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Stability against temperature of *Sulfolobus solfataricus* elongation factor 1α, a multi-domain protein

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Abstract

The elongation factors (EF-Tu/EF-1α) are universal proteins, involved in protein biosynthesis. A detailed characterization of the stability against temperature of *Ss*EF-1α, a three-domain protein isolated from the hyperthermophilic archaeon *Sulfolobus solfataricus* is presented. Thermal denaturation of both the GDP-bound (*Ss*EF-1α•GDP) and the ligand-free (nf*Ss*EF-1α) forms was investigated by means of circular dichroism and fluorescence measurements, over the 4.0–7.5 pH interval. Data indicate that the unfolding process is cooperative with no intermediate species and that the few inter-domain contacts identified in the crystal structure of *Ss*EF-1α play a role also at high temperatures. Finally, it is shown that the enzyme exhibits two different interchangeable thermally denatured states, depending on pH.

Keywords: Elongation factor; Archaea; Protein stability; Thermal denaturation; Circular dichroism; Fluorescence

1. Introduction

Protein biosynthesis is a crucial event in cellular life. Elucidation of the molecular and structural basis of this process has shown that it is accomplished by the combined actions of the ribosome and a number of ancillary proteins such as initiation, elongation, and termination factors. Ribosome provides the machinery for translation, whereas the protein factors bring all the necessary reagents for the reaction [1,2]. As other factors involved in protein biosynthesis, the elongation factors (EFs), that catalyze the binding of the aminoacyl-tRNA to the ribosome, are ubiquitous and highly conserved guanine nucleotide binding proteins, made up of a single polypeptide chain (390–460 residues) [3,4].

EFs isolated from eubacteria (EF-Tu) and from eukarya display significant differences at both sequence and structure level, whereas the archaeal ones show significant analogies only with their eukaryal counterpart. Indeed, archaeal and eukaryal EFs, collectively designated EF-1α, share sequence identities in the 50–60% range. The thermal stability of EFs is generally related to the living temperature of the host organisms. This feature has recently been used to obtain a reliable estimate of an earth temperature one billion years ago by characterizing the hypothetical EF of the common ancestor [5].

Abbreviations: EF, elongation factor; *Sc*, *Saccharomyces cerevisiae*; *Ss*, *Sulfolobus solfataricus*; nf, nucleotide-free; CD, circular dichroism; DSC, differential scanning calorimetry; ANS, 8-anilino-1-naphthalenesulfonic acid

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These enzymes exhibit a remarkable structural versatility as a consequence of their interactions with the other players of protein biosynthesis. Interactions with small and large ligands often lead to major rearrangements of EF-Tu/EF-1α inter-domain contacts [6–16]. Particularly impressive is the variation associated with the hydrolysis of the bound GTP to GDP [7,9,16]. The structures of the GDP complexes hitherto reported for eubacterial EF-Tu and for *Ss*EF-1α, isolated from the archaeon *Sulfolobus solfataricus*, present peculiar properties [9,16]. They are characterized by a very loose structure with limited inter-domain interactions. The structure of eukaryal EF-1α appears to be even more flexible; neutron scattering investigations on rabbit liver EF-1α•GDP demonstrated that the enzyme adopts an extended structure with a radius of gyration markedly different from that derived from the crystal structure of *Ss*EF-1α•GDP [17]. In order to verify the significance and the role of the limited, but significant, inter-domain contacts in *Ss*EF-1α•GDP, we have undertaken an extensive characterization of *Ss*EF-1α stability in solution. In a previous report we investigated the stability of the enzyme against chemical denaturants (urea and guanidine hydrochloride) at room temperature [18]. The temperature-induced denaturation of the GDP-bound and the nucleotide-free forms of *Ss*EF-1α are here investigated. The data show that both protein forms exhibit (a) a cooperative thermal denaturation and (b) two distinct thermally denatured states depending on pH. The finding that *Ss*EF-1α undergoes a cooperative thermal unfolding indicates that the inter-domain contacts identified in the crystallographic characterization of the enzyme [16,19] are in action and play a role at temperatures close to or above those that favour *S. solfataricus* optimal growth.

2. Materials and methods

2.1. Protein preparation

Recombinant *Ss*EF-1α was prepared by using an *Escherichia coli* expression system as previously reported [20]. The protein was stored at −20 °C in a buffer containing 20 mM Tris/HCl (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$, and 50 % (v/v) glycerol. The ligand-free form of the enzyme (nf*Ss*EF-1α) was prepared from the recombinant *Ss*EF-1α by adopting the procedure previously described [18,21]. Protein concentration was determined spectrophotometrically using an extinction coefficient of 26,025 M^{-1} cm^{-1} at 280 nm for both the GDP-bound and ligand-free forms [22]. The functionality of *Ss*EF-1α•GDP and nf*Ss*EF-1α samples used in the spectroscopic analyses was assessed by measuring both the intrinsic GTPase activity and the GDP-binding ability of the enzyme following the procedures previously described [23].

Buffers used in the experiments were acetate (pH 4.0, 5.0), cacodylate (pH 5.5, 6.0), MES (pH 6.5), MOPS (pH 7.0), HEPES (pH 7.5) and Tris (pH 7.5); all had a concentration of 10 mM.

2.2. Circular dichroism measurements

CD spectra were recorded with a Jasco J-810 spectropolarimeter equipped with a Peltier temperature control system (Model PTC-423-S), and calibrated with an aqueous solution of D-10-(+)-camphorsulfonic acid at 290 nm. Molar ellipticity per mean residue, [θ] in deg cm^2 $dmol^{-1}$, was calculated from the equation: $[\theta]=[\theta]_{obs}$ mrw $(10\ l\ C)^{-1}$, where $[\theta]_{obs}$ is the ellipticity measured in degrees, mrw is the mean residue molecular weight (111.5 Da), C is the protein concentration in g mL^{-1} and l is the optical path length of the cell in cm. Far-UV measurements (190–250 nm) were carried out at 20 °C using a 0.2 cm optical path length cell and a protein concentration of 0.15 mg mL^{-1}. CD spectra, recorded with a time constant of 4 s, a 2 nm band width, and a scan rate of 5 nm min^{-1}, were signal-averaged over at least three scans. The baseline was corrected by subtracting the buffer spectrum.

Thermal denaturation curves were recorded over the 50–105 °C temperature interval, following the CD signal at 200, 208, and 222 nm. After preliminary trials within the scan rate interval 0.5–2.0 °C min^{-1}, all curves were registered using a 0.2 cm path length cell and a scan rate of 1.0 °C min^{-1}. The protein was dissolved in a variety of buffers with pH values ranging from 4.0 to 7.5. A check of the reversibility of the denaturation was carried out by recording new spectra, after cooling to 20 °C, samples subjected to a first temperature scan.

2.3. Fluorescence measurements

Fluorescence spectra were collected using a Perkin Elmer LS50B spectrofluorimeter, using a 1.0 cm path length cell upon excitation at 295 nm. Temperature was controlled with a Perkin Elmer Peltier. In all the experiments the protein concentration was 0.15 mg mL^{-1}. Thermal denaturation curves were constructed by recording the fluorescence intensity at 340 nm upon excitation at 295 nm, over the 55–105 °C temperature range; after preliminary trials in the rate interval 0.5–2.0 °C min^{-1}, a scan rate of 1.0 °C min^{-1} was selected for all measurements.

Experiments with the fluorescent dye 8-anilino-1-naphthalenesulfonic acid (ANS) were performed at 20 °C by incubating the native and the denatured forms of the protein with the dye at 1:3 molar ratio. Fluorescence emission spectra were recorded, after 10 min of incubation, over the range 400–600 nm upon excitation at 390 nm.

3. Results

3.1. Temperature-induced denaturation over the 5.0–7.0 pH interval

To check the proximity to the thermodynamic equilibrium, we preliminarily verified that the shape and the location of both CD and fluorescence thermal denaturation curves were independent of the scan rate over the range 0.5–2.0 °C min^{-1} (Fig. 1 of Supplementary data).

Far-UV CD spectra at 20 °C of *Ss*EF-1α•GDP (Fig. 1a) and nf*Ss*EF-1α (Fig. 1b) are characterized by the presence of two minima (at 208 and 222 nm) and one maximum (at 195 nm) which are typical fingerprints of α/β proteins [24]. The temperature-induced denaturation of *Ss*EF-1α•GDP and nf*Ss*EF-1α was initially investigated at pH 5.0, 10 mM sodium acetate buffer, by recording the molar ellipticity at 200, 208 and 222 nm (Fig. 2). Acetate buffer has been selected because of its small ionization enthalpy [25], leading to a small dependence of pH on temperature. The wavelengths were chosen to obtain information on the effect of temperature on both α-helical and β-sheet regions of the protein. Secondary structure analysis by means of the self-consistent method, implemented in the Dichroweb server, resulted in 29% α-helix, 40% β-sheet and 31% unordered regions [18]; such estimates are in line with those determined from the X-ray structure of *Ss*EF-1α•GDP [16].

Thermal denaturation curves of both *Ss*EF-1α•GDP and nf*Ss*EF-1α (Fig. 2) present a sigmoidal shape with a single inflection point, corresponding to T_d values of 96–97 °C for *Ss*EF-1α•GDP and 92–93 °C for nf*Ss*EF-1α, regardless of the wavelength monitored. Far-UV CD spectra of denatured species at 105 °C (Fig. 1) are characterized by the presence of a broad minimum at 215 nm, which is indicative of the persistence of secondary structure elements in the denatured form of both