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Refolding of urea-induced denaturation of model proteins by trimethylamine N-oxide

Pankaj Attri, Pannuru Venkatesu*

Department of Chemistry, University of Delhi, Delhi 110 007, India

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ABSTRACT

The biomolecules are known to be stabilized by osmolytes, trimethylamine-N-oxide (TMAO) while urea, destabilizes the protein structures. The deleterious effect of urea on proteins has been counteracted by TMAO is well understood; nonetheless, refolding of urea-induced conformational changes of proteins by TMAO is still an active subject. To understand the refolding ability of TMAO from urea-induced denaturation of biomolecules, we have performed transfer free energy ($\Delta G'_{tr}$) and the hydrodynamic diameter (d_H) of cyclic dipeptides (CDs) such as, cyclo(Gly–Gly), and cyclo(Leu–Ala) through solubilities and dynamic light scattering (DLS) measurements, respectively. We observed positive and negative values of $\Delta G'_{tr}$ for CDs from water to TMAO and urea, respectively. Our results reveal that TMAO is a refolding additive for urea deleterious actions on CDs at 1:1 and 1:2 molar ratios of TMAO and urea. However, TMAO (1 M) fails to refolding CDs structure from the urea (3–5 M)-induced conformational changes on CDs.

1. Introduction

Proteins are multifarious system that can exist in an enormous number of conformations, exhibiting a substantial degree of structural variability in their folded state [1]. The function, structure and stability of native proteins show marginal changes in the co-solvent environment that can dramatically affect their properties and functional activity [2–8]. In the context of protein stability, co-solvents can be classified as protectants or denaturants according to their effect of folding or unfolding of the native state of proteins, respectively [2,9]. Osmolytes are small organic molecules that increase the thermal stability of biomolecules, without modulating macromolecular structure and functions [2,3,6–9]. In other words, these osmolytes, referred to as compatible or protective osmolytes, tend to stabilize the protein structure, without altering macromolecular structure and function [2,3,8–12]. The stabilization of compact native structures reveals typically the results of preferential exclusion of osmolytes from the vicinity of the biomolecule surface. Energetically, unfavorable interactions can exist between osmolyte and hydration surface of the protein that leads to stabilization of the native structure of biomolecules [6–13]. This has been depicted by preferential hydration favoring the more compact native structure of proteins. On the other hand, these protective osmolytes reduce endoplasmic reticulum stress, which is an important factor in diseases such as type II diabetes [14].

The classical chemical denaturants such as urea and guanidine hydrochloride (designated GdnHCl) are considered to act by breaking protein hydrogen bonds, and interact preferentially with the protein surface, thus appearing to be bound, and the protein is noted to be preferentially binding [15–17]. Osmolytes belong to diverse chemical families including polyols, amino acids and amino acid derivatives and methylamines [2,9,10]. Apparently, the third group of nitrogenous osmolytes, the methylamines, acts as stabilizer the folded state and counteracting the perturbations of protein structure caused by denaturants [2,9,11,13,18–26].

Among the osmolytes, trimethylamine N-oxide (TMAO) is a powerful stabilizing and most compatible osmolyte for proteins [2,8–12]. Obviously, TMAO, which is a naturally occurring osmolyte, counteracts the deleterious effects of proteins caused by denaturants [9,11], temperature [9], pressure [27], and ice [28]. The deleterious effect of denaturants and various stress on proteins has been counteracted by TMAO is well understood, although, proteins refolding of urea-induced conformational changes by TMAO is still an active subject of considerable debate. The fact that refolding conditions is critical in order to obtain satisfactory amounts of active protein. Refolding is a change in protein native structure conformation from unfolded state to folded state. The refolding process has been shown schematically in Scheme 1, which depicts a change in protein conformation from unfolded to folded state. Obviously, the role and mechanism of TMAO in protein stabilization appears to be quite clear, however its effects on refolding of urea-denature

* Corresponding author. Tel.: +91 11 2766 6646x142; fax: +91 11 2766 6605.
E-mail addresses: venkatesup@hotmail.com, pvenkatesu@chemistry.du.ac.in, pannuruv@yahoo.com (P. Venkatesu).

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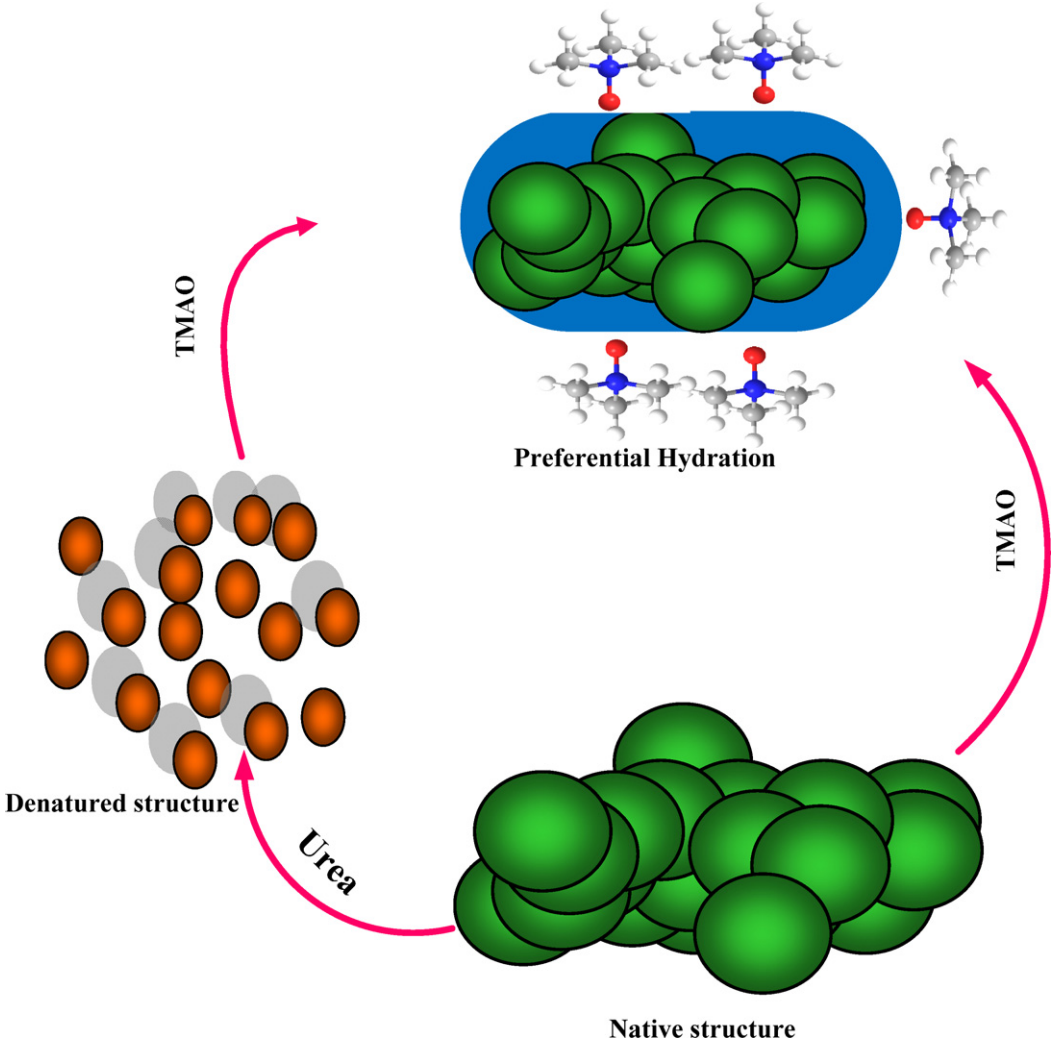

Scheme 1. Schematic illustration of the refolding of native protein structure conformation from unfolded state to folded state.

protein is poorly understood, particularly protein denatured at higher urea concentration, although some studies have been carried out [29,30]. The existing results show that TMAO may significantly enhance the refolding process [29,30], while they are not clear about the 1 M TMAO is able to refold the urea-induced denaturation at higher concentration of urea.

Cyclic dipeptides (CDs) are essentially important biomolecules because of their wide application in drug production and their use in group additivity schemes to evaluate the protein folding properties [5,10,11,31–33]. CDs with six-membered diketopiperazine ring as organic backbone, offer several advantages in the thermodynamics of protein model compounds and in the protein stability. They have no free carboxylate or amino terminus within their structures and their conformational rigidities are small. Additionally, another advantage of CDs is that they have been shown to be reasonable model compounds for the solid-like core of globular proteins. CDs are important biomolecules because of their wide application in drug production and their roles as signal transmitters in cell communications. Obviously, the refolding ability of TMAO against the urea thermal denaturation of CDs is interesting not only from an academic perspective but also from industrial point of view.

The refolding of TMAO additive for CDs from urea deleterious actions on CDs structures is unknown. To understand how TMAO is able to refold the urea-denatured CDs structure, we explore the effect of refolding of TMAO on a homologous series of CDs with aliphatic nonpolar side chains (glycine, alanine, and leucine) combinations, through transfer free energy and dynamic light scaterring (DLS) measurements. Two CDs, including cyclo(Gly–Gly) and cyclo(Leu–Ala) were investigated and these CDs are abbreviated as c(GG) and c(LA), respectively. Through this paper, we have attempted to investigate the refolding of urea-induced CDs denaturation by the addition of TMAO. These studies have attributed to study the effects of hydrophobicity, hydrogen bonding, aqueous solvation of CDs, and particle size and such parameters can be used to understand the protein folding/unfolding studies.

2. Materials and methods

2.1. Materials

The cyclic dipeptides c(GG) and c(LA) and TMAO were purchased from Sigma Chemical Co., USA. Urea obtained from Acros Organics. All these purchased materials were used as received. High purity water used for preparing the aqueous TMAO or urea solutions or their mixtures were obtained from a NANO pure-Ultra pure water system. The purified water can be distilled and deionized with a resistance of 18.3 MΩ. All solutions were prepared gravimetrically.