STRUCTURAL FEATURES OF THE GDP BINDING SITE OF ELONGATION FACTOR Tu FROM *ESCHERICHIA COLI* AS DETERMINED BY X-RAY DIFFRACTION

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1. Introduction

Guanine nucleotides play a crucial role in the control of binding of aminoacyl-tRNA (aa-tRNA) to elongation factor-Tu (EF-Tu), and positioning of aa-tRNA in the ribosomal A-site. When guanosine triphosphate (GTP) is bound as cofactor to EF-Tu, this binary complex will form a ternary complex by combining with aa-tRNA. By subsequent interaction with the ribosome and programmed by mRNA, the aa-tRNA is positioned in the A-site with concomitant hydrolysis of the GTP to GDP and the EF-Tu is hence released from the ribosome in the form of the binary complex EF-Tu:GDP (review [1]).

To understand and explain these reactions in structural terms, a crystallographic study of a modified form of EF-Tu:GDP (from *Escherichia coli*) was undertaken. Our preliminary results of this study have been published [2]. Other workers have published results of low resolution X-ray studies on similar complexes of EF-Tu:GDP [3,4], and although there are differences in the way the protein has been modified prior to and during crystallization and the way the molecules pack in the crystals, all the structures are similar, at least at low resolution. We were able to locate the bound GDP in the highly structured domain of the molecule referred to as the tight domain, and to relate the position to secondary structural elements. This location has been verified by substitution of GDP with ppGpp and difference Fourier techniques at low resolution [5]. Because of lack of information about the primary structure of EF-Tu, we were not able to correlate the secondary structure with the sequence.

With the sequence information now available [6], a reinterpretation of the tight domain has assigned the GDP binding site more convincingly to the primary structure. In the following, we report on the folding of the polypeptide chain in the tight domain, and although there remain some ambiguities in the interpretation, the proposed structure is consistent with information obtained by other means.

2. Materials and methods

X-ray data for construction of the three-dimensional electron density map are those in [2], but to improve the quality of the map, a more detailed analysis of the data was undertaken, resulting in the discovery of some systematic discrepancies. When calculating phase angles for the diffracted amplitudes using the MIR method (for explanation see [7]), it is important that the native data and the heavy atom derivative data are brought on the same scale. We noticed that for our data, the scale factors relating native amplitudes to derivative amplitudes were not constant but varied throughout reciprocal space. Similar observations were made in [8], and although the phenomenon is not fully understood, an empirical correction whereby the scale factors are computed as functions of reciprocal space coordinates removed the systematic errors in the data. Also, the internal consistency of each data set was enhanced by adopting a scaling procedure based on the method in [9]. These

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improvements in the data reduction technique resulted in a better overall agreement between the native and the derivative data and hence better determined phase angles. However, it also became evident that the lack of isomorphism at high resolution was more pronounced than previously estimated and the range of data was therefore reduced to 2.9 Å. A new electron density map was calculated, and this was significantly better (signal/noise improved by 15%). This map together with the amino acid sequence made it possible to assign and extend the previously reported model of the tight domain to cover ~180 residues from Ile 60–Glu 240.

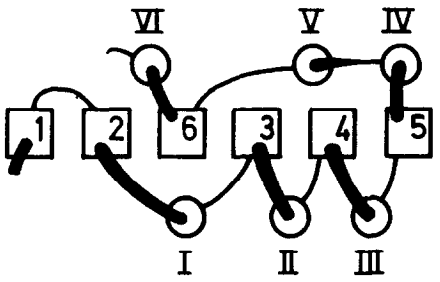

Fig.1. Packing diagram of secondary structural elements in the tight domain: (□) β-strands; (○) α-helices; the 3 βαβ units are right-handed as observed in most other α/β proteins.

3. Results and discussion

During the course of model building, evidence for the correctness of the model was substantiated by the observations:

(i) The high correlation between the nature of the amino acid side chains and the appearance of the corresponding electron densities formed the basis for the whole model building process;

(ii) The model is consistent with the observed binding sites of the heavy atom markers used in the phasing of the amplitudes. Of the 7 heavy atom positions located in the tight domain, the 2 mercury sites are located next to Cys 81 and Cys 137, the 2 platinum sites are located next to Met 139 and Met 151, and 2 lead sites are within coordination distances of Asp 141, Asp 142, Glu 143 and Glu 144, whereas the third lead site is situated between Glu 152 and Glu 155.

(iii) A comparison between predicted secondary structures and our proposed model of the tight domain shows a high degree of correlation [10].

The domain structure of the tight domain places this in the class called α/β-structures, an arrangement which is found in other nucleotide-binding proteins like dehydrogenases and kinases [11]. The folding pattern shown in fig.1 shows a 5-stranded parallel β-sheet which has been extended by a sixth antiparallel strand. The β-strands are connected by 6 α-helices via loops that are rather short except in one case. The β-sheet forms a central hydrophobic core with a relatively large left-handed twist. Surrounding this core, the α-helices provide an efficient interface to the solvent, so the whole arrangement appears very compact as shown in fig.2. This view is identical to fig.5 in [2] where we also showed the location of the bound GDP on the surface of the molecule close to helices IV and VI.

The guanine base is bound in a pocket lined with hydrophobic side chains above and below the plane of the base. There is thus no stacking interaction between the guanine base and any aromatic side chain, and this has been confirmed by NMR studies of ring current shifts in free and EF-Tu bound GDP [12]. The affinity of EF-Tu towards guanine is explained by hydrogen bond interactions between base oxygen and side chain hydrogen donors. The

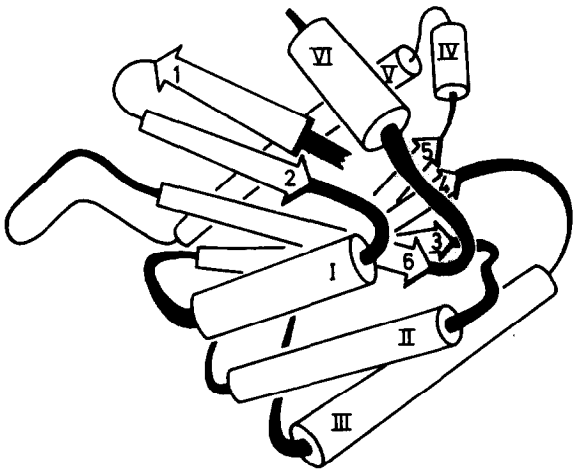

Fig.2. Structural cartoon of the tight domain with arrows representing β-strands and cylinders representing α-helices. The directions of the arrows are from the amino-ends towards the carboxy-ends of the strands. Since the central β-sheet is parallel, all the carboxy-ends of the β-strands are found at one edge of the sheet, which also contains the GDP binding site. See text for details.

ribose ring is also partly buried, but with the 2′,3′-hydroxy groups exposed to the solvent. These groups can therefore be substituted without affecting binding affinity in agreement with biochemical experiments [1].

The α- and β-phosphates are hydrogen bonded to side chain residues and backbone amide groups belonging to α-helix VI and the loop between this helix and β-strand [6]. The expected position of the γ-phosphate in GTP (assuming that GTP binds at the same site) is at the end of the cleft formed by strands 1 and 2 and helices I and II. This region of the molecule seems therefore to be important in the control of the action of the protein, specifically with respect to binding aa-tRNA when GTP is bound and releasing aa-tRNA when GTP becomes hydrolysed upon contact with the ribosome. Other evidence for the importance of this region in aa-tRNA recognition and binding comes from biochemical studies of photocrosslinking ε-bromoacetylysyl-tRNA to EF-Tu:GTP and subsequent peptide mapping of the crosslinked complex [13]. The crosslink took place at the histidine in position 66 which is located on strand 1 at the top of the cleft.

The remaining ~210 residues not belonging to the tight domain are located in two domains, one below and the other to the left of the tight domain as seen in fig.2 (see also fig.3 in [2]). The lower domain is in contact with helices I and II in the tight domain and contains ~100 residues which form a number of β-strands but no α-helices. The rest of the electron density is still difficult to trace due to the less well defined appearance of the electron density map. The rather big loop connecting helix V with strand 6 is located in this weaker electron density. We cannot rule out that the loop instead consists of two strands connecting the tight domain with the rest of the molecule.

Work is in progress to resolve this ambiguity as well as to interpret the rest of the less well ordered electron density by means of the partial structure information now available.

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