# THE PEPTIDE CHAIN OF TYROSYL tRNA SYNTHETASE : NO EVIDENCE FOR A SUPER-SECONDARY STRUCTURE OF FOUR α-HELICES

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SUMMARY

A detailed backbone model has been built for 274 residues of tyrosyl tRNA synthetase, based on an X-ray diffraction study. This includes eight helical sections and a six-stranded pleated sheet. The four helices near the carboxyl terminal end are not arranged like the helices of TMV disk protein and hemerythrin, and the structure gives no support to the idea that four antiparallel helices form a common structural unit in proteins.

## INTRODUCTION

Argos, Rossmann and Johnson (1) have proposed a new "super-secondary" structural unit composed of four "essentially parallel" alpha helices in contact. They suggest that this unit occurs in hemerythrin, tobacco mosaic virus disk protein (TMV protein), and in tyrosyl tRNA synthetase. In a private communication, Bloomer and Hendrickson (2) had already pointed out the conformational similarities of the first two proteins. Argos et al compared the TMV protein and the synthetase, whose correspondence is said to be "striking apart from the direction in which the synthetase chain has been traced."

Preliminary co-ordinates for the synthetase allow a critical evaluation of this proposal. The possible existence of such a structural unit in other protein structures is also considered.

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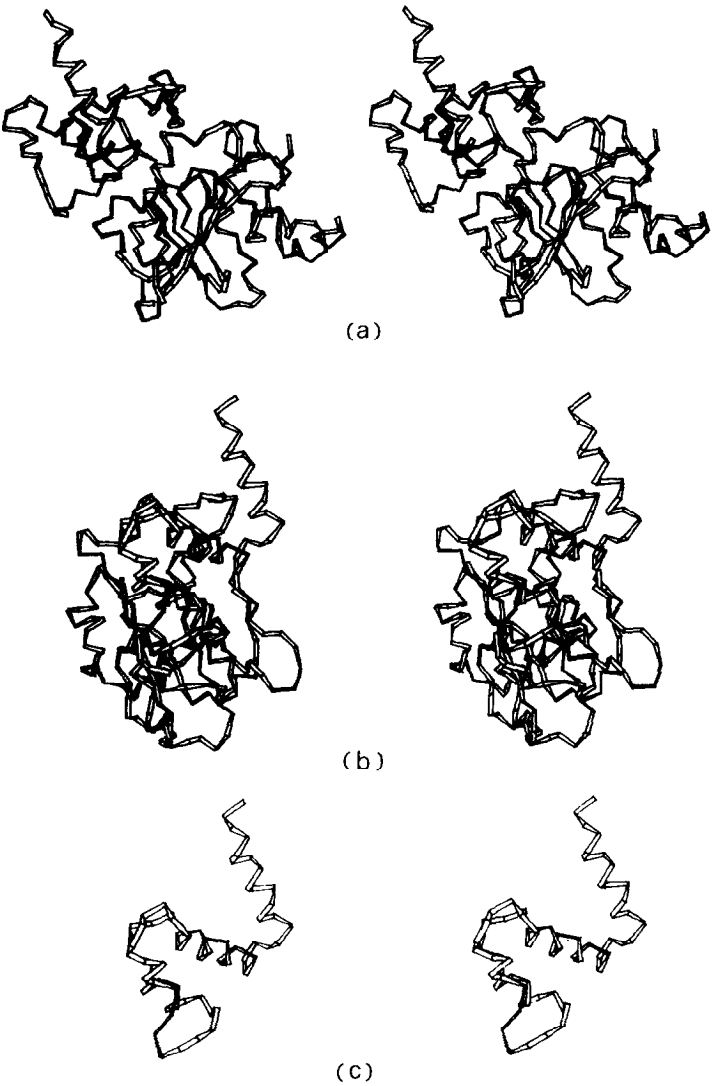


Figure 1(a) Stereoscopic view of the peptide chain of one of tyrosyl tRNA synthetase from a viewpoint similar to the illustration (Figure 5) of Ref (3). (Due to a drawing error, one chain crossing is illustrated wrongly in our earlier illustration. The chain towards the C-terminus of strand 6 of the sheet should pass behind strand 2). The four helices of the C-terminal domain are at the upper left of the subunit. (b) Peptide chain from an orthogonal viewpoint. (c) The four helices of the C-terminal domain, viewed as in (b), but separated from the rest of the subunit for greater clarity.

These drawings are produced by a computer program "Ribbons" by Dr. A.D. McLachlan.

## METHODS

Structural refinement of tyrosyl tRNA synthetase: The electron-density map of tyrosyl tRNA synthetase (E.C.6.1.1.1) at 2.7 Å resolution has been described by Irwin et al (3). This dimeric molecule crystallizes in space group $P3_121$, and the crystallographic twofold axis which passes through the origin is also a molecular twofold axis. Map sections perpendicular to this axis were contoured on transparent sheets at a scale of 2 cm/Å, with a spacing of 0.8 Å between sheets. These sheets were used in an optical comparator (4) to construct a skeletal model of the peptide chain.

It has not so far been possible to correlate the side-chain electron densities with the preliminary amino-acid sequence data available to us. At a few points the close proximity of the chains requires a particular residue to be glycine. Elsewhere the model was constructed as poly-L-alanine. Some regions of electron density are very weak, probably indicating local disorder in the structure. As mentioned by Irwin et al (3), both ends of the interpretable polypeptide chain disappear into weak, uninterpretable regions of density, so that the positions of the chain termini can not be assigned. A chain of 274 amino-acids has been built with tolerable certainty, representing about two thirds of the whole subunit. This includes five regions of weak density, where it is very possible one or two amino acids have been omitted or inserted.

The peptide chain co-ordinates were recorded directly onto a computer-linked Teletype, which was used to generate co-ordinates with standard geometry. It was generally sufficient to record the peptide chain N and O co-ordinates, the $C^\beta$ co-ordinates, and the polypeptide conformational angles $\psi$ and $\phi$ for each residue. The co-ordinates were then refined using the model-building and real-space refinement procedures of Diamond (5-7).

## DISCUSSION

Figure 1 shows the $C^\alpha$ co-ordinates of the structure obtained after one cycle of real-space refinement. Figure 1(a) shows a view broadly comparable to that shown in reference (3) Figure 5, while Figure 1(b) shows an orthogonal view. The lengths of the eight helical and six sheet regions are given in Table 1. Helix D, together with the parallel strands 5 and 6 of the sheet, form a right-handed βαβ unit (8-10).

From a view of the structures as seen in Figure 1(a), Argos et al (1) have suggested that the four C-terminal helices are in a similar conformation to the helices seen in hemerythrin (11) and TMV protein (12). They have proposed that this constellation of four antiparallel helical units forms a further "super-secondary" structure analogous to the βαβαβ unit observed in flavodoxin and in several dehydrogenases. The similarity between hemerythrin

Table I. Secondary Structure in TyrRS

| Secondary structure[a] | No. of peptides[b] | Intervening peptides before next secondary structure[b] |
|---|---|---|
| Helix A | 12 | 29 |
| Strand 1 | 7 | 12 |
| Strand 2 | 4 | 2 |
| Helix B | 5 | 3 |
| Strand 3 | 7 | 7 |
| Helix C | 15[c] | 17 |
| Strand 4 | 2 | 7 |
| Strand 5 | 5 | 4 |
| Helix D | 6 | 9 |
| Strand 6 | 6 | 33 |
| Helix E | 7[c] | 12 |
| Helix F | 6[d] | 3 |
| Helix G | 15 | 1 |
| Helix H | 15 | - |

[a] Strand arrangement is +4+1-3-5-6+2, see Irwin et al (1976).

[b] Figures are preliminary, see text. The first helix shown in Fig. 5 of Irwin et al (1976) now appears too irregular to be considered as α-helix.

[c] Includes one or two peptide bonds probably in $3.0_{10}$ conformation.

[d] Distorted helix.

and TMV protein had already been noted (2). Argos et al observed that in order to bring tyrosyl tRNA synthetase into correspondence with the others, the sense of the peptide chain would have to be reversed. From Table 2, several other differences may be noted. In the synthetase the third of the four chains (the second if the chain tracing is reversed) makes angles of

Table II Lengths and Directions of helices in five proteins

| | Hemerythrin | TMV protein[a] | T4 phage lysozyme | tyrosyl tRNA synthetase[b] | Myoglobin[c] |
|---|---|---|---|---|---|
| | Ref. (11) | | (Refs (13,14) | | Ref. (15) |
| **Helix Angles** | | | | | |
| ab | 158° | 165° | 165° | 162° | 139° |
| bc | 170° | 155° | 155° | 131° | 106° |
| cd | 163° | 165° | 172° | 138° | 162° |
| ad | 169° | 155° | 158° | 166° | 93° |
| ac | 25° | 35° | 24° | 49° | 91° |
| bd | 18° | 20° | 25° | 8° | 73° |
| **Helix lengths** (amino acid residues) | | | | | |
| a | 21 | 12 | 14 | 7 | 20 |
| ab corner | 2 | 7 | 8 | 12 | 8 |
| b | 24 | 9 | 9 | 6 | 10 |
| bc corner | 5 | 28 | 2 | 3 | 5 |
| c | 19 | >9 | 9 | 15 | 19 |
| cd corner | 5 | <29 | 8 | 1 | 6 |
| d | 20 | >20 | 13 | 15 | 26 |
| **Type** (Fig. 2) | 3 | 3 | 1 | 1?[d] | 4?[d] |

[a] Preliminary results of A.C. Bloomer and A. Klug (personal communication 1977). The "cd corner" includes a short perpendicular helix.

[b] Helices E, F, G, H, Table I.

[c] Helices E, F, G, H.

[d] Interpretation "forced", as structures do not clearly fit into the scheme.

| | | | | | |
|---|---|---|---|---|---|
| a b | a b | a d | a c | a d | a c |
| d c | c d | b c | b d | c b | d b |
| (1) | (2) | (3) | (4) | (5) | (6) |

Figure 2 Six possible ways of arranging four parallel helices in contact. Since the helices are assumed to be running in alternate directions, the letters 'a' and 'c' represent helices with their amino-terminal ends away from the observer, 'b' and 'd' with their amino-terminal ends towards the observer.

$49^{o}$, $49^{o}$ and $42^{o}$ with the other three chains. It is more nearly perpendicular to the other chains than parallel. This chain is the longest of the four α-helical segments : the first and second chains are less than half its length, and are one-third to one-quarter the length of the four chains in hemerythrin. There is, in fact, no contact at all between these two short helices and the fourth helix (Figure 1(c)), as they are separated by the length of the third helix. The structural and energetic principles which confer stability on the synthetase appear completely different from those of the hemerythrin molecule, whose main structural feature is four long, almost parallel helices arranged around a central iron atom.

If a unit contains four helical sections running approximately parallel, but in alternate directions and all in contact, then there are six different ways in which they can be arranged. If we assume that chains a and c are running towards us in Figure 2, while chains b and d run away from us, hemerythrin and TMV protein are both type (3). Tyrosyl tRNA synthetase (insofar as it can be forced into this scheme, since not all the chains are in contact) would be type (1), which is the chain reversal of type (3). The C-terminal domain of T4 phage lysozyme (13, 14) also contains four antiparallel helices in contact and these are also arranged as in type (1). The four C-terminal helices of myoglobin could possibly also be considered as fitting into this scheme (though the E and F helices are more nearly perpendicular than parallel to the G and H helices) (15). If so, it would belong to type (4).

These five structures seem to represent the most obvious cases known at present where four helices, roughly parallel, but running in alternate directions, are clustered together. This description is "forced" in two of the five cases, where one or two helices are more perpendicular than parallel to the others. Three of the six possible arrangements occur, none more than twice. The only strong similarity remains that of hemerythrin and TMV protein, though the analogy is weakened by the central iron atom in hemerythrin.

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