997; Kivi et al., 2013). This result was similar to the spectral data for acrylodan-labeled parvalbumin (λ_{max} = 498 nm) and papain (λ_{max} = 491 nm) (Prendergast et al., 1983). Therefore it could be suggested that the reporter group was only partially accessible to water if bound to these proteins. Moreover, the emission spectrum of the protein bound acrylodan was red-shifted in the presence of specific ligands of these enzymes, indicating that conformational changes induced by ligand binding partially extruded the fluorophore from the protein interior into surrounding water (Prendergast et al., 1983). This conclusion was in agreement with the fact that transfer of the acrolydan-labeled troponin into 6 M guanidine hydrochloride solution, where the protein structure was completely unfolded, shifted the λ_{max} value to 532 nm that was close to the emission spectrum of the dye in water medium (Prendergast et al., 1983). In the case of acrylodan-labeled PKAc similar shift of λ_{max} to 520 nm occurred in 4 M guanidine hydrochloride solution (our unpublished data).

In this study we performed computer modeling of the acrylodan-labeled PKAc structure and characterized positioning of the reporter group in the native enzyme, and also in protein structures modeled at high temperatures, where partial or complete destabilization of the secondary and tertiary structure elements emulated the protein unfolding process. It was found that dissolution of protein structure was, indeed, accompanied with alteration of positioning of the covalently attached acrylodan molecule, and these changes revealed formation of an intermediate state on the protein unfolding pathway, which could be characterized as a molten globule of this protein.

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2. Results and discussion

2.1. Modeling of acrylodan-labeled PKAc structure

The model of cAMP-dependent protein kinase catalytic subunit (PKAc) was built up as was described before (Izvolski et al., 2013), proceeding from the X-ray structure of the protein ternary complex with ATP analog AMP-PNP and the peptide inhibitor PKI(5–24) (Zeng et al., 1993). This structure was listed under the codename "1ATP" in the PDB database (www.pdb.org). For our analysis both bound ligands were deleted from this complex to produce an input file describing the apoenzyme.

Further, the asparagine residue in position 326 was replaced with the acrylodan-cystein conjugate to implement the fluorescence ligand into the protein, and thereafter structure of the covalently labeled enzyme was optimized by means of MD calculations taking into consideration the influence of a number of shells of surrounding water molecules. This location of the label was chosen proceeding from other studies with this fluorophore (Lew et al., 1997).

Calculations were performed at temperatures from 300 K to 700 K. It was suggested that at high temperature the energy of thermal motion overcomes interactions, which are responsible for formation of the secondary and tertiary structure of the protein, and the appropriate conformational transitions model unfolding of PKAc. The time-course of these structural transformations was characterized by alteration of the gyration radius of the protein. This approach has been successfully applied for computational modeling of different proteins as well as their unfolding process (Kaushik et al., 2011; Koshy et al., 2010; Lin et al., 2009; Paschek et al., 2008; Wang et al., 1999; Yan et al., 2010).

2.2. Model of acrylodan labeled PKAc at 300 K

The MD calculations, performed at 300 K (27 °C), revealed that "soaking" of the protein X-ray structure into water medium caused some increase in gyration radius of the protein (Fig. 1, left panel), indicating that small changes in dimensions and compactness of the protein occurred due to additional solvation. The average structure of the dye-labeled protein was obtained from these MD calculations and was shown in Fig. 1(right panel). This structure revealed no principal differences from the average structure and dynamic properties of the native enzyme as was discussed before (Izvolski et al., 2013). Analogously, similarity between the catalytic and binding properties of the native and the dye-labeled enzymes was demonstrated experimentally (Lew et al., 1997; Kivi et al., 2013).

The structure of the acrylodan-PKAc adduct revealed that the covalently attached fluorophore was posed on the surface of the protein (Fig. 1, right panel), where part of the dye molecule was embedded into the protein structure. More explicitly this positioning of the dye was characterized by the solvent accessible surface area SASA 1.64 nm^2, comprising of hydrophilic (0.39 nm^2) and hydrophobic (1.25 nm^2) components. This value was significantly smaller than the SASA value 5.0 nm^2 for the cysteine-dye conjugate in water, also comprised of hydrophobic (3.68 nm^2) hydrophilic (1.32 nm^2) components. Therefore the positioning of the reporter group on the protein surface definitely hindered its solvation by water and this result was in agreement with the blue-shifted λ_{max} value for the protein bound fluorophore at 496 nm, if compared with the λ_{max} = 540 nm for the acrylodan-thiol adduct in water (Kivi et al., 2013). On the other hand, this peripheral location of the fluorophore has made this label sensitive to the conformational changes of the protein that accompany binding of different ligands into the nucleotide binding pocket of the enzyme (Lew et al., 1997).

2.3. Acrylodan labeled PKAc unfolding at 700 K

Structure of the unfolded acrylodan-labeled PKAc was generated *in silico* proceeding from the X-ray structure of the unliganded protein as was described above and using the system temperature as high as 700 K. These extreme conditions could be applied under the virtual conditions, and by analogy with other studies (Kaushik et al., 2011; Koshy et al., 2010; Lin et al., 2009; Paschek et al., 2008; Wang et al., 1999; Yan et al., 2010) it was suggested that at this high temperature complete unfolding of the protein molecule should occur. Indeed, significant restructuring of the protein molecule was observed at this temperature, as could be concluded on the basis of significant increase in the value of the gyration radius before the new stable state of the protein was formed (see the insert, left panel of Fig. 2). This new state, shown in Fig. 2 (right panel), has lost all secondary structure elements, as could be expected in the case of the unfolded protein structure, but was still rather compact, as its gyration radius was comparable to the same parameter of the native protein.

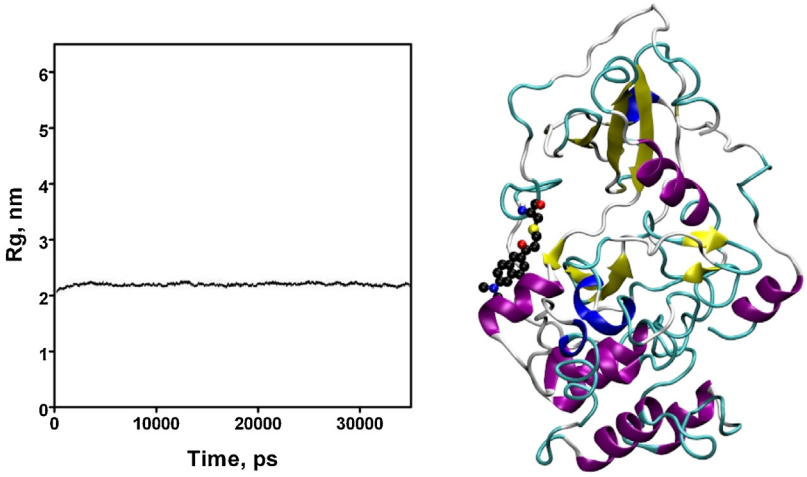

Fig. 1. Time-dependence of alteration of the gyration radius (Rg) value for acrylodan-labeled PKAc, following "soaking" of the crystalline structure of the protein into water (left panel), and the average spatial structure of the protein at 300 K (right panel). Positioning of the peptide chain attached acrylodan moiety was shown as the ball-stick model (C—black, O—red, S—yellow, N—blue, no H atoms were shown). The dye molecule was positioned on the surface of the protein and oriented towards the exterior medium. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)