Biophysical Journal Volume 93 September 2007 2170–2177

Macromolecular Crowding Induces a Molten Globule State in the C-Terminal Domain of Histone H1

Alicia Roque, Inma Ponte, and Pedro Suau
Departamento de Bioquímica y Biología Molecular, Facultad de Biociencias, Universidad Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

ABSTRACT We studied the secondary structure of the C-terminal domains of the histone H1 subtypes H1° (C-H1°) and H1t (C-H1t) in the presence of macromolecular crowding agents (Ficoll 70 and PEG 6000) by IR spectroscopy. The carboxyl-terminal domain has little structure in aqueous solution but became extensively folded in the presence of crowding agents. In 30% PEG, C-H1° contained 19% α-helix, 28% β-sheet, 16% turns, and 31% open loops. Similar proportions were observed in 30% Ficoll 70 and for C-H1t in both crowding agents. The proportions of secondary structure motifs were comparable to those of the DNA-bound domain. Kratky plots of the small-angle x-ray scattering showed that in crowding agents the C-terminus had the compaction of a globular state. Progressive dissipation of the secondary structure and a linear increase in partial heat capacity with temperature together with increased binding of ANS indicated that the C-terminus is not cooperatively folded in crowded conditions. Native-like secondary structure and compactness in absence of folding cooperativity indicate that the C-terminus in crowding agents is in a molten globule state. Folding of the C-terminus in crowded conditions may increase the rate of the transition toward the DNA-bound state and facilitate H1 diffusion inside cell nuclei.

INTRODUCTION

H1 linker histones are involved in chromatin structure and gene regulation. It is currently accepted that histone H1 could have a regulatory role in transcription through the modulation of chromatin condensation. H1 may mediate transcription on a more specific level, participating in complexes that either activate or repress specific genes (1–7). Binding to scaffold-associated regions (8) and participation in nucleosome positioning (9) are other mechanisms by which H1 could contribute to transcriptional regulation.

H1 contains three distinct domains: a short amino-terminal domain (20–35 amino acids), a central globular domain (~80 amino acids) consisting of a three-helix bundle and a β-hairpin, and a long carboxy-terminal domain (~100 amino acids) (10). The amino acid composition of the C-terminus is dominated by Lys (~40%), Ala (~17%), and Pro (~12%). The C-terminal domain is the primary determinant of H1 binding to chromatin in vivo (11,12). Several studies indicate that the ability of linker histones to stabilize chromatin folding resides in the C-terminal domain of the molecule (13,14). Preferential binding of histone H1 to scaffold-associated regions and activation of apoptotic nuclease also appear to be determined by the C-terminal domain (15,16).

In aqueous solution, the C-terminal domain is dominated by the random coil and turn-like conformations in rapid equilibrium with the unfolded state, but on interaction with DNA, it folds cooperatively (17). The DNA-bound structure is extremely stable and includes α-helix, β-sheet, turns, and open loops. The H1 carboxyl-terminal domain thus appears to belong to the so-called intrinsically disordered proteins undergoing coupled binding and folding (17–22). Like most natively disordered proteins, the C-terminus is of low hydrophobicity, large net charge, and low sequence complexity (23–25).

Proteins are usually studied in dilute solution; however, in the cellular environment, macromolecules and small-molecule solutes are present at high concentrations so that a significant fraction of the intracellular space is not available to other macromolecules. The excluded volume effects are predicted to favor the adoption of compact as opposed to expanded macromolecular conformations, resulting in a reduction of the total excluded volume. However, experimental evidence has shown that although crowding agents in general favor refolding (26–29), in some cases they appear to be ineffective (30,31). A role of molecular crowding in accelerating α-synuclein fibrillation has been described (32,33).

In this study we have used IR spectroscopy to estimate the proportions of secondary structure motifs of the C-terminus of H1° (C-H1°) and H1t (C-H1t) in the presence of Ficoll 70 and polyethyleneglycol (PEG) 6000 as macromolecular crowding agents. Our results show that crowding is highly effective in promoting folding of the C-terminal domain. In the presence of 30% PEG or Ficoll, the proportions of secondary structure motifs appear to be similar to those of the DNA-bound domain. In these conditions, Kratky plots of the x-ray scattering indicate that the compactness of the C-terminus is that of a globular state. However, binding of 1-anilinonaphthalene-8-sulfonate (ANS), thermal melting, and differential scanning calorimetry (DSC) show that in a

Submitted January 18, 2007, and accepted for publication May 10, 2007.

Address reprint requests to Pedro Suau, Departamento de Bioquímica y Biología Molecular, Facultad de Biociencias, Universidad Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain. Tel.: 34-935811391; Fax: 34-935811264; E-mail: pere.suau@uab.es.

Editor: Ruth Nussinov.

0006-3495/07/09/2170/08 $2.00

doi: 10.1529/biophysj.107.104513

crowded environment the C-terminus is not cooperatively folded. The properties of the C-terminus in crowding agents are thus those of a molten globule state.

MATERIALS AND METHODS

Cloning, expression, and purification of the C-terminal domains of histone H1 subtypes

The C-terminal domains of histones H1° and H1t were obtained from recombinant *Escherichia coli* (M15) as described previously (17).

Circular dichroism spectroscopy

The C-terminal domains were at 0.3 mg/ml in 10 mM phosphate buffer, pH 7.0, plus 140 mM NaCl and in the presence of PEG 6000 or Ficoll 70. Spectra were obtained on a Jasco J-717 spectrometer in 1-mm cells at 20°C. The results were analyzed with Standard analysis software (JACSO) and expressed as mean residue molar ellipticity [θ]. The helical content was estimated from the ellipticity value at 222 nm (θ_{222}), according to the empirical equation of Chen et al. (34): percentage helical content = $100[\theta_{222}/-39{,}500 \times (1 - 2.57/n)]$, where n is the number of peptide bonds.

Infrared spectroscopy measurements

The C-terminal domains were at 5 mg/ml in 10 mM HEPES, pH 7.0, plus 140 mM NaCl and in the presence of PEG 6000 or Ficoll 70 at 300 g/L. Data were collected on an FT600 Bio-Rad spectrometer equipped with an MCT detector. Typically, 1000 scans for each background and sample were collected, and the spectra were obtained with a nominal resolution of 2 cm^{-1} at 20°C. Secondary structure content was determined by curve fitting to the original spectrum using the component band positions identified by Fourier self-deconvolution as previously described (35,36). The baseline contributed by the solvent (10 mM HEPES, 140 mM NaCl, pH 7.0 in H_2O or D_2O and with or without PEG 6000 or Ficoll 70) was removed before fitting. Spectra were recorded both in H_2O and D_2O to distinguish between the overlapping contributions in D_2O of random coil and open loops. The vibrations of loops and the α-helix are found at similar positions in H_2O and D_2O, the α-helix at ~1652 cm^{-1} and loops at ~1643 cm^{-1}. In contrast, deuteration has a major effect on the position of the random coil so that in H_2O it overlaps with the α-helix, whereas in D_2O it overlaps with loops. The α-helix was, therefore, estimated directly in D_2O, whereas loops were estimated directly in H_2O. When both α-helix and loops are present in the protein structure, the percentage of random coil can be obtained either from the difference of the components around 1552 cm^{-1} in H_2O and D_2O or from the difference of the components around 1643 cm^{-1} in D_2O and H_2O, as previously described (17).

Small-angle x-ray scattering

Measurements were performed in an MBraun instrument equipped with a Siemens Kristalloflex (Graz, Austria) 760 (K-760) generator, producing radiation with a wavelength of 1.54 Å (CuK_α), operating at 50 kV and 40 mA, and a Kratky Hecus camera. The collimator was a slit window, and the scattering was detected with a linear position sensitive detector OED-50M. The sample-to-detector distance was 268 mm. Samples were measured at 25°C. All samples were placed in glass capillaries of 1 mm diameter and 10 μm wall thickness. The background was subtracted by measuring blanks without protein. Both the samples and blanks were measured for 4 h unless otherwise stated. The concentration of protein was 4–10 mg/ml.

The scattering intensity $I(Q)$ is represented as a function of the momentum transfer Q; $Q = 4\pi \sin\theta/\lambda$, where λ is the wavelength of x-rays and 2θ the scattering angle. The inner part of the scattering profile can be described by the Guinier approximation, $I(Q) = I_0 e Q^2 R_g^2/3$ (37). The scattering at zero angle, I_0, is proportional to the molecular mass of the particle and to the square of the contrast between the particle and the solvent. The radius of gyration, R_g, which is the root mean-square of the distances of all volume elements to the center of mass of the electronic volume of the particle, was obtained from the slope of a Guinier plot of $\ln I(Q)$ versus Q^2.

The R_g can also be measured with the Debye equation (38), $P_D(x) = I(Q)/I_0 = 2(x - 1 + e^{-x})$, where $x = Q^2R_g^2$, using the approximation given by Calmettes et al. (39): $[P_D(x)]^{-1} = 1 + 0.359\, x^{1.103}$. The R_g is given by $R_g = (a/0.359b)^{0.453}$, where a is the slope and b the intercept of the straight line.

Thermal melting

For the analysis of the thermal stability of C-H1t in 30% Ficoll 70, the temperature of the sample was adjusted using a cover jack connected to a circulating thermostatic bath and monitored with a fitted external probe. Thermal analyses were performed by heating from 20 to 85°C at a rate of 1°C/min. For each degree of temperature interval, 400 interferograms were averaged, Fourier-transformed, and ratioed against background. Thermal analyses were performed in D_2O. The Ficoll contribution at each temperature was subtracted using a Ficoll sample at the same temperature and concentration.

ANS fluorescence assays

1-Anilinonaphthalene-8-sulfonate (ANS) binding assays were performed in a Cary Eclipse spectrofluorometer. The spectra were measured in 140 mM NaCl, 10 mM phosphate buffer, pH 7.0, at 20°C. The concentration of ANS was 0.3 mM, and the concentration of protein was 10 mM. The ANS emission was scanned between 400 and 650 nm with an excitation wavelength of 380 nm.

Differential scanning calorimetry

DSC measurements were carried out on a Microcal MC-2 calorimeter supplied with an Origin software package for data analysis and curve fitting. Protein and background scans were performed at a scan rate of 1.5°C/min between 20 and 100°C. We used RNase A as a control at a concentration of 150 μM. C-H1t was analyzed at a concentration of 100 μM in buffer and in buffer plus 30% Ficoll 70. The buffer was 10 mM phosphate, pH 7.0, plus 140 mM NaCl.

RESULTS

CD and IR spectroscopy analysis of the C-terminal domain of histone H1 in the presence of crowding agents

We used CD to explore the effects of different concentrations of Ficoll 70 and PEG 6000 (10, 20, and 30%) on the secondary structure of the C-terminal domains of the histone H1 subtypes H1° and H1t in physiological salt (0.14 M NaCl) (Fig. 1). We found that in 30% of both crowding agents C-H1° and C-H1t became significantly folded. In 30% PEG, the estimated values of α-helix were 16% for C-H1° and 13% for C-H1t. In 30% Ficoll, the estimated helical content was 16% for both C-terminal domains.

IR spectroscopy was used to further characterize the secondary structure of the C-terminal domains in crowding agents. In the absence of crowding agents, the amide I (D_2O)