Carbohydrate Research 345 (2010) 243–249

Contents lists available at ScienceDirect

Carbohydrate Research

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

Chaperone-like activity of α-cyclodextrin via hydrophobic nanocavity to protect native structure of ADH

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ARTICLE INFO

Article history:
Received 21 August 2009
Accepted 5 November 2009
Available online 10 November 2009

Keywords:
α-Cyclodextrin
Hydrophobic nanocavity
Microenvironment
Aggregation
Blind docking
Molecular dynamic simulations

ABSTRACT

The chaperone action of α-cyclodextrin (α-CyD), based on providing beneficial microenvironment of hydrophobic nanocavity to form molecular complex with alcohol dehydrogenase (ADH) was examined by experimental and computational techniques. The results of UV–vis and dynamic light scattering (DLS) indicated that the chaperone-like activity of α-CyD depends on molecular complex formation between α-CyD and ADH, which caused to decrease the amount and size of polymerized molecules. Computational calculations of molecular dynamic (MD) simulations and blind docking (BD) demonstrated that α-CyD acts as an artificial chaperone because of its high affinity to the region of ADH's two chains interface. The hydrophobic nanocavity of α-CyD has the ability to form inclusion complex due to the presence of phenyl ring of aromatic phenylalanine (Phe) residue in the dimeric intersection area. Delocalization of ADH subunits, which causes the exposure of Phe110, takes part in the enzyme polymerization and has proven to be beneficial for aggregation inhibition and solubility enhancement within the host α-CyD-nanocavity.

1. Introduction

Alcohol dehydrogenases catalyze the reversible oxidation of the alcohols to aldehydes or ketones. Alcohol dehydrogenases, from a wide variety of organisms, have been studied extensively.[1] The potential biotechnological application of alcohol dehydrogenase enzymes has long been recognized.[2,3] Homodimeric horse liver alcohol dehydrogenase (ADH) is the representative of alcohol dehydrogenase family.[4] In spite of the fact that ADH has potential biotechnological applications, it is a mesophilic enzyme and can easily be aggregated under thermal stress,[5] which limits its applications in biotechnological industry.

The α-cylcodextrin (α-CyD) acts as an 'artificial chaperone' to decrease the thermal aggregation of ADH with conformational stabilization.[6] Better separation of hydrophilic and hydrophobic domains is the common feature of chaperones, which leads to the increased solubility of hydrophobic substrates.[7] Possession of two distinct regions, including hydrophobic cavity and hydrophilic surfaces, makes the α-CyD important in preventing protein aggregation meanwhile it provides the capability to encapsulate poorly soluble drugs in biotechnology and drug delivery.[8,9] The most remarkable characteristic of CyDs is the ability to form rigid host–guest inclusion complexes with a wide variety of molecules. The guest molecule is held within the hydrophobic nanocavity of host CyD molecule via a dimensional fit between them.[10] Complexation in CyD exerts a profound effect on the physicochemical properties of guest molecules as they are locked or caged within the host cavity giving rise to benefits such as solubility enhancement, stabilization of labile guests, and controlled release of drugs.[10,11] Table 1 shows the main properties of α-CyD.

Herein, we have focused on the mechanism of α-CyD action in controlling the ADH aggregation process (chaperone action) with the help of experimental and theoretical approaches of molecular modeling/docking. Understanding the physicochemical determinants, underlying the artificial chaperone action, is fundamental in the rational design of nanostructures for new drugs that are able to interfere with harmful aggregation process. The combined efforts of experimental and theoretical approaches have been useful in expanding the understanding of molecules' physiochemical properties. Molecular docking is one of the most powerful techniques for the structure-based drug design and ligand/macromolecule interaction studies.[12,13] Using the docking tools gives valuable information on the unknown binding regions of the macromolecules.

2. Results and discussion

2.1. Artificial chaperone activity of α-CyD

Chaperones provide considerable protection against protein misfolding and aggregation.[5] Different protein classes play the role of chaperone to protect the other protein molecules from

Abbreviations: MD, molecular dynamic; BD, blind docking; CyD, cyclodextrin; Phe, phenylalanine; RMSD, root mean square deviations; ADH, alcohol dehydrogenase; DLS, dynamic light scattering; *I*, aggregation prone state intermediate.

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doi:10.1016/j.carres.2009.11.008

Table 1
Properties of α-CyD[8]

Property	
Number of glucopyranose units	6
Molecular weight (g/mol)	972
Solubility in water at 25 °C (%, w/v)	14.5
Outer diameter (Å)	14.6
Cavity diameter (Å)	4.7–5.3
Height of torus (Å)	7.9
Cavity volume (Å^3)	174

unfolding/aggregation. Amphiphilic non-protein molecules, such as RNA and phospholipids, have also been shown to function as the molecular chaperones.[14] Figure 1 indicates that the OD measured at a wavelength range far from λ_{max} (290–370 nm) sequentially increases for scanned wavelengths of ADH during the incubation period. Absorbance changes at the wavelengths far from protein's λ_{max} ($\geq$280 nm) are purely related to the scattering, derived from aggregation. Hence, ADH easily aggregates under thermal pressure. Figure 1B depicts the chaperone action of α-CyD in decreasing and moderating the ADH aggregation phenomenon, as it can be inferred that the presence of free Phe suppressed this activity during the same conditions (Fig. 1C). Analyzing the aggregation size distributions by DLS in Figure 2A confirms the results of UV–vis studies. Therefore, α-CyD helps in decreasing the size and amount of aggregated ADH molecules. Thus, the UV–vis and DLS results indicate that the free Phe prevents the chaperone action of α-CyD.

Cyclodextrins (CyDs) possess hydrophobic nanocavity that binds to the aromatic amino acids with high affinity.[15,9] Interestingly for the different complexes of CyDs, the ones in which aromatic amino acids are involved of attention as a model for enzyme–substrate-specific binding. The molecular dynamics of particular α-CyD/Phe complexes have been studied by ^{13}C NMR spectroscopy[16] and MD simulation techniques.[15,17] In agreement with other reports, an easy construction of the α-CyD/free-Phe inclusion complexes culminates the prevention of α-CyD/ADH complex formation, which protects the enzyme from aggregation. Some critical regions of ADH should be important in thermal aggregation phenomenon, where the presence of α-CyD leads to the formation of hydrophilic mask that controls the enzyme aggregation process.

Recently, we have shown that the hydrophobic interactions between ADH molecules are the main factors of its aggregation.[6] The α-CyD possesses a hydrophobic nanocavity (diameter = 0.5 nm; Table 1) and hydrophilic surface. It can spontaneously form inclusion complexes with the exposed hydrophobic parts of ADH, leading to the formation of hydrophilic cover, which increases the solubility of protein. Role of free Phe in suppressing the α-CyD-stabilizing action can be detected by observing the ADH's UV–vis absorption spectra where they explain the critical role of Phe residues during this phenomenon (Fig. 2B). Usually, proteins have λ_{max} at 280 nm because of the presence of tyrosine (Tyr) and especially the tryptophan (Trp) residues in their primary structure. Whereas, Figure 2B indicates that the ADH is an unusual protein because it does not show its particular λ_{max} at 280 nm. Primary structure of ADH contains a large number of Phe residues, which are ninefolds of its Trp residues (Phe36, Trp4 and Tyr8). The presence of a large number of Phe residues which have high affinity for α-CyD to form inclusion complexes, may contribute to protect the ADH from thermal polymerization/aggregation. To understand how α-CyD forms the inclusion complexes and which part/residues of ADH molecule are involved in this phenomenon, we have focused on the following computational studies.

2.2. MD simulations and molecular docking of ADH/α-CyD

Chaperone-like activity of α-CyD cannot be clearly understood by the experimental approaches. So, the recent study has been carried out by combining the experimental data with the molecular modeling/docking computational calculations. The MD simulation attempts to release strain from the highly strained region of a molecule, leading to an equilibrium native conformation that is possible to be evaluated by changes in root mean square deviations (RMSD).[18] Figure 3 shows the RMSD of the ADH backbones under MD simulation times. After the simulation of about 5000 ps, the RMSD of the ADH backbone was kept at 0.38 nm (±0.02 nm) for 1000 ps. It indicates that after the elimination of unfavorable strains from the X-ray geometry, ADH acquired a rather stable conformation that was suitable for molecular docking studies.

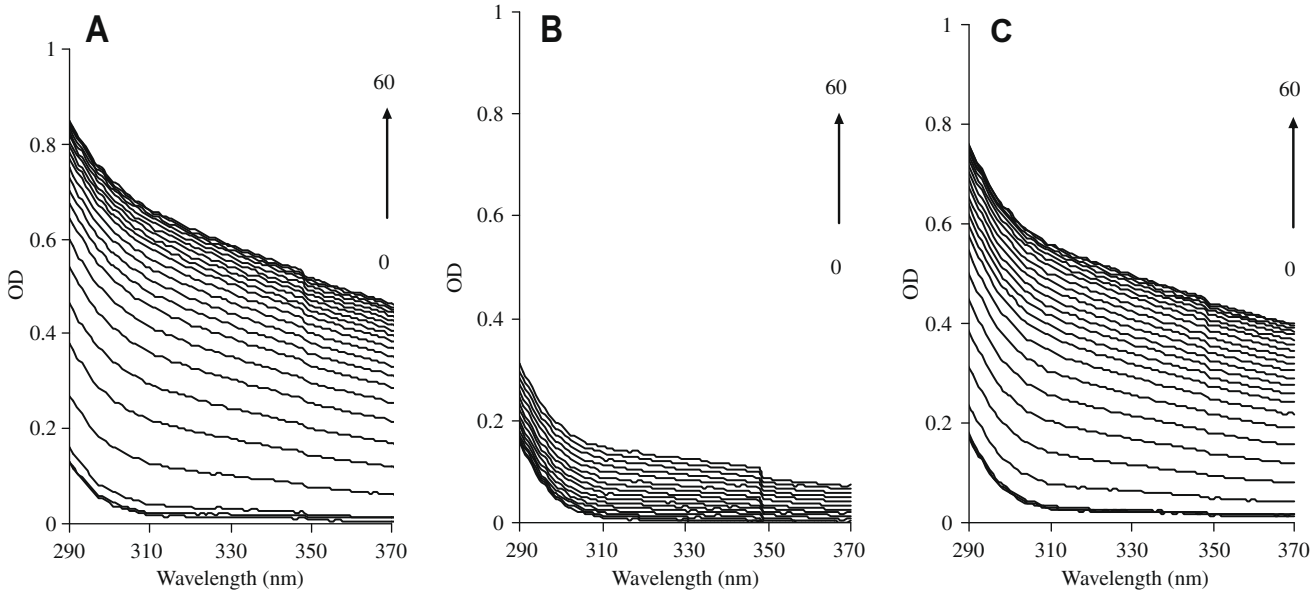

Figure 1. UV–vis scattering spectra of ADH under thermal stress. The sequential increase in each spectrum was recorded at 3 min intervals during the incubation of ADH enzyme at 50 °C for 60 min. The panels denote A; control (ADH alone), B; the presence of 100 mM α-CyD, C; the presence of 100 mM α-CyD with 30 mM Phe. (Free Phe had negligible changes in the scattering spectra without α-CyD).