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Structure of a methionine-rich segment of *Escherichia coli* Ffh protein

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Abstract The methionine-rich segments of the Ffh protein of *Escherichia coli* and its eukaryotic counterpart SRP54 are thought to bind signal sequences of secretory proteins. The structure of a chemically synthesized 25-residue-long peptide corresponding to one of the proposed methionine-rich amphiphilic helices of Ffh was determined in water and in aqueous trifluroethanol (TFE) solution using CD and NMR. An appreciable α-helix conformation exists even in water and this peptide assumes a stable α-helix along most of its length in aqueous TFE solution. It is clear that this segment of Ffh protein has a very strong propensity to form α-helical structure.

Key words: Ffh protein; Peptide; NMR; CD; α-Helix; Translocation

1. Introduction

It was recently proposed that a targeting system very similar to the eukaryotic SRP/SRP receptor is also involved in some protein translocation in *Escherichia coli* [1–4]. This concept originated mainly from data base searches which revealed that mammalian SRP54 and SRP receptor have the corresponding counterparts in *E. coli*, Ffh and FtsY, respectively [1,2]. SRP54 and Ffh are the units which bind directly with the signal sequences [5,6]. From the sequence analysis, Bernstein et al. [2] proposed that SRP54 and Ffh have GTP binding domains (G-domains) and methionine-rich domains (M-domains). The secondary structure prediction of the M-domain suggested that there are two possible helices in Ffh and three in SRP54 which are rich in Met [2]. These putative helices, with hydrophobic residues including Met, clustered on one face, are thought to form a groove in each M-domain. Signal sequences may bind with this Met-rich groove through hydrophobic interactions and their widely differing primary sequences may be accommodated by the flexible Met side chains [2,7,8].

Since the signal peptides have a propensity to form α-helical structures [9–12], it is important to determine whether they interact with Met-rich segments of the Ffh protein in the form of α-helices. As an initial study along this approach, we have investigated here the structure of a synthesized 25 amino acid peptide, Ffh(410–434), shown below which corresponds to one of the proposed Met-rich amphiphilic helix (410–434 region) of Ffh.

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410                 414                 419                 424                 429
V Q D V N R L L K Q F D D M Q R M M K K M K K G G
1                   5                   10                  15                  20                  25
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It is likely that this segment and the other Met-rich segments have similar structural characteristics. The structural study was carried out in water and in aqueous TFE solutions using CD and NMR.

2. Materials and methods

The Ffh(410–434) peptide was synthesized by the solid-phase method on a MilliGen 9050 automated peptide synthesizer. The peptide was purified by reverse phase HPLC using a Phenomenex w-porex C8 column (150 mm×10 mm, 10 μm). Elution was performed with a linear water-acetonitrile gradient containing 0.1% trifluoroacetic acid. The N-terminal sequence and molecular weight of the purified peptide were determined using an ABI protein sequencer and fast atom bombardment mass spectroscopy (JEOL JMS-HX110/110A), respectively.

CD spectra were obtained on a Jasco J-720 spectropolarimeter with a Neslab RTE-210 temperature controller using a 1 mm pathlength cell. The peptide concentration, as determined by quantitative amino acid analysis, was 32.3 ± 2.5 μM. The TFE solutions were prepared by dissolving varying amounts of TFE in phosphate buffer. All CD spectra obtained were the average of five scans and were base-line corrected.

NMR spectra were obtained on a Bruker AMX 500 spectrometer or on a Bruker DMX 600 spectrometer at 25°C. The peptide concentration used was approx. 2.5 mM in water (90% H_2O, 10% D_2O) and 1.5 mM in a mixed solvent of 50% TFE-d_3 (Cambridge Isotope Laboratories) and 50% unbuffered water (by volume). The TOCSY [13] and NOESY [14] spectra were acquired with 512 (t_1)×2048 (t_2) data points and the DQF-COSY [15] spectra were acquired with 512 (t_1)×4096 (t_2) data points. All 2D data sets were collected in the phase-sensitive mode, using the time-proportional phase incrementation (TPPI) method [16], and processed with FELIX2.30 (Biosym, Inc.) on an INDY work station (Silicon Graphics, Inc.). In the TOCSY experiments, an MLEV-17 composite pulse [17] was used for spin locking with a mixing time of 75 ms. NOESY spectra were collected with a mixing time of 100, 150, 200 and 240 ms. The water resonance was suppressed by irradiation of water frequency during the relaxation delay (1.3 s) as well as during the mixing time in the NOESY experiments. The hydrogen-deuterium (H/D) exchange rates of backbone amide protons were measured by 1D and 2D NMR after the dissolution of lyophilized sample into TFE-d_3/D_2O (1:1, v/v) at pH 3. Sequential assignments were achieved by standard procedures [18] using TOCSY, DQF-COSY and NOESY spectra.

3. Results and discussion

Fig. 1 shows CD spectra of Ffh(410–434) peptide in water at pH 7 and at several temperatures. The spectrum at 1°C shows a minimum at 206 nm with a shoulder around 222 nm suggesting the presence of an α-helical structure. The α-helix content decreased with increasing temperature and the isodichroic point observed at 201–202 nm is consistent with helix-random coil interconversion. The spectra obtained with the peptide concentration ranging from 12 to 205 μM in water were the same within the experimental errors, which indicate

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Abbreviations: Ffh, fifty-four homologue; SRP, signal recognition particle; SRP54, 54 kDa subunit of signal recognition particle; CD, circular dichroism; TFE, 2,2,2-trifluroethanol

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no oligomer formation (data not shown). The CD spectra collected at different TFE concentrations showed an isodichroic point near 203 nm which, again, indicates a transition between random coil and α-helical conformations (data not shown). The $[\theta]_{222}$ increased with increasing TFE concentrations, approaching saturation at 30% TFE (by volume).

The estimated α-helix contents from curve fitting [19] the spectra of the Ffh(410–434) peptide in water were 30 ± 4 and 25 ± 1% at 1 and 25°C, respectively. Even at higher temperatures of 50 and 75°C (pH 7), the α-helix content obtained was appreciable at approx. 10% in each case after proper high temperature corrections [20,21]. In TFE/water (1:1, v/v) at 25°C, the estimated α-helix content was 75 ± 10% at pH 7.

The NOESY spectrum in water (Fig. 2) shows that Ffh(410–434) has α-helical conformation in water. Consecutive NH(*i*)/NH(*i*+1) crosspeaks were observed from Asn-5 to Lys-23 as shown in Fig. 2B, the exception being Lys-19/Lys-20 and Met-21/Lys-22 (Table 1). There were a large number of medium-range interactions as shown in Fig. 2A. CαH(*i*)/CβH(*i*+3) and consecutive CβH(*i*)/NH(*i*+1) crosspeaks were also observed (data not shown). CαH(*i*)/NH(*i*+3), CαH(*i*)/CβH(*i*+3) and consecutive NH(*i*)/NH(*i*+1) and CβH(*i*)/NH(*i*+1) crosspeaks are consistent with the presence of an α-helix. A schematic diagram summarizing the various connectivities observed in the NOESY spectrum and the $^3J_{NH\alpha}$ coupling constants is shown in Fig. 4A. Although it is difficult to locate exact N-terminal and C-terminal ends of the helix due to fraying effects [22], the NOE pattern may be interpreted as at least the region between Asn-5 and Met-21 forming α-helical conformation. The chemical shift index [23] shown in Fig. 5 also supports such α-helix stretch.

The NMR experiments in TFE/water (1:1, v/v), which include H/D exchange experiments, were performed at pH 3 because the amide exchange rate with H_2O is very high at pH 7. It was observed that the CD spectra obtained at pH 7 and 3 are identical within the estimated error. The NOESY spectrum shows a network of medium-range NOEs (Fig. 3A) and consecutive NH(*i*)/NH(*i*+1) crosspeaks (Fig. 3B). The

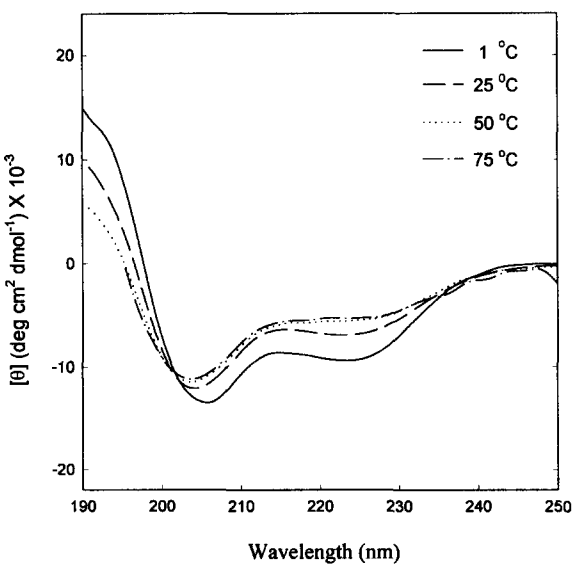

Fig. 1. CD spectra of Ffh(410–434) peptide in 25 mM potassium phosphate buffer (pH 7). (———) 1°C, (— — —) 25°C, (· · ·) 50°C, (– · –), 75°C.

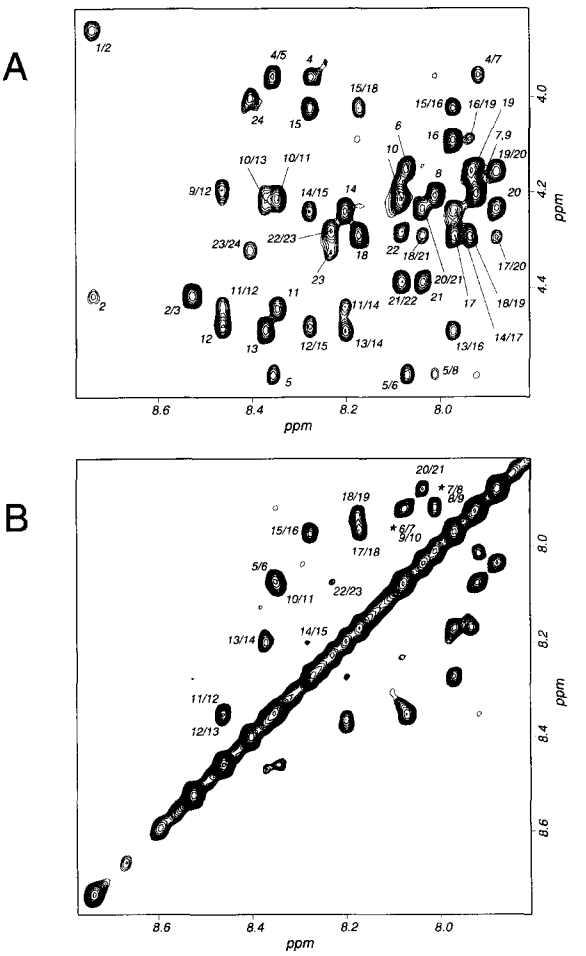

Fig. 2. NOESY spectrum of Ffh(410–434) peptide in water, pH 7 at 25°C. The mixing time used for this spectrum was 200 ms. (A) Fingerprint region. The CαH(*i*)/NH(*i*), CαH(*i*)/NH(*i*+1), CαH(*i*)/NH(*i*+2), CαH(*i*)/NH(*i*+3) and CαH(*i*)/NH(*i*+4) crosspeaks are labeled with the sequence numbers of interacting amino acids. (B) NH-NH region. The crosspeaks between two NHs are marked by their sequence numbers. The symbol (★) represents the possible overlapped peaks. Because the chemical shift of the NH proton of Arg-6 is very close to that of Gln-10 and those of Leu-7 and Lys-9 are identical, it is expected that the crosspeak 6/7 overlaps with crosspeak 9/10 while 7/8 crosspeak is superimposed with the 8/9 crosspeak.

CαH(*i*)/NH(*i*+4) crosspeaks, which were detected only in the stable α-helix, were observed from Asn-5 to Asp-12 and also between Met-21 and Gly-25. All this information suggests that an α-helix spans from Val-4 to the C-terminal end in aqueous TFE solution. Schematic diagrams summarizing these NOE data, the $^3J_{NH\alpha}$ coupling constants and H/D exchange rate are shown in Fig. 4B. The chemical shift index (Fig. 5) and H/D exchange experiments also suggest the existence of stable α-helix.

A large number of short peptides of 5–30 residues were investigated in buffer solutions, TFE solutions and phospholipid vesicle suspensions. For most of these peptides, no appreciable structure was observed in buffer solutions but they form predominantly α-helical structures in TFE solutions [24–26] and in the suspensions containing amphiphilic surfaces [9,10]. In this regard, the Ffh(410–434) peptide is somewhat atypical in forming an appreciable α-helix in the water. Although CD data give 25% of α-helix content in water,

Table 1
Chemical shift values of Ffh(410–434) protons in water and in aqueous TFE solution

Residue	NH	αH	βH	γH	Others
Val-1		3.87 (3.81)	2.24 (2.20)	1.03 (1.02)	
Gln-2	8.74 (8.60)	4.46 (4.42)	2.01, 2.08 (2.11, 2.00)	2.37 (2.37)	
Asp-3	8.52 (8.53)	(4.42)	2.71, 2.83 (3.00, 2.87)		
Val-4	8.27 (8.08)	3.96 (3.87)	2.13 (2.09)	0.95 (0.94, 0.99)	
Asn-5	8.35 (8.05)	4.59 (4.44)	2.84 (2.81)		γNH_2 7.71, 6.91 (7.43, 6.63)
Arg-6	8.07 (7.74)	4.15 (4.02)	1.86 (1.89)	1.62, 1.69 (1.72, 1.66)	δCH_2 3.23 (3.21) εNH 7.28 (7.20)
Leu-7	7.92 (7.61)	4.21 (4.11)	1.70 (1.73, 1.67)	1.59 (1.58)	δCH_3 0.85 (0.85)
Leu-8	8.01 (8.04)	4.21 (4.14)	1.72 (1.79)	1.63 (1.67)	δCH_3 0.85 (0.91)
Lys-9	7.92 (7.84)	4.21 (4.05)	1.88 (1.94)	1.44 (1.45)	δCH2 1.67 (1.71) εCH2 3.01 (3.02) ζNH 7.52
Gln-10	8.08 (7.94)	4.21 (4.11)	2.10 (2.27, 2.21)	2.31, 2.39 (2.47, 2.41)	δNH_2 7.31, 6.78 (6.53, 6.94)
Phe-11	8.34 (8.61)	4.44 (4.39)	3.14, 3.24 (3.26)		ring H 7.25, 7.26, 7.28 (7.25, 7.20)
Asp-12	8.46 (8.84)	4.48 (4.37)	2.91, 2.82 (3.12, 2.93)		
Asp-13	8.37 (8.53)	4.49 (4.42) (3.20, 2.87)	2.87, 2.78		
Met-14	8.20 (8.43)	4.24 (4.16)	2.15 (2.24)	2.53, 2.66 (2.54, 2.72)	
Gln-15	8.28 (8.25)	4.03 (3.93)	2.06 (2.11, 2.07)	2.19, 2.31 (2.24)	δNH_2 7.15, 6.81 (6.26, 6.41)
Arg-16	7.97 (7.99)	4.10 (3.96)	1.91 (1.91, 1.97)	1.77 (1.62)	δCH_2 3.24 (3.20) εNH 7.31 (7.12)
Met-17	7.97 (8.08)	4.28 (4.19)	2.16 (2.21)	2.58, 2.67 (2.65, 2.57)	
Met-18	8.17 (8.41)	4.29 (4.17)	2.09 (2.20)	2.54, 2.63 (2.72, 2.54)	
Lys-19	7.93 (7.92)	4.15 (4.00)	1.86 (1.93)	1.44, 1.53 (1.43)	δCH2 1.70 (1.65) εCH2 3.01 (2.95) ζNH 7.52
Lys-20	7.87 (7.86)	4.23 (4.06)	1.85, 1.90 (1.97)	1.45, 1.52 (1.44)	δCH2 1.70 (1.55), εCH2 2.97 (3.01) ζNH 7.52
Met-21	8.04 (8.15)	4.39 (4.26)	2.09 (2.15, 2.18)	2.58, 2.67 (2.58, 2.71)	
Lys-22	8.08 (8.00)	4.29 (4.29)	1.82, 1.87 (1.92, 1.87)	1.44, 1.47 (1.51, 1.48)	δCH2 1.69 (1.69) εCH2 3.02 (2.99) ζNH 7.52
Lys-23	8.23 (8.00)	4.32 (4.19)	1.88, 1.81 (1.91)	1.45 (1.54, 1.46)	δCH2 1.70 (1.69) εCH2 3.02 (2.99) ζNH 7.52
Gly-24	8.40 (8.15)	4.00 (3.97)			
Gly-25	(8.10)	(3.92)			

The values in parentheses are chemical shifts in water/TFE solution (1:1, v/v). The NMR experiments were performed at 25°C, pH 7 in water and at 25°C, pH 3 in aqueous TFE solution. The chemical shift values are referenced to sodium 3-(trimethylsilyl)propionic acid (0 ppm) in water and are to trifluroethanol methylene resonance (3.88 ppm) in aqueous TFE solution. The estimated errors are within ±0.02 ppm.

NMR experiments suggested the α-helix stretching from Asn-5 to Met-21. This inconsistency may be due to dynamic equilibrium between α-helical structures and random coils.

As of now, we do not have a ready answer for this unusual propensity of secondary structure formation. It is tempting to speculate that charge-charge interactions between Asp-3 and Arg-6 and those of Lys-9 and Arg-16 with either Asp-12, Asp-13 or both may be important. The hydrophobic interactions of the side chains aligned on one surface of the amphiphilic helix may also contribute to the stability of the α-helix.

Although the high propensity of this peptide to form a stable α-helix is in agreement with the proposed targeting [2], it still does not provide any mechanism. Recently, the structures of a number of isolated signal peptides and their mutants in TFE solution were determined and these were compared with their function [9–12]. The general consensus from these studies is that the signal peptides must form a certain minimum length of stable α-helix for efficient translocation of the corresponding precursor proteins. How the α-helix of the Ffh protein segments and the α-helix of the signal peptides may become structurally compatible during targeting remains an important question.

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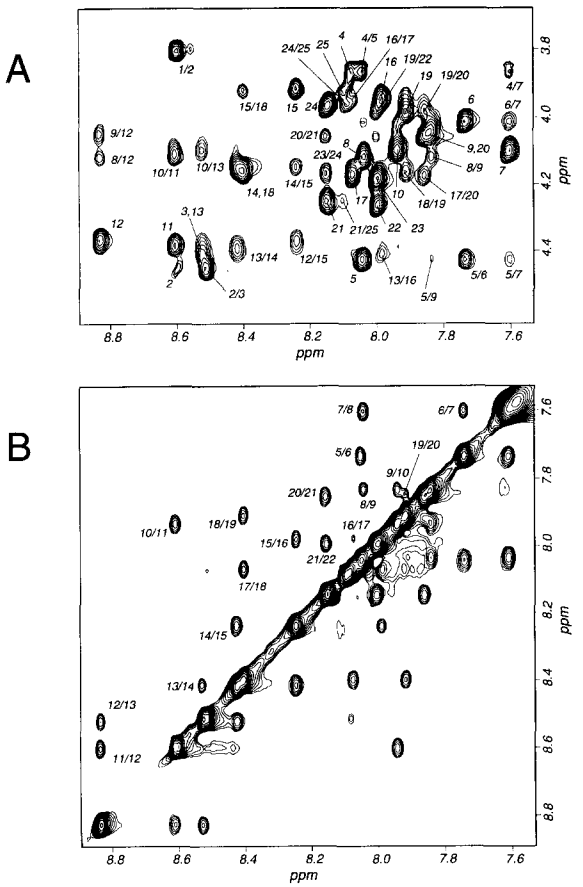

Fig. 3. NOESY spectrum of Ffh(410–434) in TFE/water (1:1, v/v), pH 3 at 25°C. The mixing time used for this spectrum was 200 ms. (A) Fingerprint region. (B) NH-NH region. Notation of crosspeaks as in Fig. 2.

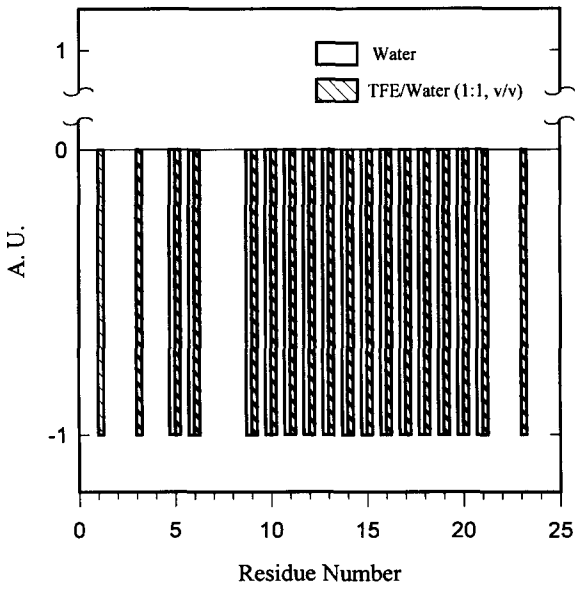

Fig. 5. Chemical shift index of the α-protons of Ffh(410–434). The values were calculated according to the method of Wishart et al. [23]. Open bar and hatched bar represent the chemical shift index in water, pH 7 and in TFE/water (1:1, v/v), pH 3 at 25°C, respectively. No corrections were introduced for pH and solvent differences.

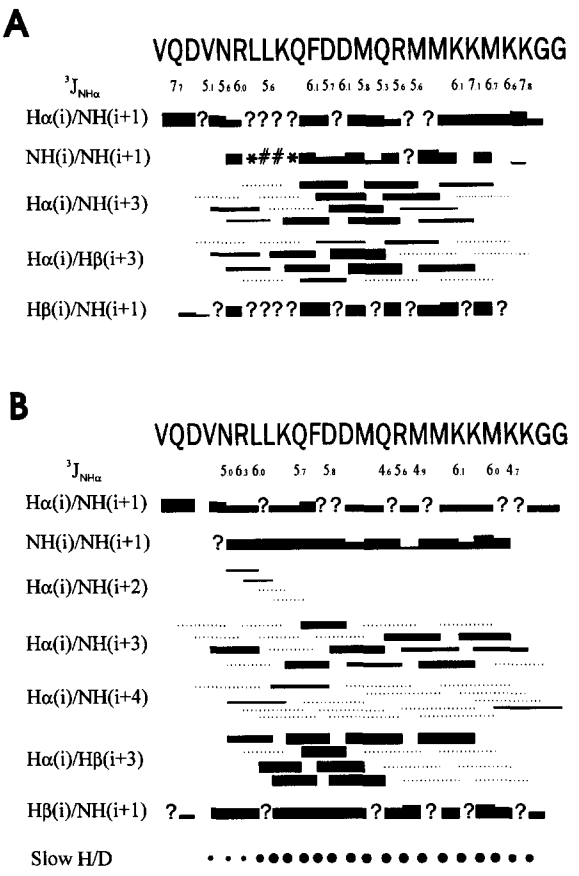

Fig. 4. Schematic representation summarizing the $^3J_{NH\alpha}$ coupling constants, NOE connectivities and slowly exchanging amide protons in water (A) and in TFE/water (1:1, v/v) solution (B). The thickness of the boxes represents the relative intensity of NOEs. The question marks in the sequential NOEs and the dashed lines in the medium NOEs indicate the possible crosspeaks overlapped by the other crosspeaks. The backbone amide protons, which exchange slowly with solvent, are indicated by circles. In (A), * and # represent the crosspeaks which may be overlapped with the other crosspeaks designated by the same notation. Notation * represents a crosspeak which can be assigned to 6/7, 9/10 or both and in the same way, # represents a crosspeak which can be assigned to 7/8, 8/9 or both.

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