Volume 17 Number 10 1989 Nucleic Acids Research

Ribosomal protein L7/L12 has a helix-turn-helix motif similar to that found in DNA-binding regulatory proteins

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Received January 23, 1989; Revised and Accepted April 17, 1989

ABSTRACT

Inspection of the structure of the C-terminal domain of ribosomal protein L7/L12 (1) reveals a helix-turn-helix motif similar to the one found in many DNA-binding regulatory proteins (2−5). The 19 α-carbon atoms of the L7/L12 α-helices superimpose on the DNA binding helices of CAP and cro with root-mean-square distances between corresponding alpha carbons of 1.45 and 1.55 Å, respectively. These helices in L7/L12 are within a patch of highly conserved residues on the surface of L7/L12 whose role is as yet uncertain. We raise the possibility that they may constitute a binding site for nucleic acids, most probably RNA. Consistent with this hypothesis are calculations of the electrostatic charge potential surrounding the protein, which show a region of positive potential centered on the first of these helices.

INTRODUCTION

Ribosomal protein L7/L12 is present in the *E. coli* ribosome in four copies and is generally thought to form the long stalk protruding from the large subunit. This protein is a dimer in solution and is attached to the 23S RNA via protein L10, with which it forms a pentameric complex consisting of 4 copies of L7/L12 and one of L10 (for reviews, see 6 & 7).

L7/L12 consists of two domains: An N-terminal domain which is necessary for binding to L10 and for dimerization and a C-terminal domain which is important in the binding of elongation factors Tu and G, and in factor-dependent GTP hydrolysis (8,9). Analysis of mutant proteins has also shown that L7/L12 plays a role in the control of translational accuracy (10,11).

NMR linewidth measurements have shown that the C-terminal region of L7/L12 has a mobility much higher than that of the rest of the ribosome (12). This supports models of the protein that place the N-terminal domain bound to the rest of the ribosome via L10 with the C-terminal region connected by a flexible hinge region (6). While no direct interaction between L7/L12 and RNA has been reported, transient interactions or interactions dependent on the presence of other factors may be difficult to detect.

The structure of the C-terminal region of L7/L12 has been determined at 1.7 Å resolution by Leijonmark and Liljas (1). On examining this structure, we noticed that residues 69−87 form a helix-turn-helix motif strikingly similar to those found in many DNA-binding regulatory proteins.

The helix-turn-helix motif consists of two approximately perpendicular helices connected by a distinctive short turn first recognized from a comparison of the structures of CAP and cro (2). It has been seen in the crystal structures of CAP(13,14),

λ cI repressor (15), λ cro (16,17), 434 repressor (18), 434 cro (19), and trp repressor (20,21), and in the NMR structure of the lac repressor headpiece (22). The degree of similarity among the bihelical motifs in these structures is striking: for example, the α-carbons of the bihelical motif of trp repressor can be fit to those of cro, CAP and λ cI repressor with rms distances between corresponding atoms of 0.68 Å, 0.98 Å, and 0.96 Å, respectively (21). Many other proteins, such as the DNA invertases, the resolvases, and the homeobox proteins, contain sequences homologous to these and are expected to contain a similar helix-turn-helix structure (23,24).

When these peptides bind B-form DNA, the amino end of the second helix penetrates into the major groove while the first helix lies across the groove. Positively charged and other amino acid side chains contact phosphates on both sides of the major groove, while side chains, primarily from the second helix, generally make specific base contacts within the major groove (25).

MATERIALS AND METHODS

The coordinates of the α-carbon atoms used for comparisons were available either in the protein data bank or in the literature. They were: CAP (Brookhaven protein data bank code 2GAP) (14), cro (16), L7/L12 C-terminal fragment (1CTF) (1), hen egg white lysozyme (6LYZ)(26), crambin (1CRN) (27), glutathione reductase (2GRS)(28), and myoglobin (2MBN)(29). Lysozyme was included here because a section of it was found to be the closest structural match to the CAP and cro helices when Steitz *et al.* (2) searched the protein data bank for such fits in 1982. Crambin, glutathione reductase and myoglobin were also examined because they were included, along with the more usual helix-turn-helix structures above and L7/L12, as part of a class of proteins containing α-α corners with short connections in Efimov's study of α-α supersecondary structures (30).

The following residues were chosen by visual examination as corresponding to the 19 residues of the helix-turn-helix motif of L7/L12 (residues 69−87): CAP A169−A187, cro 16−32, lysozyme 14−32, crambin 12−30, myoglobin 90−108, and glutathione reductase 446−464. Minimum rms distances between corresponding α-carbons were calculated using a least-squares fitting algorithm. (31)

The electrostatic potential surrounding the L7/L12 C-terminal fragment was calculated by J. Warwicker (32,33).

RESULTS

The α-carbon atoms of the 19 residues that constitute the 2 helix motif in L7/L12 (residues 69−87) superimpose on the 2 helix motifs of CAP and of cro with root-mean-square distances between corresponding α-carbons of 1.45 and 1.55 Å, respectively (Figure 1). These three peptides fit on one another much better than they do on any of the other four peptides included in this study, which were chosen to be the most likely to match well to a helix-turn-helix motif (Table 1). The next best fit to the 2 helix motif of L7/L12 are residues in crambin with an rms difference of 2.20 Å.

Calculation of the electrostatic potential surrounding the L7/L12 protein shows that while most of the protein is electrostatically negative, there is a prominent region of positive electrostatic potential centered on the first of these two helices (Figure 2).

Surprisingly, while some of the general features of the sequence fingerprint of the helix-turn-helix motif are present in the L7/L12 peptide (the glycine at the beginning of the

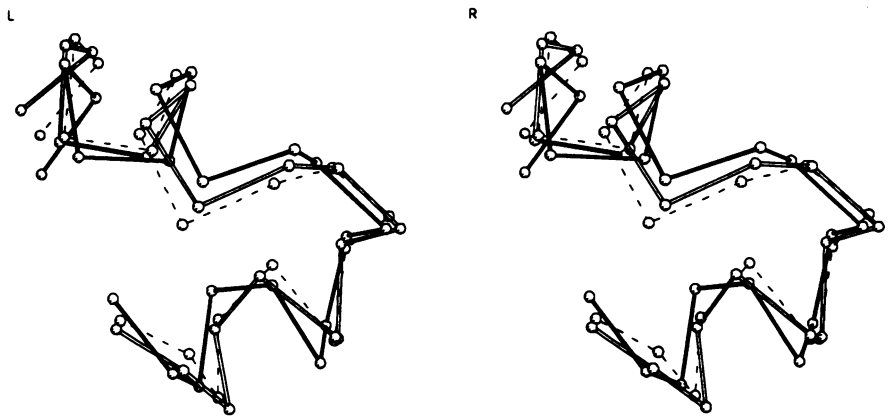

Figure 1. Stereo pair showing the α-carbons of the 2 helix motifs of CAP, cro and L7/L12 superimposed on one another. The CAP helices are drawn with dashed bonds, cro with open lines and L7/L12 with solid lines.

turn and a group of interacting hydrophobic side chains) (3,4,5,21), no statistically significant sequence similarity between L7/L12 and the sequences of other proteins known to contain helix-turn-helix motifs could be identified.

DISCUSSION

The possible significance of the helix-turn-helix motif in protein L7/L12 is supported both by the degree of its structural similarity to the 2-helix DNA binding motifs of CAP and cro and by its positive electrostatic potential. In 1982, as a test of the significance of the

Table 1. Root-mean-square distances (in Å) between corresponding α-carbons of the 2-helix motifs of CAP, cro and L7/L12, and similar peptides from lysozyme, glutathione reductase, myoglobin and crambin.

	CAP	cro	L7/L12	lysozyme	glutathione reductase	myoglobin	crambin
CAP		0.74	1.45	2.57	3.07	2.95	2.62
cro			1.55	2.29	2.89	2.72	2.70
L7/L12				2.98	2.87	3.05	2.20
lysozyme					3.43	2.86	3.53
glutathione reductase						3.02	2.29
myoglobin							4.04
crambin							

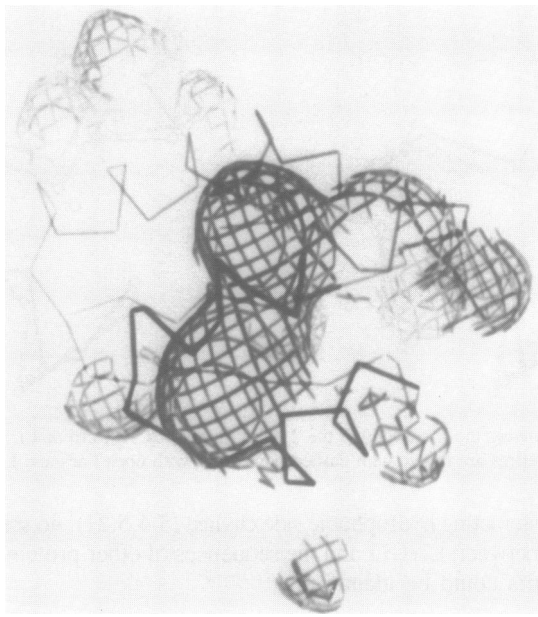

Figure 2. Electrostatic potential surrounding the C-terminal fragment of L7/L12. Only the positive electrostatic contours are shown. Residues 69–87, which form a helix-turn-helix structure, are highlighted and account for most of the positive electrostatic potential around the molecule.

fit between the two helix motifs of CAP and cro, Steitz *et al.* (2) undertook a systematic search of the protein data bank using as a template the 24 residues comprising the cro two helix motif. The best fit found (after CAP) was a section of lysozyme, with an rms Cα-Cα distance of 2.8 Å (2). If the 19 residues of cro corresponding to the L7/L12 helices are fit to this piece of lysozyme, the rms Cα-Cα distance is 2.3 Å, still significantly larger than the fit to L7/L12, which was 1.55 Å. Furthermore, according to the empirical structure agreement plot of Remington & Matthews (34), an α-carbon fit of 1.5 Å between two 19-residue peptides is about 3σ above the average expected distance. Calculation of the electrostatic potential surrounding the protein shows that while the protein is predominantly negative, there is a prominent region of positive potential centered on the first of these two helices (Figure 2). This is as would be expected if the protein were to bind some highly negatively charged ligand, such as DNA or RNA. In fact, a similar region of positive potential over the two-helix motif was seen in CAP (35).

While the role of this region of L7/L12 is as yet undetermined, it is centered in a patch of residues on the surface of the protein which are highly conserved across several species, implying that this region is responsible for some important function of the protein (36). In the crystal structure this region of the protein is involved in making dimer contacts, but the relationship of the crystal dimer to the solution dimer and/or tetramer are uncertain.

The helix-turn-helix motifs of CAP, cro, and the other repressor proteins of known structure are responsible for a large part of the sequence specific DNA binding of these regulatory proteins. The amino end of the second helix penetrates into the major groove and the first helix lies across the groove making interactions with the sugar-phosphate backbone (25). By analogy one is led to wonder whether L7/L12 uses its bihelical motif

in a similar manner to interact with nucleic acid.

This motif has only been known so far to bind duplex B-form DNA. Although interaction between L7/L12 and duplex DNA has not been ruled out, it seems much more likely that this protein might interact with RNA. The B conformation is in general unavailable to RNA, however, this same motif might prove equally useful in recognizing the edges of base pairs exposed in the shallow or minor groove of A-form RNA. While the major (deep) groove of RNA is too narrow to accommodate an α-helix, the minor (shallow) groove can do so in a manner that is similar to the fitting of an α-helix into the major groove of B-DNA. The crystal structure of a complex between $tRNA^{Gln}$ and its cognate synthetase shows an α-helix penetrating into the minor groove of the acceptor stem and making a sequence specific interaction with the exposed edges of base pairs (37).

ACKNOWLEDGEMENTS

We thank Dr. James Warwicker for electrostatic calculations. This work was supported in part by NIH grant GM-22778 to T.A.S. and a NSF fellowship to P.A.R. A preliminary presentation of this work was given at the RNA-Protein Interaction workshop in Urbino, Italy in June, 1987.

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