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Solution Structure of the Basic Region from the Transcriptional Activator GCN4

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ABSTRACT: The structure of the basic region (i.e., the region responsible for sequence-specific binding to DNA) of the transcriptional activator GCN4 was studied. Two peptide fragments containing either the basic region alone (residues 240–280) or the basic and the dimerization leucine zipper domains (220–280) were synthesized and investigated by nuclear magnetic resonance and circular dichroic spectroscopy. The basic region in the absence of DNA appears as a mobile flexible segment folded into a loose helix. The helical stability increases upon addition of trifluoroethanol and/or lowering of the temperature. Dimerization via the leucine zipper does not affect the three-dimensional structure of the basic region. Possible consequences for the binding to DNA are discussed.

A class of transcriptional activators called the "leucine zipper" family has recently been discovered [Landschulz et al., 1988; for a review see, e.g., Busch and Sassone-Corsi (1990)]. These proteins are active as dimers and contain two functionally distinct regions necessary for DNA binding: One (the leucine zipper domain) is responsible for the formation of the active dimer. The other (basic region) is required for the sequence-specific binding to DNA (Hope & Struhl, 1987).

Considerable evidence, coming from molecular biology (Turner & Tjian, 1989), chemical studies (O'Shea et al., 1989), secondary structure predictions (Busch & Sassone-Corsi, 1990), and circular dichroic spectroscopy (O'Shea et al., 1989), indicates that the conformation of the leucine zippers is an amphiphilic α-helix. It was suggested that the dimerization was based on the hydrophobic interaction of the side chains and the arrangement of the helices was head to head, probably forming a coiled coil (O'Shea et al., 1989). A so-called "scissors-grip" model of the structure has been presented (Vinson et al., 1989), where the leucine zippers bring together the adjacent basic regions such that they can wrap around the DNA in the major groove. According to this model, the basic regions are also folded predominantly in an α-helical conformation which is broken at an asparagine residue.

An interesting representative of this family of transcriptional activators is GCN4, a yeast protein whose sequence was de-

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Table I: Proton Chemical Shifts of BD Peptide in Aqueous Solution at 37 °C and pH 3.7

amino acid	chemical shift (ppm)						
	HN	HA	HB	HG	HD	HE	others
1-Val	nd	4.18	2.31	1.90, 1.00			
		4.09	1.94	0.91			
2-Pro							
trans		4.49	2.33, 2.07	1.98, 2.03	3.77, 3.63		
cis		4.40	2.43, 2.01	2.05	3.88, 3.40		
3-Glu	8.56	4.37	2.12, 2.03	2.53			
4-Ser	8.37	4.43	3.91, 3.82				
5-Ser	8.31	4.48	3.85				
6-Asp							
cis	8.38	4.96	2.96, 2.82				
trans		4.68	2.79				
7-Pro							
cis		4.32	2.37, 1.88	2.06	3.85		
trans		nd	2.43, 1.83	2.21	3.60, 3.69		
8-Ala	8.18	4.18	1.41				
9-Ala	7.87	4.18	1.43				
10-Leu	7.83	4.22	1.70	1.62	0.92–0.88		
11-Lys	8.01	4.18	1.83, 1.67	1.38	1.48	2.99	7.53
12-Arg	8.02	4.24	1.83, 1.81	1.68	3.21		6.68, 7.21
13-Ala	8.16	4.18	1.43				
14-Arg	8.25	4.25	1.85, 1.62	1.64	3.20		6.68, 7.20
15-Asn	8.40	4.70	2.82, 2.92		7.49, 6.85		
16-Thr	8.17	4.32	4.27	1.22			
17-Glu	8.33	4.23	2.09	2.41			
18-Ala	8.23	4.18	1.42				
19-Ala	8.08	4.22	1.42				
20-Arg	8.13	4.22	1.85, 1.73	1.63	3.20		6.68, 7.18
21-Arg	8.23	4.23	1.85, 1.73	1.64	3.20		6.68, 7.20
22-Ser	8.22	4.37	3.93, 3.90				
23-Arg	8.24	4.22	1.85, 1.73	1.63	3.20		6.68, 7.20
24-Ala	8.13	4.20	1.42				
25-Arg[a]	8.29	4.30	1.85, 1.73	1.63	3.20		6.68, 7.20
26-Lys[a]	8.27	4.23	1.85, 1.78	1.42	1.68	2.99	7.50
27-Leu	8.20	4.30	1.72	1.60	0.91, 0.83		
28-Gln	8.37	4.35	2.13, 2.03	2.03, 2.09		6.88, 7.63	
29-Arg[a]	8.18	4.23	1.85, 1.73	1.63	3.20		6.68, 7.20
30-Met	8.31	4.43	2.03, 2.05	2.55, 2.62	2.03		
31-Lys[a]	8.20	4.24	1.85, 1.78	1.42	1.68	2.99	7.50
32-Gln[a]	8.24	4.27	2.02, 2.09	2.38			
33-Cys	8.46	4.47	2.84			6.88, 7.63	
34-Glu[a]	8.42	4.41	2.14, 2.00	2.48			
35-Asp	bleached	4.67	2.93, 2.78				
36-Lys[a]	8.23	4.38	1.85, 1.78	1.42	1.68	3.00	7.53
37-Val	8.15	4.23	2.18	0.94			

[a] The spin system is assigned correctly; the sequential assignment is tentative.

rived from the cloned gene (Hinnebusch et al., 1984; Hope & Struhl, 1987). It is a very suitable material for conformational analysis because it forms an active homodimer (in contrast to most proteins in this family). Initial NMR[1] study of the GCN4 leucine zipper domain (residues 249–282) concentrated on its secondary structure and confirmed that it is indeed a helical peptide, forming a symmetrical parallel dimer (Oas et al., 1990).

We are currently investigating a 61-residue fragment of GCN4 (LZ-BR, residues 220–280) containing both the leucine zipper (248–280) and the basic region (227–247). In our first paper we described its solution structure using NMR spectroscopy (Saudek et al., 1990). Our analysis showed that the functionally distinct leucine zipper and basic regions are also structurally very distinct. The leucine zipper is extremely rigid and conformationally well-defined whereas the basic region is very flexible and mobile.

In the present paper we concentrate on the basic region of GCN4. We synthesized a 37 amino acid peptide corresponding to residues 220–256 in GCN4. It contains the basic region proper, responsible for the DNA binding interactions of the native protein, flanked by 6 and 10 residues at the N- and C-termini, respectively. We henceforth call this peptide BR (the sequence is given in Table I). We describe here its structure as obtained by NMR and CD spectroscopy. We shall compare the conformational behavior in the presence and absence of the leucine zipper and discuss the possible consequences for structural stabilization upon DNA binding.

Materials and Methods

Materials. BR (peptide consisting of residues 220–256 of GCN4 and Leu 257 replaced by Cys, Table I) and LZ-BR (peptide consisting of residues 220–280) were prepared by

[1] Abbreviations: NMR, nuclear magnetic resonance; CD, circular dichroism; LZ-BR, peptide corresponding to residues 220–280 in GCN4, i.e., containing the basic region and the leucine zipper [for sequence, see Saudek et al. (1990)]; BR, peptide corresponding to residues 220–256 in GCN4, i.e., containing the basic region and 6 and 10 flanking residues (sequence in Table I); 2D, two dimensional; COSY, 2D correlated spectroscopy; HOHAHA, 2D homonuclear Hartmann–Hahn spectroscopy; NOE, nuclear Overhauser enhancement; NOESY, NOE 2D spectroscopy; ROESY, rotating-frame NOESY; $^3J_{HN/HA}$, coupling constant of HN and HA resonances; ppm, parts per million; TFE, trifluoroethanol. The Brookhaven database notation is used for the hydrogens of the amino acids; e.g., HA, HB, etc. stand for CHα, CHβ, etc. An index identifies the position in the primary sequence; if no index is specified, all hydrogens belong to one residue.

solid-phase synthesis and purified by HPLC as described previously (Saudek et al., 1990). They were dissolved in H_2O or D_2O, and the pH was adjusted with NaOH or NaOD. The pH values given in D_2O or in aqueous TFE are the uncorrected direct pH meter readings. Unless otherwise stated, the concentration of the sample was 6×10^{-2} mM for the CD measurements and 4 mM for the NMR spectroscopy. The exact concentration of the peptides was determined by quantitative amino acid analysis.

Circular Dichroism. The spectra were measured with an Aviv Model 62D spectropolarimeter using a 1.5-nm bandwidth, 0.5-nm step, and a time constant of 5 s. Circular cells with a 0.183-nm path length were used. The spectrometer was calibrated with (+)-10-camphorsulfonic acid. The estimation of the secondary structure was made with the Aviv program PROSEC and the standard spectra of Chang et al. (1978).

NMR Spectroscopy. Spectra were acquired on a Bruker AM 500-MHz spectrometer equipped with an Aspect 3000 computer and a digital-phase shifter. By use of standard methods (Wüthrich, 1986) a set of 2D spectra was accumulated in the phase-sensitive mode (Redfield & Kunz, 1975; Marion & Wüthrich, 1983) with time proportional phase incrementation: COSY (Marion & Wüthrich, 1983), double quantum filtered COSY (Rance et al., 1983), NOESY (Jeener et al., 1979; Macura et al., 1981), HOHAHA (Bax & Davis, 1985; Rance, 1987), and ROESY (Bothner-By et al., 1984). NOE buildup was followed in the NOESY spectra with mixing times in the range of 100–600 ms. Spin-locking experiments used a radio-frequency field strength between 7 and 8 KHz with mixing times of 18, 36, 100, and 120 ms for HOHAHA and 100 and 200 ms for ROESY. Solvent suppression was achieved by irradiating the H_2O peak during the relaxation delay (1.5 s) and in NOESY experiments during the mixing time as well. Data were accumulated with 16 transients and 4 dummy transients in 2048 data points in the second time domain (t_2) and in 512 in the first one (t_1). For H_2O solutions, the transmitter offset was placed on the H_2O resonance; a spectral width of 5 KHz and an incrementation in t_1 of 100 μs were applied. For D_2O solutions, the transmitter was placed in the middle of the spectrum, and a spectral width of 2.5 KHz and an incrementation in t_1 of 200 μs were applied. Data processing took place off-line on a Bruker station X32, with the manufacturer's program UXNMR. Prior to Fourier transformation, the free induction decays were multiplied with a Gaussian or a shifted sine-bell weighting function and a cosine-bell function in t_2 and t_1, respectively. Zero filling led to the digital resolution of 2.44 Hz/point and 4.88 Hz/point in the second (F_2) and first (F_1) frequency domains, respectively. The base line was fitted with a third-degree polynomial in both frequency dimensions and subtracted from the spectra. The horizontal and vertical axes of the spectra in the figures represent F_2 and F_1, respectively. Further experimental details can be found in our previous paper (Saudek et al., 1990).

Results

Circular Dichroism. Spectra of BR under various conditions are shown in Figures 1 and 2. Such a profile with two minima at about 222 and 208 nm and a maximum at about 193 nm is characteristic for an α-helix. According to a computational fit blending ideal α-helix and random coil spectra of poly(lysine), the amount of the α-helix varies between 15% (aqueous solution at pH 3.2 and 37 °C) and 50% [50% (w/w) aqueous TFE]. The estimates are, of course, only approximate, as we lack more suitable standard spectra. A simulation of the spectra employing more parameters (blending β-sheet, α-helix, β-turn, and random coil spectra) did not provide a

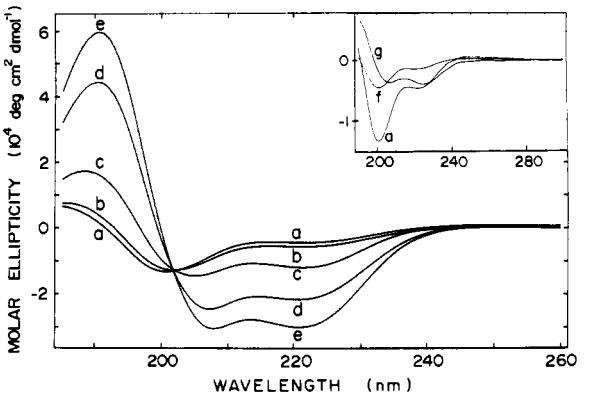

FIGURE 1: CD spectra of BR peptide (concentration 6×10^{-2} mM) at various conditions: (a) 37 °C, pH 3.5; (b) 37 °C, pH 7.0; (c) 5 °C, pH 3.5; (d) TFE/H_2O (1/1 v/v), pH 3.5, 37 °C; (e) as (d) but at 5 °C. (Inset) spectrum (a) compared with the effect of added heparin: (f) BR/heparin (10/1, w/w); (g) BR/heparin (1/1 w/w).

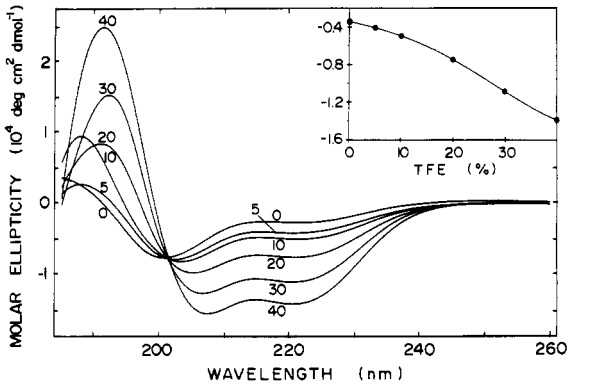

FIGURE 2: CD spectra of BR peptide (concentration 3×10^{-2} mM, pH 3.5, 37 °C) at different concentrations of TFE (the numbers refer to the percentage in v/v). Inset: molar ellipticity at 222 nm as a function of TFE added.

better fit than the two-parameter one. CD investigation of the basic region in the LZ-BR peptide is precluded as its spectrum is overshadowed by the intense spectrum of the helical leucine zipper portion of the molecule.

NMR Spectra. The influence of temperature was followed in the range of 5–37 °C, and pH was varied between 3.5 and 7; the best conditions for the spectral resolution were found to be 37 °C and pH 3.5. The HN hydrogens were almost completely exchanged at pH >4.5 while the aliphatic part did not change significantly within the pH range studied. Lowering the temperature changed the chemical shifts slightly, and although the intensities of some NOEs showed a moderate increase, no new ones appeared. No effect of concentration changes in the range between 1 and 10 mM was observed.

BR as well as LZ-BR provided COSY, HOHAHA, and NOESY spectra of sufficient quality (e.g., Figure 3) to permit a full NMR analysis. However, due to the absence of aromatic residues, the spread of the chemical shifts was significantly smaller than is usually observed with peptides of this size. The HA region is particularly narrow, making the resolution of some NOEs impossible. For the same reasons, some of the HA/HN COSY cross-peaks in the fingerprint region could not be distinguished individually. On the other hand, the amide region provided good resolution of the resonances and was fully explored for the spectrum assignment and NOE delineation. In order to observe the NOEs in the spectra of BR clearly, longer mixing times than in our previous work with LZ-BR or the isolated leucine zipper (50–200 ms; Saudek et al., 1990) were required. However, the increase of the NOE

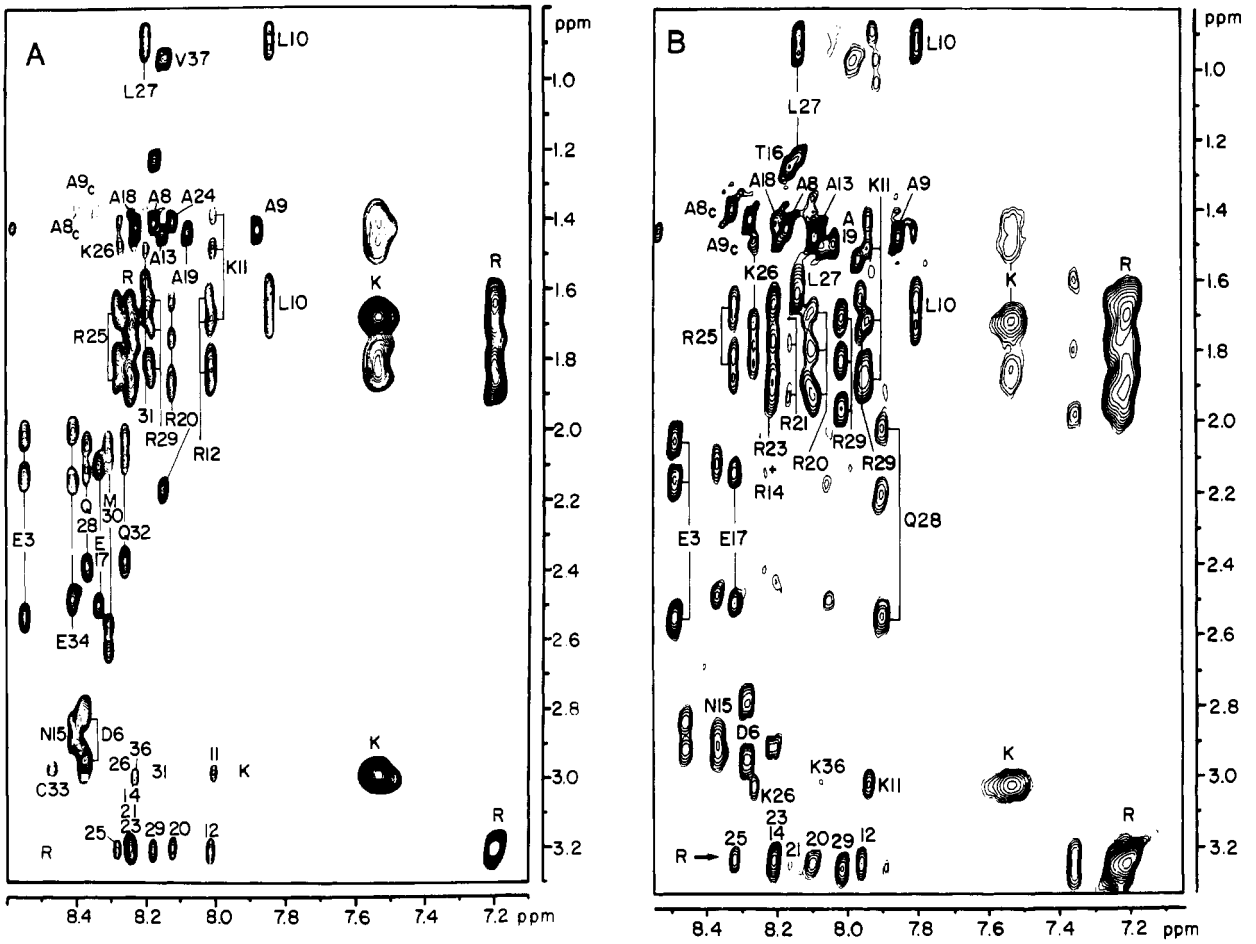

FIGURE 3: Part of the HOHAHA spectra of (A) BR peptide and (B) LZ-BR peptide: pH 3.5; 37 °C; mixing time 120 ms. The peaks belonging to the basic region are labeled according to the assignments in Table II.

intensities was nearly linear within the range between 100 to 500 ms. Mixing times of 200 and 300 ms were used for the assignment and the structure determination. No additional peaks appeared in the ROESY spectra, which were not also detected in the NOESY spectra.

Assignment. The amino acid spin systems of the BR peptide were assigned fully in the HOHAHA and COSY spectra. The AMX systems were identified in the COSY spectrum, whereas the amino acids with the long side chains were discerned in the HN part of the HOHAHA spectrum. Glutamines and glutamic acids were recognized by their HN/HG cross-peaks. Asparagine and glutamines were distinguished from aspartic and glutamic acids by their HB/HD and HG/HE cross-peaks in the NOESY spectra. Arginines and lysines were first identified according to the cross-peaks of the HE or HZ resonance and then were localized by the HD/HN or HE/HN cross-peaks, respectively. Leucine spin systems gave strong HN/HD and HA/HD cross-peaks. The two prolines provided four independent spin systems discernible in the aliphatic region of the spectra. The NOESY spectra then revealed that this corresponded to the cis and trans conformations of Pro 2 and Pro 7, as they both provided HD_{i-1}/HA_i or HA_{i-1}/HA_i connectivities to their respective preceding amino acid in the sequence. This cis/trans isomerism complicated the spectra somewhat because some resonances of the residues even sequentially remote from the prolines yielded several cross-peaks (the effect was most clearly observed on strong CH_3 HB resonances of alanines; see Figure 3).

Residues 1–8 were assigned sequentially via their sequential HA/HN (or HA/HD for prolines) NOEs. The following residues, up to Ala 24, could then be determined in the NH

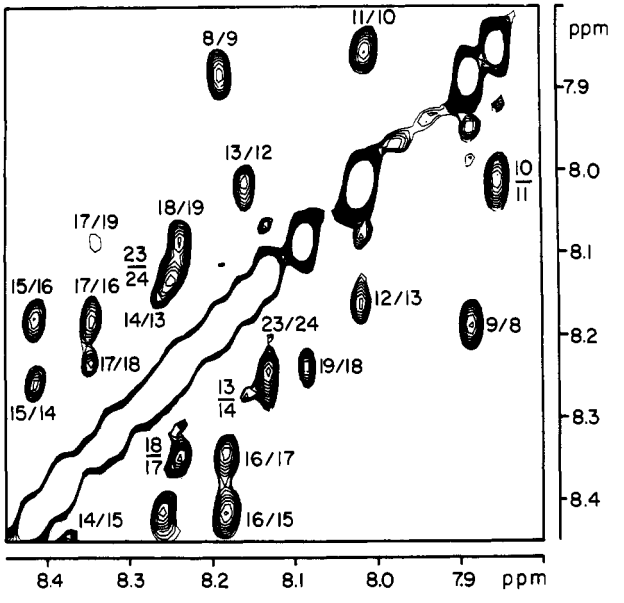

FIGURE 4: Diagonal part of the NOESY spectrum of BR peptide showing the sequential connectivities between the main-chain amides: mixing time 200 ms; other conditions as in Figure 3. The numbers refer to the residues involved.

diagonal region through NH_i/NH_{i+1} connectivities (Figure 4). Residues 24–27 provided no interresidue NOEs.

The assignment of both LZ-BR and BR peptides is summarized in Tables I and II. The sequential assignment of seven nonunique residues in the region 25–36 of BR remains tentative due to the lack of an interresidue NOEs. This un-

Table II: Proton Chemical Shifts of LZ-BD Peptide in Aqueous Solution at 37 °C and pH 3.1

amino acid	chemical shift (ppm)						
	HN	HA	HB	HG	HD	HE	others
1-Val							
trans	nd	4.20	2.33	1.13, 1.03			
cis	nd	4.11	2.52	0.97			
2-Pro							
trans		4.52	2.33, 2.00	2.08, 1.98	3.77, 3.63		
cis		4.42	2.47, 2.08	2.18	3.41, 3.45		
3-Glu	8.47	4.38	2.15, 2.03	2.53			
4-Ser	8.29	4.45	3.85, 3.83				
5-Ser	8.26	4.48	3.93, 3.85				
6-Asp							
trans	8.25	4.93	2.92, 2.77				
cis	nd	4.72	3.07, 2.63				
7-Pro							
trans		4.33	2.37, 2.01	2.08	3.87		
cis		nd	2.43, 1.82	2.22	3.58, 3.68		
8-Ala	8.15	4.20	1.42				
9-Ala	7.83	4.18	1.43				
10-Leu	7.77	4.23	1.71	1.63	0.90, 7.50		
11-Lys	7.82	4.10	1.82, 1.65	1.41	1.48	2.99	7.50
12-Arg	7.84	4.25	1.85, 1.62	1.63	3.22		7.18
13-Ala	8.06	4.17	1.46				
14-Arg	8.18	4.25	1.83, 1.62	1.64	3.21		7.17
15-Asn	8.34	4.75	2.88, 2.92		7.42, 6.79		
16-Trh	8.13	4.37	4.25	1.27			
17-Glu	8.29	4.22	2.11	2.50			
18-Ala	8.17	4.17	1.46				
20-Arg	8.07	4.22	1.80, 1.78	1.65	3.22		7.30
21-Arg	8.18	4.25	1.83, 1.74	1.64	3.22		7.30
22-Ser	8.14	4.35	3.89, 3.97				
23-Arg	8.18	4.25	1.83, 1.74	1.64	3.21		7.30
24-Ala	8.08	4.19	1.42				
25-Arg	8.29	4.45	1.84, 1.78	1.66	3.21		7.30
26-Lys	8.24	4.17	1.81, 1.77	1.46	1.66	3.00	7.40
27-Leu	8.11	4.40	1.60	1.23	0.90, 0.85		
28-Gln	7.85	4.43	2.52	2.17, 2.98		7.42, 6.78	
29-Arg	7.98	4.23	1.75, 1.80	1.68	3.24		7.30
30-Met	8.37	4.05	2.35, 2.36	2.58, 2.53	2.52, 2.58	2.07	
31-Lys	nd	4.07	1.98	1.55	1.69	3.03	
32-Gln	7.98	4.11	2.15, 2.28	2.54, 2.42		7.42, 6.78	7.42, 6.78
33-Leu	8.22	4.18	1.82, 1.77	1.40			
34-Glu	8.42	3.96	2.28, 2.12	2.65, 2.43			
35-Asp	8.46	4.45	3.03, 2.88				
36-Lys	8.05	4.18	2.07	1.57	1.59	2.95	
37-Val	8.37	3.43	2.30	1.05, 0.88			

certainty in the assignment does not however impair the following discussion.

Discussion

Comparison of the Basic Region in BR and LZ-BR Peptides. The peptide containing both the basic and the leucine zipper domains, LZ-BR, has been studied and its spectrum assigned in our previous paper (Saudek et al., 1990). We observed a striking difference between its NOESY and HOHAHA spectra. Whereas the NOESY spectra contained mainly resonances of the leucine zipper part, the HOHAHA spectra reflected above all the basic region of the peptide. This can now be confirmed by comparing the HOHAHA spectra of the basic region with and without the attached leucine zipper (peptides BR and LZ-BR; Figure 3). The spectra of the free basic region and basic region bound to the leucine zipper domain are indeed very similar. The comparison of the assignment of both peptides (Tables I and II) shows only minor differences in the chemical shift for all the resonances of residues 1–26. (This fact helped us to improve our previous assignment of LZ-BR.) The divergence in the chemical shifts starts only from Leu 27 onward, the region which would be a part of the leucine zipper in the native protein. The near identity of the chemical shifts of residues 1–26 in the two peptides is a strong indication that the basic region assumes nearly identical three-dimensional structure irrespective of the presence or absence of the neighboring leucine zipper domain. Also, the dimerization does not alter the three-dimensional structure of the basic region. In agreement, domain swapping experiments have shown that the biological activity of the DNA binding region is independent of the leucine zipper domain (Agre et al., 1989).

Secondary Structure. The basic region is obviously a very mobile region lacking any rigid, well-defined secondary structure such as is observed in well-packed globular proteins. However it is clearly not entirely in a random-coil conformation. This is indicated by CD spectra and by several NMR parameters, namely, the NOEs, coupling constants, and secondary chemical shift (i.e., the difference between the chemical shift of a residue in a random coil and the measured value).

CD studies show the presence of some helical conformation (Figures 1 and 2). Its extent and/or stability depends on various conditions. The helicity is only slightly influenced by pH in the range of 3–10, whereas the effect of temperature is more pronounced. A well-known helix promoter, trifluoroethanol (TFE), raises the amount of helix significantly. Addition of the strong polyanion heparin changes the spectra drastically.

The observation of strong sequential HN/HN NOEs for residues 7–21 (Figure 4) indicates that this fraction of the

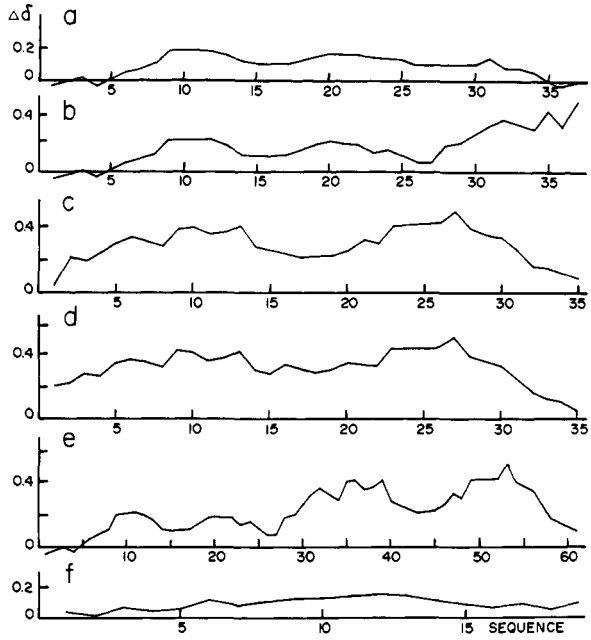

FIGURE 5: Secondary chemical shift ($\Delta\delta$) profiles of basic region in different environments compared to those of some other helical peptides: (a) BR peptide; (b) basic region of LZ-BR peptide; (c) leucine zipper peptide [peptide consisting of residues 248–280 of GCN4; see Saudek et al. (1990)]; (d) leucine zipper part of LZ-BR; (e) LZ-BR; (f) C-helix of myohemerythrin (Dyson et al., 1988). $\Delta\delta$ is defined as the difference between the chemical shift of HA of a particular residue in the sample and in a random coil conformation; the values for three consecutive residues are averaged (Pastore & Saudek, 1990).

peptide is folded into a regularly repeated turning conformation, bringing the HN hydrogens in this sequence to a close spatial proximity. Such an arrangement is typical for an helix. The spectra contain also some other typical helical NOEs, e.g., HN_{17}/HN_{19} or HA_{17}/HB_{19}, but in general most of them are difficult to detect because they are either weak (HN region) or in the regions with a very narrow spread of the chemical shift (aliphatics).

All the HN/HA COSY cross-peaks which are sufficiently resolved to allow the estimation of the apparent coupling constant $^3J_{HA/HN}$ (i.e., residues 9, 10, 11, 15, 17, 18, and 30) are noticeably smaller ($\leq$5 Hz) than those at the beginning of the sequence ($\approx$9 Hz). A small $^3J_{HA/HN}$ is indicative of a small dihedral angle Φ as expected for a helix (Pardi et al., 1984; Wüthrich, 1986).

It has been shown that the secondary chemical shift of the HA resonance is sensitive to the dihedral angle Ψ and consequently to the secondary structure (Clayden & Williams, 1982). A positive value is indicative of a helical conformation (Szilagyi & Jardetzky, 1989). The effect is especially clear if an averaging of the values over several residues is applied (Pastore & Saudek, 1990). Figure 5 shows such a secondary shift profile for the basic region (free, as well as bound, to the leucine zipper). For comparison the profiles of the GCN4 leucine zipper domain and the C-terminal peptide from myohemerythrin (Dyson et al., 1988) are shown. The first is an example of a peptide which is essentially 100% helical; the second, a peptide which assumes a helical conformation in the native protein but is almost completely unfolded as an isolated peptide. Figure 5 reflects the gross helical content of the peptides. According to these plots the helicity of the basic region is lower than that in the well-structured leucine zipper but significantly higher than that in a loosely folded peptide. Note that the profiles of the free basic region and that bound to the leucine zipper are nearly identical.

The C-terminal segment (from residue 22 on) did not provide any interresidue NOEs, and the COSY HA/HN cross-peaks are all crowded into a narrow, mainly unresolved area. Nevertheless, the only measurable coupling constant ($^3J_{HA/HN}$ for Met 30) is smaller than 5 Hz, the secondary chemical shifts are negative, and the HA profile (Figure 5) is shifted slightly upfield.

The conformation of the residues between the two prolines toward the N-terminus is clearly irregular. Strong sequential HA_i/HN_{i+1} and the coupling constant $^3J_{HA/HN} \approx 9$ HZ are typical for an extended conformation (Pardi et al., 1984; Wüthrich, 1986). The presence of both cis and trans forms of both prolines reveals that these residues are not fixed in any stable secondary structure. Furthermore, the secondary chemical shift of residues 1–7 approaches zero.

Average Three-Dimensional Structure of the Basic Region. The analysis of the secondary structure showed that although the basic region is a mobile segment, it exhibits a pronounced helical tendency. Its conformational behavior is independent of the neighboring leucine zipper. Addition of TFE reveals that some other conditions can exist under which the helical conformation is much more stable. Its N-terminal segment is disordered. While the amino acids at the C-terminus (22–37) form a very stable leucine zipper helix in LZ-BR, the helix is almost completely destroyed in BR by truncation at position 37, although some residual helical tendency may remain.

The average structure of a loosely folded peptide corresponding to the C-helix in myohemerythrin has been studied in detail by CD and NMR spectroscopy by Dyson et al. (1988). It has been shown that there is a conformational ensemble of rapidly interconverting turn-like structures in the isolated peptide in solution. The structure, named *nascent helix*, can be stabilized by addition of TFE. In the native protein the same sequence assumes a well-defined helix. Applying the same criteria as Dyson et al. (1988), we can define the average structure of the basic region as a nascent helix as well. We would add as an additional criterion the secondary shift of the HA resonances (Figure 5). The helicity of the basic region as compared to the myohemerythrin C-helix is distinctly more pronounced. It can be detected, for instance, even at 37 °C (as compared to 5 °C) and converted with the TFE to much higher values. The secondary HA chemical shift is also more distinct.

The stability equilibria of a helix may be described by Zimm–Bragg (1959) theory according to which short helices are unstable because the initiation of a helix is thermodynamically unfavorble (nucleation parameter σ_a). Helix formation is promoted by certain amino acids (propagation parameter τ_α), and due to its cooperativity the effect becomes significant only for long peptides. The theory does not take into account specific side-chain interactions along the helix which could further contribute to the stability [e.g., Maxfield and Scheraga (1975) and Finkelstein and Ptitsyn (1976)] but in general predicts correctly that the equilibrium in short isolated helices is shifted toward the unfolded state (Finkelstein et al., 1990). Helices are largely stabilized in globular proteins where they pack with the rest of the molecule (Chothia, 1981).

Considering its small size, the basic region in isolation forms a rather well-pronounced helix. Its helicity is higher than that in the isolated C-helix of myohemerythrin (Dyson et al., 1988) and comparable with that of C-peptides of RNase, which have been found to be exceptionally helical (Shoemaker et al., 1987), or with the polymers of so-called "helix-making" amino acids (Sueki et al., 1984). Many other helical peptides of a

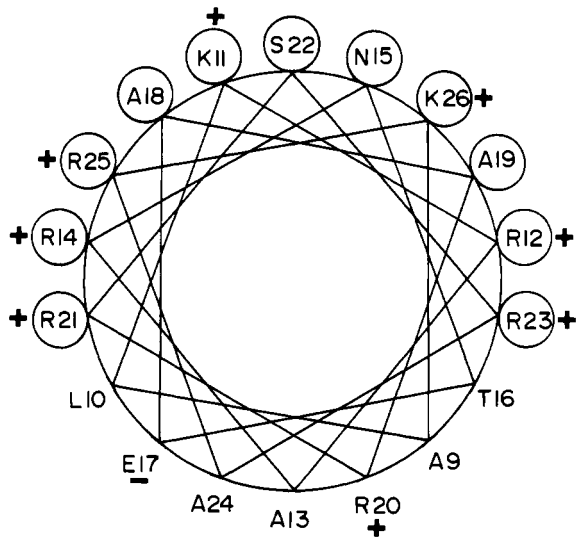

FIGURE 6: Schematic helical wheel of the basic region of GCN4 folded in an idealized helix. Encircled residues are conserved in the following homologues: VAP-1, C/ERB, CREB, CRE-BP1, c-Jun, JunB, JunD, FosB, Fra-1, c-Fos [reviewed by Busch and Sassone-Corsi (1990)]. (+) and (−) indicate the charged amino acids.

size similar to that of the basic region do not form any detectable amount of helix in water, e.g., calcitonin (Moe & Kaiser, 1985; Meyer et al., 1989), melittin (Bazzo et al., 1988), and β-endorphin (Taylor & Kaiser, 1986). On the other hand, small helical peptides whose structures are stabilized by packing of the side chains at the interphase of a dimer, e.g., neuropeptide Y (Minakata et al., 1989; Saudek et al., 1989), leucine zipper peptides (Saudek et al., 1990), and ROP protein (Eberle et al., 1990), fold into well-structured helices. Their conformations are stable in aqueous solution and detectable by NMR spectroscopy, giving a wealth of measurable intramolecular constraints.

We can speculate that this degree of stability could be achieved when the basic region binds to DNA, similarly as when helices in proteins pack with other secondary structural units. An idealized helical wheel of such a stabilized helix is shown in Figure 6. We observe that the conserved and variable residues separate on opposite sides of the helix. Moreover, all but one of the positively charged residues are located on the conserved side. The conserved side of the helix is very likely the region interacting with the DNA. The clustering of the positive charges must be unfavorable for the helix formation. This effect will be suppressed upon binding to DNA, which almost certainly utilizes ionic interactions of the arginines and lysines with the phosphates. Indeed, we observed a radical change toward greater helicity in the CD spectra of the basic region in the presence of heparin, a strong polyanion (inset of Figure 1).

Conclusions

The structure of the basic region of GCN4 in the absence of the DNA may be regarded as nascent helix in the sense of Dyson et al. (1988). Our data are in broad agreement with the scissors-grip model of Vinson et al. (1989), which predicts conformational flexibility for the engagement and disengagement of the basic region from the DNA cognate sequence. However, the break in the basic region helicity at Asn 15 in the model of Vinson et al. is not supported by our data since the sequential HN connectivities are observed to include this residue. Indeed, so far we have not observed any breaks in the helical structure of the basic region under our experimental conditions. Future studies should now concentrate on the binding interaction with DNA.

Added in Proof

Recent studies with fragments of leucine zipper proteins indicate that the basic domain indeed stabilizes upon binding to DNA in a helical conformation [e.g., Weiss et al. (1990) and Talanian et al. (1990)].

Acknowledgments

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Registry No. GCN4 residues 220–256, 131152-49-3.

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Tryptophan Fluorescence in Electron-Transfer Flavoprotein:Ubiquinone Oxidoreductase: Fluorescence Quenching by a Brominated Pseudosubstrate†

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ABSTRACT: We have studied the intrinsic fluorescence of the 12 tryptophan residues of electron-transfer flavoprotein:ubiquinone oxidoreductase (ETF:QO). The fluorescence emission spectrum (λ_{ex} 295 nm) showed that the fluorescence is due to the tryptophan residues and that the contribution of the 22 tyrosine residues is minor. The emission maximum (λ_m 334 nm) and the bandwidth ($\Delta\lambda_{1/2}$ 56 nm) suggest that the tryptophans lie in hydrophobic environments in the oxidized protein. Further, these tryptophans are inaccessible to a range of ionic and nonionic collisional quenching agents, indicating that they are buried in the protein. Enzymatic or chemical reduction of ETF:QO results in a 5% increase in fluorescence with no change of λ_m or $\Delta\lambda_{1/2}$. This change is reversible upon reoxidation and is likely to reflect a conformational change in the protein. The ubiquinone analogue $Q_0(CH_2)_{10}Br$, a pseudosubstrate of ETF:QO (K_m = 2.6 μM; k_{cat} = 210 s^{-1}), specifically quenches the fluorescence of one tryptophan residue (K_d = 1.6–3.2 μM) in equilibrium fluorescence titrations. The ubiquinone homologue UQ-2 (K_m = 2 μM; k_{cat} = 162 s^{-1}) and the analogue $Q_0(CH_2)_{10}OH$ (K_m = 2 μM; k_{cat} = 132 s^{-1}) do not quench tryptophan fluorescence; thus the brominated analogue acts as a static heavy atom quencher. We also describe a rapid purification for ETF:QO based on extraction of liver submitochondrial particles with Triton X-100 and three chromatographic steps, which results in yields 3 times higher than previously published methods.

Electron-transfer flavoprotein:ubiquinone oxidoreductase (ETF:QO) is an iron–sulfur flavoprotein located on the inner face of the inner mitochondrial membrane. This integral membrane protein catalyzes the oxidation of electron-transfer flavoprotein (ETF)[1] and the reduction of ubiquinone. It thus functions with ETF as an intermediate electron carrier between eight primary flavoprotein dehydrogenases and the ubiquinone pool (Frerman 1987). These primary dehydrogenases are the three acyl-CoA dehydrogenases of β-oxidation, isovaleryl-CoA dehydrogenase, 2-methylbutyryl-CoA dehydrogenase, glutaryl-CoA dehydrogenase, sarcosine dehydrogenase, and dimethylglycine dehydrogenase (Frerman, 1988).

ETF:QO contains a single noncovalently bound molecule of FAD together with a $[4Fe\text{-}4S]^{1+,2+}$ cluster (Ruzicka & Beinert, 1977; Beckmann & Frerman, 1985a) and can exist in four oxidation states. Enzymatic reduction yields a two-

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[1] Abbreviations: deazaflavin, 10-*N*-methyl-3-*N*-(sulfopropyl)-5-deazaflavin; ETF_{ox}, electron-transfer flavoprotein (oxidized); ETF_{1e^-}, electron-transfer flavoprotein (one electron reduced); ETF_{2e^-}, electron-transfer flavoprotein (two electron reduced); Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; MCAD, medium-chain acyl-CoA dehydrogenase; Q_0, 2,3-dimethoxy-5-methyl-1,4-benzoquinone; $Q_0(CH_2)_{10}Br$, 6-(10-bromodecyl)ubiquinone; $Q_0(CH_2)_{10}OH$, 6-(10-hydroxydecyl)ubiquinone; $Q_0(CH_2)_7H$, 6-heptylubiquinone; $Q_0(CH_2)_9H$, 6-nonylubiquinone; UQ-1, ubiquinone 1; UQ-2, ubiquinone 2.

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