Tryptophan tRNA of *Escherichia coli*

THE tryptophan tRNA of CAJ64, a UGA suppressing strain of *Escherichia coli*[1], has an altered structure compared with that isolated from the wild type (CA244). A complete account of this work will be published elsewhere, but the sequence shown in Fig. 1 has two points of immediate interest. First, both the su$^+$ and su$^-$ structures contain an adenosine at position 15 and a uridine at position 48. In most other tRNAs guanosine and cytosine, respectively, occupy homologous positions in the sequence. Another exception with adenosine and uridine in these positions has recently been found in a leucine tRNA of *E. coli*[2]. The three dimensional model proposed by Levitt[3] has hydrogen bonds between the bases in these positions; these coordinate base changes are consistent with the structure and lend support to this feature of it.

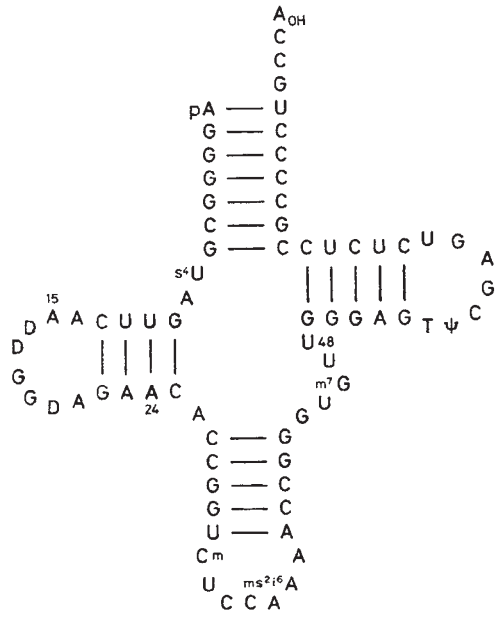

Fig. 1. Nucleotide sequence of *E. coli* (strain CAJ64) tryptophan tRNA. The unusual nucleoside 3′-phosphates are abbreviated as s^4U for 4-thiouridine, D for dihydrouridine, Cm for 2′-O-methyl cytidine, Ms^2i^6A for 2-methylthio-6-isopentenyl adenosine, m^7G for 7-methyl guanosine, T for ribosyl thymine, and Ψ for pseudouridine (5-ribosyl-uracil).

Second, both the su$^+$ and su$^-$ sequences have the same anticodon sequence, CCA, which according to the predictions of the wobble hypothesis[4] should recognize the codon UGG alone. The only difference between the two sequences is that the su$^-$ has a guanosine instead of an adenosine at position 24. Evidence has been obtained to show that there is no other tryptophan tRNA (less than 3 per cent of the amount found) in the su$^+$ strain, that both the su$^+$ and su$^-$ tryptophan tRNAs can read UGA, and that the mutant alteration allows the su$^+$ sequence to do this more efficiently.

DAVID HIRSH

MRC Laboratory of Molecular Biology,
Hills Road,
Cambridge, England.

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Amino-acid Composition of Crayfish Trypsin

THE homology in primary structure of mammalian pancreatic serine proteases is well established[1,2]. Progress has now been made in the elucidation of the evolutionary history of this group of proteases. Neurath and his coworkers[3] studied the amino-acid composition of trypsinogen isolated from the pancreas of the spiny Pacific dogfish and presented a preliminary examination of the primary structure of this enzyme. From these data a high degree of homology as compared with bovine trypsinogen can be inferred. It is now of interest what will be found beyond the gap that separates vertebrate and invertebrate animals. Invertebrate proteases are difficult to investigate because of the small amounts of material obtainable from these animals. In the crayfish *Astacus leptodactylus* we found an enzyme resembling mammalian trypsin in many respects[4,5]. It exhibits a distinct tryptic cleavage specificity when tried on the oxidized B-chain of insulin and is inhibited by all natural and synthetic trypsin inhibitors including TLCK. It is a serine protease and possesses the sequence –Asp–Ser–Gly– around the active centre serine residue[6]. In addition, extended immunological studies have been carried out on the crayfish trypsins of three species which revealed that they do occur as isoenzymes varying in number and electrophoretic mobility[7].

Table 1. AMINO-ACID COMPOSITION OF TRYPSINS

	Invertebrate	Vertebrate	
	Crayfish	Dogfish[3]	Bovine[11,12]
Lysine	5	5	14
Histidine	5	8	3
Arginine	2	7	2
Aspartic acid	30	24	22
Threonine	15*	7	10
Serine	17*	17	33
Glutamic acid	21	16	14
Proline	10	10	9
Glycine	28	28	25
Alanine	16	16	14
Half-cystine	6†	12	12
Valine	18‡	17	17
Methionine	2	8	2
Isoleucine	15‡	14	15
Leucine	16	15	14
Tyrosine	11	12	10
Phenylalanine	7	1	3
Tryptophan	3§	5	4
NH_3‖	17		
No. of residues	227	222	223

* Calculated from 20 h and 70 h hydrolysates by first order approximation to zero time.

† Determined after performic acid oxidation[9].

‡ Values for 70 h hydrolysis.

§ Determined spectrophotometrically[10].

‖ An approximative value for the amide content of crayfish trypsin was derived from the 20 h ammonia value by subtraction of the ammonia peak from a blank hydrolysate, the ammonia being the result of the decomposition of serine and threonine.

Purified *Astacus leptodactylus* trypsin is composed of two closely related bands which are both active against BAEE. They were separated in a final purification step by disc electrophoresis[8] and the amino-acid content of each component was analysed quantitatively. Four analyses of component I (which moved fastest to the anode) were done from 20 h and 70 h acid hydrolysates at each time interval. An analysis of bovine trypsin which was run for comparison in the same conditions gave correct values. The examination of component II did not reveal significant differences in amino-acid composition. This is in concordance with our observation that both components are cross-reacting immunologically[7].

Crayfish trypsin was shown to have 227 amino-acid residues (dogfish and bovine trypsin have 222 and 223 residues respectively). This accounts for a molecular weight of 24,060.