Biophysical Chemistry 137 (2008) 105–109

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Structure-based analysis reveals hydration changes induced by arginine hydrochloride

Makoto Nakakido [a], Yoshikazu Tanaka [a,b], Mariko Mitsuhori [a], Motonori Kudou [a], Daisuke Ejima [c], Tsutomu Arakawa [d], Kouhei Tsumoto [a,*]

[a] Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa 277-8562, Japan
[b] Creative Research Institute Sousei, Hokkaido University, Sapporo 001-0021, Japan
[c] Amino Science laboratories, Ajinomoto Co., Inc., Kawasaki 210-8681, Japan
[d] Alliance Protein laboratories, Thousand Oaks, CA 91360, United States

ARTICLE INFO

Article history:
Received 3 July 2008
Received in revised form 28 July 2008
Accepted 30 July 2008
Available online 9 August 2008

Keywords:
Arginine hydrochloride
Protein aggregation
Refolding
Crystal structure
Lysozyme
Hydration water molecules

ABSTRACT

Arginine hydrochloride has been used to suppress protein aggregation during refolding and in various other applications. We investigated the structure of hen egg-white lysozyme (HEL) and solvent molecules in arginine hydrochloride solution by X-ray crystallography. Neither the backbone nor side-chain structure of HEL was altered by the presence of arginine hydrochloride. In addition, no stably bound arginine molecules were observed. The number of hydration water molecules, however, changed with the arginine hydrochloride concentration. We suggest that arginine hydrochloride suppresses protein aggregation by altering the hydration structure and the transient binding of arginine molecules that could not be observed.

1. Introduction

Solvents play a major role in refolding of proteins [1–6]; arginine hydrochloride has been one of the frequently used co-solvents [7–13]. Arginine hydrochloride is considered to improve the refolding efficiency of recombinant proteins by its ability to suppress aggregation of the proteins [14–16]. Moreover, arginine hydrochloride is now used in various other applications, e.g., solubilization of proteins from loose "flocculate-type" inclusion bodies [17,18], milder elution of antibodies from protein-A affinity resins [19,20], and improved separation and recovery of proteins in various chromatographies [13,21–24]. Thermodynamic interactions of proteins with solvent components, both water and arginine in this case, determines the effects of the solvent additives on proteins. Although small arginine binding has been suggested from thermodynamic measurements (e.g. equilibrium dialysis), the physical state of both arginine and water binding has never been experimentally determined.

Recently, a great deal of attention has been given to the structure of water and the assistive at high additive concentrations [25]. X-ray crystallography is useful for high-resolution structural analysis and for characterizing the interactions between water and proteins [26–30]. We investigated arginine binding and hydration and their effects on the structures of hen egg-white lysozyme (HEL). This is the first report examining the physical state of protein solution in aqueous arginine solution.

2. Materials and methods

2.1. Materials

Hen egg-white lysozyme was purchased from Seikagaku Corp. (Tokyo, Japan). Crystallization buffer was purchased from Hampton Research (Aliso Viejo, CA, USA). All other reagents were of biochemical research grade.

2.2. Crystallization of HEL in presence of arginine hydrochloride, NaCl, betaine, and sucrose at various concentrations

Hen egg-white lysozyme was dissolved with arginine hydrochloride at 25, 50, 100, 125, 150, 200, 250, 375, 500, 750, and 1000 mM in 20 mM Tris–HCl buffer (pH 6.9) and diluted to 20 mg mL^{-1}. The HEL solution was dialyzed against the buffer for 12 h at 4 °C and then subjected to crystallization by the oil-batch method [31] to prevent the arginine hydrochloride concentration from being changed by vapor diffusion. Two microliters of HEL was mixed with an equal

Abbreviations: HEL, hen egg-white lysozyme; B-factor, relative temperature; DMSO, dimethyl sulfoxide; GdnHCl, guanidine HCl.

* Corresponding author. Tel.: +81 4 7136 3600; fax: +81 4 7136 3601.
E-mail address: tsumoto@k.u-tokyo.ac.jp (K. Tsumoto).

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doi:10.1016/j.bpc.2008.07.009

volume of 100 mM HEPES (pH 7.5) in 1.4 M sodium citrate on 72-microwell plates, and then covered with 10 μl of mineral oil. As a reference, crystals of HEL were also prepared in solutions containing NaCl at different concentrations.

2.3. Diffraction data collection and processing

The high-resolution diffraction data were collected on a beamline BL6A, NW12, or BL17A diffractometer (Photon Factory, Tsukuba, Japan) under cryogenic conditions. Each crystal was soaked for 1 min in a buffer containing 25% glycerol in addition to the crystallization reagents and arginine hydrochloride, and then mounted on a goniometer. The diffraction data were indexed, integrated, scaled, and merged by using the HKL2000 software package [32]. All crystals used were isomorphous crystals whose space group was $P4_32_12$, with unit cell parameters $a=b=c.a.$ 77 Å and $c=37$ Å. The data collection statistics are shown in Supplementary Tables 1 and 2.

2.4. Structure solution and refinement

The structures in each condition were refined from the crystal structure of HEL (PDB ID: 1BWI), which is an isomorphous crystal, as described above. The complete atomic model with a total of 129 residues, including side chains, was rebuilt manually with the molecular graphics program XtalView [33]. Positional and individual temperature factor (B-factor) refinement was carried out with the program REFMAC5 [34]. To monitor the refinement, a 5% subset of all reflections, which was unified like those in the initial mode, was set aside for calculation of the free R factor (Rfree). After iterative cycles of refinement and manual model fitting, water molecules were located by using an Fo–Fc map. At an electron density that was obviously redundant despite location of the water molecules, sodium or chloride ions were placed instead of the water molecules on the basis of the magnitude of the redundancy map and the surrounding conditions. The stereochemical quality of the final refined models was analyzed with the program PROCHECK [35]. The parameters of the data collection and reduction are shown in Supplementary Tables 1 and 2.

3. Results

With a hope to observe both arginine binding and hydration, we crystallized HEL in a solution containing arginine hydrochloride at various concentrations. The oil-batch method was used for crystallization to keep the concentration of arginine hydrochloride constant. Diffraction-quality crystals were obtained in the identical crystallization buffer, regardless of the arginine hydrochloride concentration. Under all conditions, high-resolution diffraction data were collected, and the structures of HEL containing arginine hydrochloride at various concentrations were determined at resolutions higher than 1.65 Å. We examined the backbone and side chain structure of lysozyme in the presence of arginine hydrochloride at various concentrations (Fig. 1). Neither the backbone nor the side-chain structure of HEL was altered by the presence of arginine hydrochloride. Moreover, the relative values of the B-factor of the residues of HEL in the presence of arginine hydrochloride at various concentrations did not change (Fig. 2).

Then we investigated the solvent molecules around HEL in the crystal structure. Even at high concentrations of arginine hydrochloride, no bound arginine molecule was observed around HEL. We

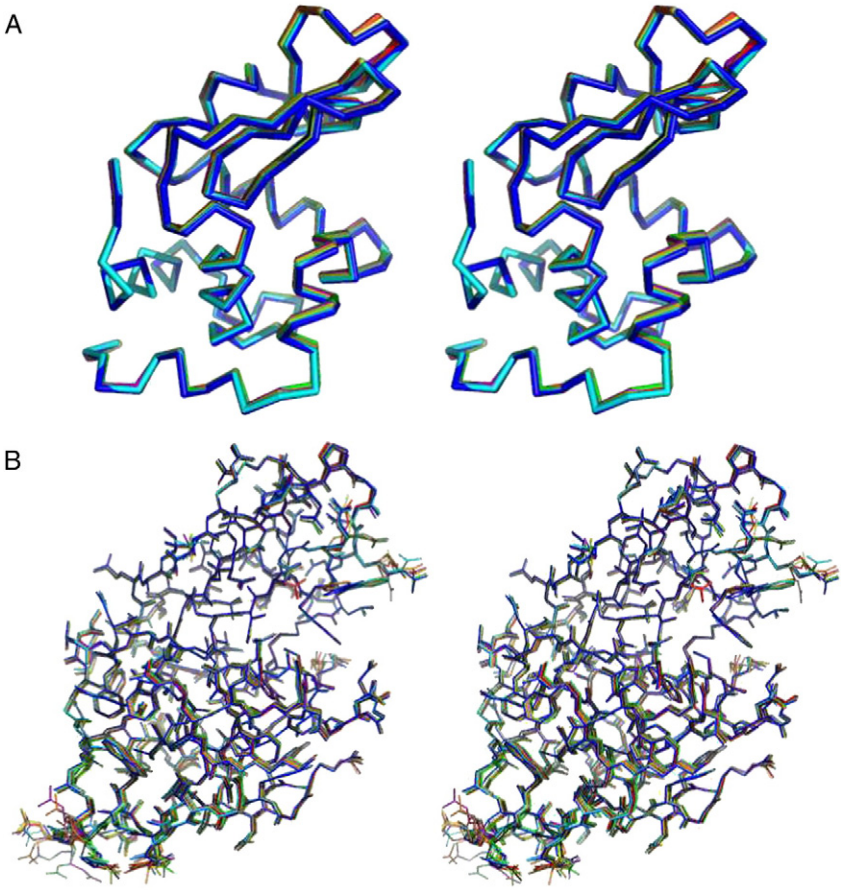

Fig. 1. Crystal structure of HEL. Structures in the presence of 0, 25, 50, 100, 125, 150, 200, 250, 375, 500, 750, and 1000 mM arginine hydrochloride are drawn in blue, cyan, gray, purple, light green, green, yellow, orange, magenta, black, brown, and red, respectively. (A) Backbone structure of HEL in the presence of arginine hydrochloride at the concentrations listed above. (B) Crystal structure of HEL shown with side chains.