The Infrastructure of Valyl-Transfer Ribonucleic Acid Synthetase from Yeast

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Valyl-tRNA synthetase from yeast is shown to contain a significant proportion of repeated sequence, similar to the enzyme from bacterial sources.

It has recently been shown that those aminoacyl-tRNA synthetases of bacterial origin that have polypeptide chains greater than about 50000 daltons usually contain a significant proportion of repeated sequence (Kula, 1973; Koch *et al.*, 1974; Waterson & Konigsberg, 1974; Bruton *et al.*, 1974), which probably arises from gene duplication and fusion rather than post-transcriptional or post-translational fusion (Bruton *et al.*, 1974). The aminoacyl-tRNA synthetases from yeast have similar structural features, in terms of their quaternary structure and protomeric molecular weights, to those of bacterial origin, and it is of considerable interest to ascertain whether this feature is preserved.

The simplest indication of such repeated sequences comes from counting peptides on 'fingerprints' and counting unique sequences around specific residues. A more rigorous approach is to demonstrate the

existence of peptides in yields equivalent to more than one copy within the polypeptide chain. In this paper I describe both types of experiment, which show that valyl-tRNA synthetase from yeast, like the enzyme from *Bacillus stearothermophilus*, contains repeated sequences.

A typical 'fingerprint' of the performic acid-oxidized protein [valyl-tRNA synthetase purified as described by Lagerkvist & Waldenstrom (1967) was a gift from Professor U. Lagerkvist, University of Gothenberg, Sweden] is shown in Fig. 1. The composite staining procedure used enables one to obtain a considerable amount of information from one single digest of 2mg of protein. All the peptides are detected by the initial fluorescamine stain (Udenfriend *et al.*, 1972), those containing arginine by a second dip in the phenanthroquinone reagent (Yamada & Itano, 1966) and finally a Pauly (Easley

Fig. 1. Tryptic 'fingerprint' of valyl-tRNA synthetase

Protein (2mg) was oxidized with performic acid. The freeze-dried material was digested with 20 μ g of trypsin (2 $\times$ recrystallized; Worthington Biochemical Corp., Freehold, N.J., U.S.A.) dissolved in 50mM-NH₄HCO₃ at 37°C for 4h and freeze-dried again. The entire digest was dissolved in 1M-pyridine acetate, pH6.5, and applied as a 1.5cm band to the centre of a sheet (10 cm $\times$ 57 cm) of Whatman 3MM chromatography paper. The paper was subjected to ionophoresis at pH6.5 for 1h at 3kV. The neutral band was excised, stitched to a fresh sheet (10 cm $\times$ 57 cm) of 3MM paper and resubjected to ionophoresis at pH2.1 for 1h at 3kV. Both bands were stitched to fresh sheets of 3MM paper and chromatographed overnight in BAWP. (a) Acidic and basic peptides, (b) neutral peptides. ○, Fluorescamine; ●, arginine; ⊙, histidine; ⊙, tyrosine.

Table 1. Yields of some tryptic peptides from valyl-tRNA synthetase

Peptide	Composition	Yield (mol/113000g)
V1	Asn+Asp (2.02), Ala (0.97), Val (1.69), Lys (1.00)	1.15
V2	Ser (0.89), Glu (1.03), Gly (1.00), Ala (0.96), Lys (1.00)	1.25
V3	Asn (1.04), Val (0.87), Tyr (0.93), Lys (1.00)	1.20
V4	Asp (1.02), Leu (0.91), Met (0.84), Arg (1.00)	1.10
V5	Asn (1.97), Ser (0.93), Pro (0.94), Val (1.71), Leu (0.91), Phe (0.92), Arg (1.00)	1.15
V6	Asp (0.99), Thr (0.90), Gly (1.01), His (1.00), Arg (1.00)	1.20
V7	Ser (0.94), Pro (0.98), Ile (0.99), Lys (1.00)	1.25
V8	Thr (0.95), Pro (0.98), Gly (1.02), His (1.00), Lys (1.00)	1.15
V9	Gly (1.04), Tyr (1.81), Lys (1.00)	1.20
V10	Thr (0.98), Gly (1.10), Leu (0.98), Arg (1.00)	1.15

et al., 1969) stain indicates the peptides which contain histidine and, less well, tyrosine. The amino acid composition of the protein was determined after acid hydrolysis and is in good agreement with that published (Rymo *et al.*, 1972). On the basis of a unique polypeptide chain of 113000 daltons one would expect 132 tryptic peptides, 34 containing arginine, 24 histidine and 30 tyrosine. However, only 70 peptides, 19 containing arginine, 12 histidine and 14 tyrosine were observed. Thus in every case about half of the expected number of peptides is observed, probably indicating repeated sequences.

A more positive demonstration was obtained from an experiment designed to isolate tryptic peptides in yields equivalent to more than 1 mol/113000 daltons. A 5.3mg portion of performic acid-oxidized protein was digested with trypsin exactly as described previously (Bruton *et al.*, 1974). The digest was freeze-dried and the peptides were separated on a column of Locarte L1/5 resin. The effluent was monitored by the ninhydrin reaction after alkaline hydrolysis. All peaks were analysed by high-voltage paper ionophoresis at pH6.5 and 2.1 and paper chromatography in BAWP* (Waley & Watson, 1953). Samples of those peaks which appeared to contain a single pure peptide were hydrolysed and the amino acid analysis was used as a measure of the yield of the peptide. The compositions and yields of ten peptides with corrected yields above 1.10 per molecule of mol.wt. 113000 are given in Table 1. Norleucine was used as internal standard.

Unfortunately there was insufficient material to prove homogeneity by determining the amino acid

sequences of the peptides, but the discriminating screening procedure used makes it most unlikely that these peptides are mixtures. In each case only one basic amino acid was found and the molar proportions of the amino acids present were good. This purification procedure is designed to give pure peptides with the minimum of manipulation, so that the yields may be determined as accurately as possible. Unfortunately, only neutral and basic peptides, which tend to be fairly small from tryptic digests, are obtained, but there is no good general method for purifying the larger acidic peptides in high yields. The probability that all ten of these peptides could be duplicated by random chance is negligible.

These results indicate very strongly that the infrastructure of valyl-tRNA synthetase from yeast is very similar to that of valyl-tRNA synthetase of bacterial origin.

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* Abbreviation: BAWP, butan-1-ol-acetic acid-water-pyridine (15:3:12:10, by vol.).