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Proteolytic signal sequences (PEST) in the mammalian aminoacyl-tRNA synthetase complex

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Eight aminoacyl-tRNA synthetases together with three unidentified proteins are associated as a multi-enzyme complex in mammalian cells. Partial peptide sequences for lysyl- and aspartyl-tRNA synthetases are determined and no highly hydrophobic peptides are found. The partial amino acid sequences for two of the unidentified proteins in the complex are shown to have substantial homology and each has a number of unique sequences. The results suggest that the two unidentified proteins are fragments of synthetases. The partial sequences revealed the presence of PEST sequences in at least three proteins. Inasmuch as PEST sequences are signals for intracellular degradation, the mammalian synthetase complex may have evolved to protect these synthetases against intracellular proteolysis.

Aminoacyl-tRNA synthetase; Multi-enzyme complex; Amino acid sequence

1. INTRODUCTION

Eight mammalian aminoacyl-tRNA synthetases (Arg, Asp, Gln, Glu, Ile, Leu, Lys and Met) are associated as a synthetase complex as demonstrated by the purification of the synthetase complex [1] and the identification of the proteins as specific aminoacyl-tRNA synthetases [2]. The association of mammalian aminoacyl-tRNA synthetases as a multi-enzyme complex has raised the question as to the function of the synthetase complex and the molecular mechanism of their association. The particular set of amino acids specified in the synthetase complex does not show any correlation with respect to their genetic codons, amino acid transport systems or essential amino acids. Association of the synthetases is apparently not required for the enzymatic activities [3–5], and each synthetase is capable of catalyzing a reaction independently in the complex [6,7]. Furthermore, hydrophobic structural domains in the synthetases have been suggested to play major roles in the association of synthetases [8–10].

The aminoacyl-tRNA synthetase complex contains eleven proteins as analyzed by SDS-polyacrylamide gel electrophoresis. Proteins corresponding to bands I through to VIII (in the order of descending subunit molecular masses) have been identified as synthetases [1,2]. The identity of proteins corresponding to band IX (M_r 43000), band X (M_r 38000) and band XI (M_r 18000) is not known. A number of enzymatic activities are reported as being associated with the synthetase complex [11], including elongation factors [12] and casein kinase [13].

In view of the reactions catalyzed by the synthetases, and their role in protein metabolism, possible association with enzymes such as inorganic pyrophosphatase, DNA polymerase α and tRNA modification enzymes [11] has been suggested. However, no definitive evidence on the identity of the non-synthetase proteins has been established thus far.

In the present investigation, the peptide sequences of the two non-synthetase components are analyzed in an attempt to seek sequence homology

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with proteins or motif of known structure and function, which in turn may lead to the elucidation of the functional significance of the synthetase complex. Partial sequences of aspartyl- and lysyl-tRNA synthetases are also determined in an attempt to isolate the 'hydrophobic structural domains' involved in the association of synthetases.

2. MATERIALS AND METHODS

The synthetase complex was purified from Sprague-Dawley young male rats according to Kellermann et al. [1], with modifications [14]. The synthetase complex was pyridine ethylated [15]. Component proteins were purified by SDS-polyacrylamide gel electrophoresis and electrophoretically eluted [16]. The eluted proteins were precipitated by adding 10 vols of acetone, kept at −20°C overnight and dried under vacuum. The purity and identity of the eluted proteins were confirmed by SDS-polyacrylamide gel electrophoresis. The purified protein (0.2 nmol each) was resuspended in 0.1 M ammonium bicarbonate (50 μl). Freshly dissolved TPCK-trypsin (Sigma) was added to 2.7% (w/w) and the mixture was incubated at 37°C. Two additional aliquots of the same amount of TPCK-trypsin were added at the end of 1 and 2 h, respectively, and digestion was continued for another 2 h. The peptides were dried, redissolved in 1% TFA and then purified by chromatography on a Vydac 214 TP column (RPC C4) using a Hewlett-Packard HP-1090 HPLC system. Well-resolved peptides with good recovery were sequenced using the Applied Biosystem model 470A gas-phase amino acid sequencer [17] with an on-line PTH amino acid analyzer (Applied Biosystem model 120A).

3. RESULTS AND DISCUSSION

Lysyl-tRNA synthetase was electrophoretically eluted from the SDS-polyacrylamide gels of the purified synthetase complex. Lysyl-tRNA synthetase did not yield PTH amino acid during sequencing due to a blocked N-terminus. Similarly, glutamyl-, methionyl-, arginyl- and aspartyl-tRNA synthetases were also N-terminal blocked. When the synthetase complex with eleven proteins was loaded directly onto the polybrene membrane to detect any unblocked N-terminal sequences, no N-terminal amino acids or sequences were obtained. It appears that all proteins in the synthetase complex have blocked N-termini.

On one occasion, the extracted aspartyl-tRNA synthetase showed an identifiable sequence (namely A(S)XKGQEKPX(E/V)IVDAAEDYAX-EXYXSXXI) (cf. footnote to table 1). Since the yields of PTH amino acids are rather low, it is uncertain whether it is the N-terminal sequence of intact or slightly degraded aspartyl-tRNA synthetase.

The procedure described in section 2 was adopted to analyze the amino acid sequences of the component proteins. The proteins were completely digested with trypsin and the resulting polypeptides were purified on an RPC C4 column. The HPLC profiles for protein band IX and band X are strikingly similar, as shown in fig.1. Most of the peptide peaks have identical retention times. Possible cross contamination was excluded by SDS-polyacrylamide gel electrophoresis analysis. These results suggest that these two proteins are structurally related. Furthermore, as shown in table 1, three of the peptides have identical sequences, and one is unique. On the basis of the HPLC profiles of the tryptic peptides and partial sequences, protein bands IX and X evidently exhibit high sequence homology. However, protein band X which has a slightly lower molecular mass than band IX appears to contain at least six additional peptides. It is possible that band IX may contain partial sequence repetition. Alternatively, band X may contain two different proteins. However, the number of peptides appears insufficient to account for two proteins. Finally, protein IX and protein X may be large fragments with an overlapping sequence of one of the synthetases. Some of the synthetases are frequently present in substoichiometric amounts. The synthetase complex is devoid of bands IX and X, when additional steps were taken to inhibit proteolysis during purification. It is thus likely that bands IX and X are fragments of synthetases. However, since the 37 kDa protein has been suggested to be an inactive form of casein kinase [13], our data should be of interest to provide a comparison of the amino acid sequences when the sequence of casein kinase becomes available. No significant amino acid sequence homology of these peptides can be found in the NBRF protein data base. No homology was found with the α-subunit of G-proteins.

Aspartyl- and lysyl-tRNA synthetases display different peptide fragment profiles. Representative peptides with long retention times were selected in an attempt to isolate more hydrophobic peptides. As shown in table 1, most of the peptides are acidic. Eight of the amino acids in one of the octadecamer (peptide no.2) in aspartyl-tRNA synthetase are either Glu or Asp which appear to be

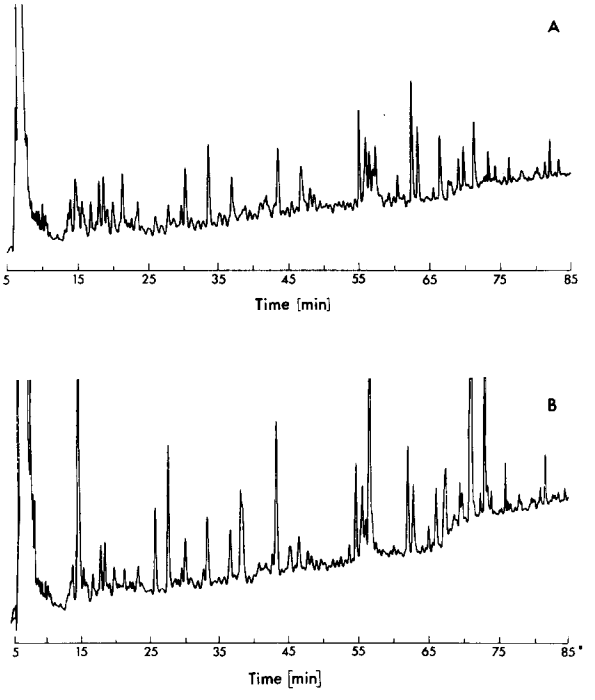

Fig.1. The chromatograms of tryptic peptides of (A) protein band IX and (B) protein band X. The tryptic digest of the protein was loaded directly onto a Vydac 214TP column at 0.5 ml/min, 40°C and 200 lb/inch^2 using a HP1090 HPLC system. The column was developed by gradient elution where solvent A is 0.1% TFA in water and solvent B is 0.1% TFA in 70% acetonitrile and 30% water. The percentage of solvent B is programmed as follows: 13% (0–5 min), 15–35% (5–60 min), 35–60% (60–85 min), 60–100% (85–95 min), 100% (95–100 min), 100–10 min). Absorbance at 214 nm was monitored as shown in the chromatograms. The retention times of the sequenced peptides are shown in table 1.

Table 1

Peptide sequences from aminoacyl-tRNA synthetase complex[a]

Retention time (min)[b]	Sequence
Lysyl-tRNA synthetase (band VI)	
1. 22.77	ETATATETPESTEASPXV
2. 36.60	KGELSIVPR
3. 52.63[c]	LPETSLFETEET (major)
	VLLFPAMKP (minor)
4. 70.99	TYHPDGPEGQAYEIDF
Aspartyl-tRNA synthetase (band VIII)	
1. 32.87	EIVDAAEDYAK
2. 33.97	EAGVEMDDEEDLSTPNEK
3. 44.69	ADEVVWVR
4. 64.38[c]	ESIIDVEGIVR (major)
	QQQFNVQALVAVGDHASK (minor)
5. 68.81	LPLQLDDAI(S)PEVEGEEDGR
Protein band IX	
1. 33.59	QV(P/A)ATDAGHVQEPSEPSL
2. 43.53[c]	AVYNSWMK (major)
	ILQATL (minor)
3. 66.55	GAEADQIIEYLK
Protein band X	
1. 43.54[c]	AVYNSWMK (major)
	ILQATL (minor)
2. 66.37	GAEADQIIEYLK
3. 71.21	NFTIPDHDNWPVGAGDS

[a] The single letter codes of amino acids are used. 'X' stands for unidentified amino acids. PTH amino acids in low yields are shown in parentheses

[b] All retention times are obtained under the conditions described in the legend to fig.1

[c] Peptide peaks gave simultaneously two sequences, based on the clear difference in the relative amounts

related to the 'PEST' sequences. PEST sequences which are rich in Pro, Glu, Ser, Thr and bound by charged amino acids have been hypothesized as signals for rapid intracellular turnover [18]. PEST sequences are also found in lysyl-tRNA synthetase (peptides nos 1 and 3, major), protein band IX (peptide no.1) and no.5 in aspartyl-tRNA synthetase. In contrast, examination of histidyl-tRNA synthetase sequence [19] which occurs exclusively as a free synthetase did not reveal any PEST sequences. It appears that one of the possible functions of complex formation of mammalian synthetases is to protect those synthetases with PEST sequences against proteolyses. This is in accord with one of the earlier hypothesis that the multimeric proteins may confer stability against degradation [20]. None of the peptides are highly hydrophobic, although hydrophobic interaction has been suggested as the interaction which accounts for the association of the synthetases [8–10]. Complete sequence analysis of these synthetases may ultimately lead to the elucidation of the structure of the domains involved in the association of synthetases.

In summary, the peptide sequence analyses of the synthetase complex revealed sequences of a number of PEST sequences. The two previously unidentified proteins are likely fragments with overlapping sequences of one of the synthetases in the complex.

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