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# REVERSED-PHASE LIQUID CHROMATOGRAPHY OF PEPTIDES FOR DIRECT MICRO-SEQUENCING

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## SUMMARY

Tryptic and cyanogen bromide peptides derived from yeast aspartyl-tRNA synthetase and from *Escherichia coli* ribosomal proteins were separated by reversed-phase liquid chromatography, employing volatile buffers of low ionic strength. The conditions used allow the performance of micro-sequencing without desalting or extensive lyophilization, and can therefore be applied to peptide mixtures containing hydrophobic fragments which tend to precipitate. To prevent losses of peptides, direct ultra-violet detection of the peptides was preferred, to detection by post-column derivatization with an additional stream splitting device. Preparative separations were performed with 5–10 nmol of peptide mixture; analytical runs were made with 5–10 μg of protein hydrolysate.

## INTRODUCTION

Over the past few years high-performance liquid chromatography (HPLC) has become an essential tool in protein chemistry. Indeed many recent publications describe its use for peptide separation. In many cases non-volatile buffers or solvents have been proposed, *e.g.*, trialkylammonium phosphate buffers, perchloric acid or sulph(on)ate buffers[1–3] which must be removed prior to sequence determination, especially when applying micro-methods such as the manual DABITC/PITC* double coupling method[4] or solid-phase sequencing[5]. Desalting is usually achieved by gel-filtration, but with inevitable loss of material. Some authors have employed volatile aqueous solvents such as pyridine formate, acetate buffers or dilute ammonium hydrogen carbonate[6–9] which can be removed to a certain extent by repeated lyophilization. However, traces of salt disturb the peptide sequence determination, and the repeated dryings frequently result in insoluble peptides. Further, these buffers have

* DABITC = 4-N,N-Dimethylaminoazobenzene-4′-isothiocyanate; PITC = phenylisothiocyanate.

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the great disadvantage of a high absorption in the 206–230 nm range which is widely used for peptide detection. To overcome these difficulties, monitoring techniques which divert a fraction of the column effluent to an analytical system where it reacts with ninhydrin, fluorescamine or *o*-phthalaldehyde have been designed[7,10]. Again this will result in losses of sample, *e.g.*, 1/2–1/10 of the total peptide amount when less than 5 nmol are injected. For these reasons peptide separations by HPLC were recently made in aqueous trifluoroacetic acid (TFA)[11,12].

In this paper we describe the use of volatile aqueous solutions of low ionic strength, such as 0.0027 *M* ammonium formate buffer pH 4.2 (or pH 7.5) or 0.05% (v/v) TFA combined with either acetonitrile or methanol. The ammonium formate buffer enabled us to purify most of the tryptic peptides from the *Escherichia coli* ribosomal proteins, whereas dilute trifluoroacetic acid proved very useful for the isolation of many hydrophobic peptides arising from cyanogen bromide cleavage of yeast aspartyl-tRNA synthetase. Both aqueous solvents have a very low absorbance at 210–220 nm, thus allowing easy and quick monitoring of the column effluent at these wavelengths.

Yeast aspartyl-tRNA synthetase has been purified and crystallized in our laboratory. Biochemical as well as crystallographic data support a dimeric structure of the $\alpha_2$ type ($\alpha$, $M_r$ 60,000)[13]. Crystallographic studies are underway on both the enzyme and a complex ($tRNA^{Asp}$, aspartyl-tRNA synthetase). Therefore, the determination of the enzyme sequence is an essential step in both the elucidation of its tertiary structure and the characterization of the regions involved in tRNA recognition.

## MATERIALS AND METHODS

### *Materials*

Ribosomal proteins S2, L9 and L17 were isolated from *E. coli* 30S subunits (strain K12) in the presence of urea[14] and were kindly provided by Dr. H. G. Wittmann.

Yeast aspartyl-tRNA synthetase was purified as described[15,16]. Trypsin (TPCK*-treated) was from Worthington, cyanogen bromide from Merck-Schuchardt. Methanol was of spectroscopic grade (Merck) and acetonitrile of chromatographic grade (Merck). All other chemicals were from Merck and of analytical grade.

### *Methods*

*Tryptic digestion of ribosomal proteins.* The ribosomal proteins were digested in 0.1 *M* N-methylmorpholine acetate buffer pH 8.1 or in 0.07% ammonia for 4–6 h at 37°C at a ratio of enzyme to protein of 1:50. Samples (1 mg each) were digested in 0.2–1.0 ml of the buffer for preparative runs; for analytical separations 200 μg protein were cleaved in 30–200 μl buffer in the glass tubes (volume 400 μl) used for the automatic HPLC injector (WISP sample injector, Waters).

*HPLC separation of the tryptic peptides derived from ribosomal proteins.* The digests were (a) lyophilized and dissolved in buffer A (used in the gradient of the HPLC run and if necessary 1–2 μl of 98% formic acid were added) and centrifuged (Beckman microfuge) prior to injection, or (b) directly injected into the HPLC col-

* TPCK = L-1-Tosylamido-2-phenylethylchloromethyl ketone.

umn without any drying step. Any visible sediment (in digests of 1 mg protein) was centrifuged off and treated separately, *i.e.*, dissolved under different conditions *(e.g.*, in a buffer of different pH or in TFA–water, 50:50) and these conditions were used for the separation. In no case was undissolved material applied to the HPLC columns. 5–10 $\mu$g protein digests in 5–10 $\mu$l volumes (for analytical runs) and 250-$\mu$g digests in 20–30 $\mu$l volumes (for preparative runs) were injected. The HPLC separation was performed on a Dupont 850 instrument (for details see Fig. 1) with flow-rates of 1.5–2.0 ml/min at 40°C and linear gradients of methanol (from 0 to 80% in 60–90 min) or acetonitrile (from 0 to 60% in 60 min).

The following buffers were used as buffer A: 0.0027 *M* ammonium formate, pH 4.2 (0.25 ml 98% formic acid + 0.4 ml 25% $NH_4OH$ + 2 l water); 0.01 *M* ammonium formate, pH 7.8 (1.6 ml 25% $NH_4OH$ + 0.25 ml 98% formic acid + 2 l water); 0.05% TFA. Buffer B was 80% methanol (or 80% acetonitrile) + 20% buffer A.

The following supports were used: Hypersil ODS ($C_{18}$), 5 $\mu$m (Shandon) or LiChrosorb RP-8 and RP-18, 5–10 $\mu$m (Merck). The columns were filled with the aid of a Shandon filling apparatus as described below. The column effluents were monitored at 210–220 nm. For preparative runs 0.6–1 ml fractions were collected manually. Peptide-containing fractions were dried under vacuum (Speed Vac Concentrator, Savant) and used directly for micro-sequencing or amino acid analysis.

*Column filling procedure.* The filling column (Shandon) was mounted directly on top of the experimental column (250 × 4.6 mm, Knauer), the bottom of which was closed with a filter and adapter. 3.3 g support in 30 ml *n*-heptane were degassed by sonication for 5 min and poured into the filling column, which was then connected to the solvent inlet. The filling column with the experimental column were turned through 180° and the support pumped in under a pressure of 300 bar (4285 p.s.i.) first with *n*-heptane for 10 min, and then with methanol for 10 min. The columns were turned to their original position and washed for another 10 min with methanol (to remove air-bubbles). The experimental column was then closed with a filter and adapter, ready for use. The back-pressure of the Hypersil ODS column at a flow-rate of 1.5 ml/min was 60 bar, that of the LiChrosorb columns was 80–100 bar.

*Cyanogen bromide cleavage of yeast aspartyl-tRNA synthetase.* About 150 nmol enzyme were dissolved in 2 ml 70% (v/v) formic acid; 75 mg of cyanogen bromide were added and the mixture was incubated in the dark for 48 h, at room temperature, in the presence of 6 *M* urea.

*Gel filtration of the cyanogen bromide fragments.* The cyanogen bromide digest was loaded onto a column of Sephadex G 50 (100 × 2 cm) equilibrated and eluted with 30% (v/v) acetic acid, at a flow-rate of 9 ml/h. Fractions of 1 ml were collected. Peptides were monitored by analysing aliquots of the fractions, by chromatography on a cellulose thin-layer plate.

*HPLC separation of cyanogen bromide fragments and tryptic peptides of yeast aspartyl-tRNA synthetase.* Pooled fractions from the above column were lyophilized, dissolved in 0.05% (v/v) TFA; if necessary, a few drops of concentrated TFA were added and injected into a Waters Assoc. apparatus with a two-pump solvent delivery system (Model 6000 A), a manual injector (Model U6k) and a UV detector (Model 450, variable wavelength). The column ($C_{18}$ $\mu$Bondapak, 30 × 0.4 cm I.D., particle size 10 $\mu$m) was eluted with a gradient made up of two solvents: A, 0.05% (v/v) TFA; B, acetonitrile or methanol. The gradient was linear from 0 to 80% B in 43

min or exponential (curve 8 of the gradient delivery system). The flow-rate was 2 ml/min at room temperature.

One of the purified BrCN peptides, BrCN-1 (Fig. 4), was further digested with trypsin and the digest fractionated on an Altex RP-18 column (25 cm × 0.46 cm I.D., particle size 5 μm). The column was eluted at a flow-rate of 1.5 ml/min with the same linear or exponential gradient as described above for the $C_{18}$ μBondapak column.

## RESULTS AND DISCUSSION

### *Ribosomal proteins*

The tryptic peptides generated from three *E. coli* ribosomal proteins, namely S2 ($M_r$ 26,600), L9 ($M_r$ 16,500) and L 17 ($M_r$ 14,500), have been separated by HPLC. As an example, the separation of the tryptic peptides of S2, which is one of the large proteins in the ribosome of *E. coli*, is presented in Fig. 1. This protein generates 30 tryptic peptides some of which are rather hydrophobic and tend to precipitate, *e.g.*, peptides T26, T27, T28 and T16. Sequence analysis has shown that these peptides are difficult to isolate by conventional techniques, such as Sephadex gel filtration or thin-layer chromatography (TLC)[17]. For comparison with the HPLC trace, the thin-layer fingerprint of protein S2 is shown in Fig. 2. The hydrophobic peptides T26 and T27 occur as long trailing bands of low resolution, and insoluble peptides are visible

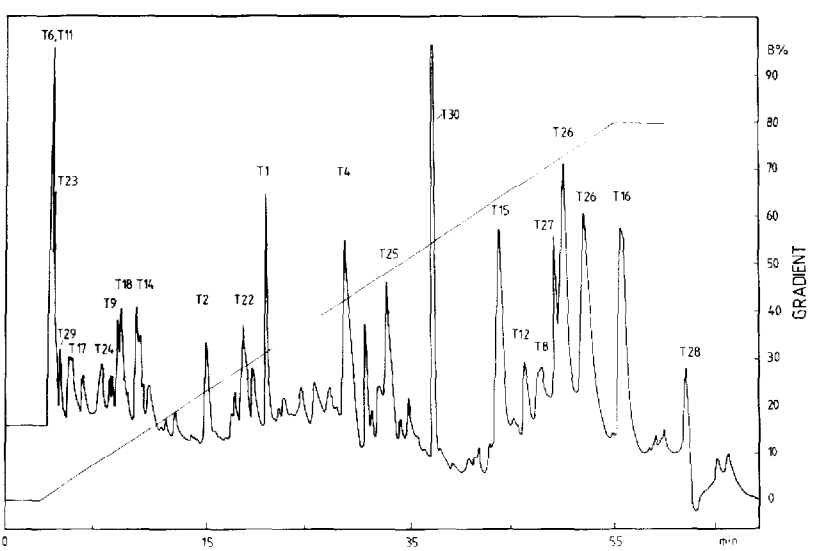


Fig. 1. Separation of the tryptic peptides from ribosomal protein S2 by reversed-phase HPLC. Column: LiChrosorb RP-18, 5 μm (Merck), packed with a Shandon filling apparatus (as in Methods); 250 × 4.6 mm I.D., stainless steel (Knauer). Equipment: Dupont 850, variable wavelength detector 852 monitored at 220 nm, scale 0.16, sampler WISP 710 A (Waters). Conditions: flow-rate 2 ml/min, 40°C, pressure 220 bar. Buffer A: 0.0027 *M* ammonium acetate, pH 4.2. Buffer B: 80% methanol (spectroscopic grade) + 20% buffer A. Gradient: 0–50% B (35 min, linear), 50–80% B (20 min, linear), held at 80% B for 15 min, 80–0% B (2 min, linear), held at 0% B for 30 min prior to next injection. Sample: 15 nmol of tryptic digest of protein S2 were dissolved in 50 μl buffer A (with addition of 2 μl 98% formic acid) and centrifuged; 40 μl were injected, fractions of 0.3–0.5 ml were collected; one fraction from each peptide peak was used for amino acid analysis, two for micro-sequencing as detailed in the text.

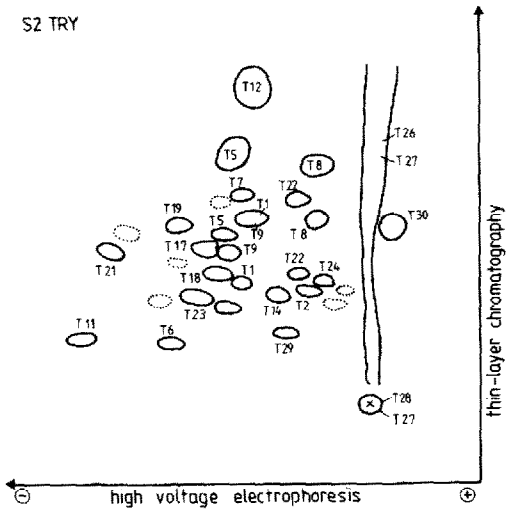


Fig. 2. Separation of the tryptic peptides of *E. coli* ribosomal protein S2 by fingerprinting on cellulose thin-layer sheets (for details see ref. 18). Major peptides are circled with solid lines, minor ones with dotted lines.

at the origin. Furthermore, peptide T16 containing two tryptophan residues could not be eluted from fingerprints in significant yields[17]. The reversed-phase HPLC separation on $C_{18}$ support was carried out employing a volatile ammonium formate buffer of low ionic strength, at pH 4.2, in a linear gradient with methanol. Compared to the fingerprinting, all peptides of S2 were obtainable in one HPLC run (Table I). Peptide T16 was found by monitoring at 294 nm, as in Fig. 3. In the HPLC separation the di- and tripeptides were not well resolved (peak 1) as the conditions were designed to resolve the hydrophobic peptides. Their recovery varied between 20 and 60% as estimated from the released endgroups.

70% of the peptide-containing fractions were pure enough for direct microsequencing. Two adjacent fractions were used for degrading the peptide by the manual DABITC/PITC double coupling method[4], or for the carboxyl attachment of the peptide to amino glass beads for solid-phase sequencing[5]. With methanol as organic modifier the peptide fractions were dried quickly (in a Speed Vac Concentrator directly in the glass tubes taken for the degradation), avoiding harsh drying conditions. Peptides T27 and T26 which occurred as an incompletely resolved double peak (Fig. 1) were sequenced with fractions from the ascending and descending shoulders of the peak.

The elution pattern showed that peptides containing hydrophobic residues had greater retention times, increasing mainly with the number of valine, isoleucine, phenylalanine and tryptophan residues. Similar results were obtained in HPLC separations of other ribosomal proteins. The tryptic peptides of protein L17 were very well resolved under similar conditions to those in Fig. 1, and all of them could be sequenced from the HPLC fractions. For solubility reasons, the tryptic peptides from protein L9 were separated in ammonium formate buffer at pH 7.8. After separation, the column was rinsed with methanol–water (70:30) to prevent destruction of the support which is more labile at this pH. All tryptic peptides, some of which could

TABLE I

ELUTION PATTERN OF TRYPTIC PEPTIDES DERIVED FROM RIBOSOMAL PROTEIN S2 BY HPLC SEPARATION ON RP-18

| *Fraction* | *Peptide* | *Positions in the sequence* | *Sequence* | *Charge* |
|---|---|---|---|---|
| 5 | T10 | (63, 110, 131) | Lys | Basic |
| | T13 | (73, 112) | Arg | Basic |
| | T6 | (35, 36) | Asn-Lys | Basic |
| | T11 | (64, 65) | Gly-Lys | Basic |
| | T23 | (137–138) | Thr-Arg | Basic |
| 10 | T29 | (222–224) | Glu-Gly-Arg | Neutral |
| 16 | T19 | (113, 114) | Leu-Lys | Basic |
| 18 | T17 | (105–107) | Thr-Val-Arg | Basic |
| 24 | T2 | (7–10) | Asp-Met-Leu-Lys | Neutral |
| 31 | T24 | (139–142) | Glu-Leu-Glu-Lys | Acidic |
| 35 | T21 | (128–130) | Leu-Thr-Lys | Basic |
| 38 | T9 | (59–62) | Ile-Ala-Ser-Arg | Basic |
| 40 | T18 | (108–110) | Gln-Ser-Ile-Lys | Basic |
| 42 | T14 | (74–80) | Ala-Ala-Ser-Glu-Ala-Val-Lys | Neutral |
| 61 | T2 | (7–10) | Asp-Met-Leu-Lys | Neutral |
| 72 | T22 | (132–136) | Glu-Ala-Leu-Met-Arg | Neutral |
| 75 | T1 | (1–6) | Ala-Thr-Val-Ser-Met-Arg | Basic |
| 83 | T20 | (115–127) | Asp-Leu-Glu-Thr-Gln-Ser-Gln-Asp-Gly-Thr-Phe-Asp-Lys | Acidic |
| 88 | T3 | (11–20) | Ala-Gly-Val-His-Phe-Gly-His-Gln-Thr-Arg | Basic |
| 99 | T4 | (21–25) | Tyr-Trp-Asn-Pro-Lys | Basic |
| 104 | T5CT | (30–34) | Ile-Phe-Gly-Ala-Arg | Basic |
| 106 | T20 | (115–126) | Asp-Leu-Glu-Thr-Gln-Ser-Gln-Asp-Gly-Thr-Phe-Asp-Lys | Acidic |
| 109 | T5NT | (26–29) | Met-Lys-Pro-Phe | Basic |
| 113 | T25 | (143–151) | Leu-Glu-Asn-Ser-Leu-Gly-Gly-Ile-Lys | Neutral |
| 135 | T30 | (225–240) | Ser-Gln-Asp-Leu-Ala-Ser-Gln-Ala-Glu-Glu-Ser-Phe-Val-Glu-Ala-Glu | Acidic |
| 164 | T15 | (81–94) | Asp-Ala-Ala-Leu-Ser-Cys-Asp-Gln-Phe-Phe-Val-Asn-His-Arg | Neutral |
| 179 | T12 | (66–72) | Ile-Leu-Phe-Val-Gly-Thr-Lys | Basic |
| 190 | T7 | (37–44) | Val-His-Ile-Ile-Asn-Leu-Glu-Lys | Basic |
| 196 | T8 | (45–58) | Thr-Val-Pro-Met-Phe-Asn-Glu-Ala-Leu-Ala-Glu-Leu-Asn-Lys | Acidic |
| 203 | T27 | (174–207) | Glu-Ala-Asn-Asn-Leu-Gly-Ile-Pro-Val-Phe-Ala-Ile-Val-Asp-Thr-Asn-Ser-Asp-Pro-Asp-Gly-Val-Asp-Phe-Val-Ile-Pro-Gly-Asn-Asp-Asp-Ala-Ile-Arg | Acidic |
| 205 | T26 | (152–173) | Asp-Met-Gly-Gly-Leu-Pro-Asp-Ala-Leu-Phe-Val-Ile-Asp-Ala-Asp-His-Glu-His-Ile-Ala-Ile-Lys | Acidic |
| 220 | T16 | (95–104) | Trp-Leu-Gly-Gly-Met-Leu-Thr-Asn-Trp-Lys | Basic |
| 233 | T28 | (208–221) | Ala-Val-Thr-Leu-Tyr-Leu-Gly-Ala-Val-Ala-Ala-Thr-Val-Arg | Basic |

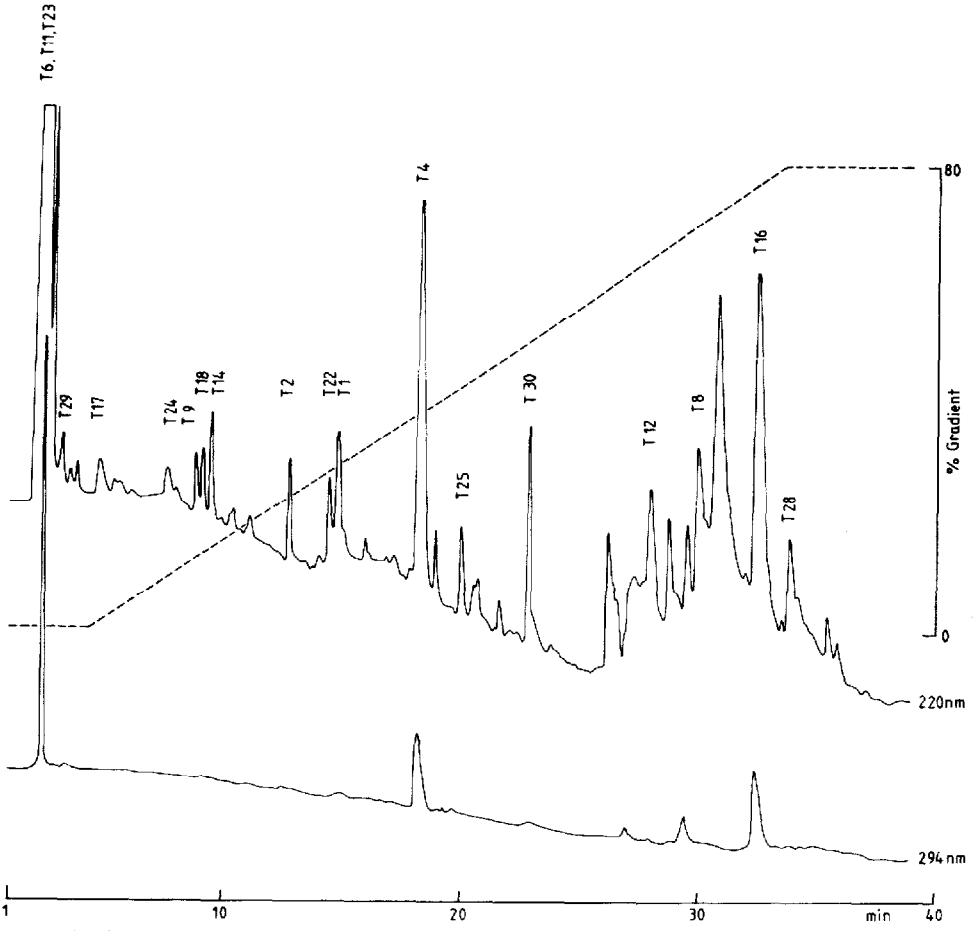


Fig. 3. Analytical separation of the tryptic peptides from *E. coli* ribosomal protein S2 employing 294 nm (lower trace) and 220 nm (upper trace) for peptide detection. Conditions are similar to those of Fig. 1; however, buffer B was methanol without addition of buffer A, and 2.5 nmol were injected (detector scale 0.04). In protein S2 only two peptides contain tryptophan, peptide T4 and T16, as listed in Table I.

not be isolated at all by any of the conventional procedures, were obtainable in this way, and this has recently allowed the complete sequence analysis of this ribosomal protein[19].

*Aspartyl-tRNA synthetase*

The structural data available for aspartyl-tRNA synthetase suggest the presence of two important domains in the protein: one is characterized by a high number of acidic and basic amino acids and is therefore hydrophilic and very sensitive to proteolysis; the other is hydrophobic and may have a rather compact structure since it is not easily hydrolysed by proteolytic enzymes, *e.g.*, trypsin. Therefore trypsin generates mainly small acidic and basic peptides. These peptides have been purified by conventional methods including gel filtration and TLC on cellulose plates. Some of the corresponding sequences are shown in Table II: they show remarkable features, such as clusters of acidic and basic residues adjacent to one another.

TABLE II

SEQUENCES DERIVED FROM TRYPTIC PEPTIDES OF YEAST ASPARTYL-tRNA SYNTHETASE

| |
|---|
| Glu-Glu-Glu-Lys-Lys-Glu-Lys-Lys-Arg |
| Lys-Lys-Glu-Glx-Arg |
| Ala-Leu-Gln-Lys-Lys-Gln-Leu-Lys-Lys |
| Glu-Gln-Glu-Lys-Lys |
| Lys-Glu-Arg-Glu-Ala-Arg |

Cyanogen bromide cleavage of this enzyme yielded some hydrophobic peptides. These have been purified by a combination of gel filtration on Sephadex columns and HPLC. It must be emphasized that classical techniques of peptide purification, such as ion-exchange chromatography or TLC, did not prove to be suitable for these large hydrophobic peptides, whereas they could be obtained pure and in high yields with the described HPLC method.

As examples we present three different elution profiles of peptide mixtures: the first (Fig. 4) corresponds to cyanogen bromide peptides of about $M_r$ 4000–8000, the second (Fig. 5) to the tryptic peptides generated from BrCN peptide 1 shown in Fig. 4 and the last (Fig. 6) to cyanogen bromide peptides ranging from $M_r$ 1500 to 2500. Experimental conditions are described in *Methods*. These profiles show that large peptides can give rise to slightly asymmetrical peaks (*e.g.*, peptides 1 and 2 from Fig.

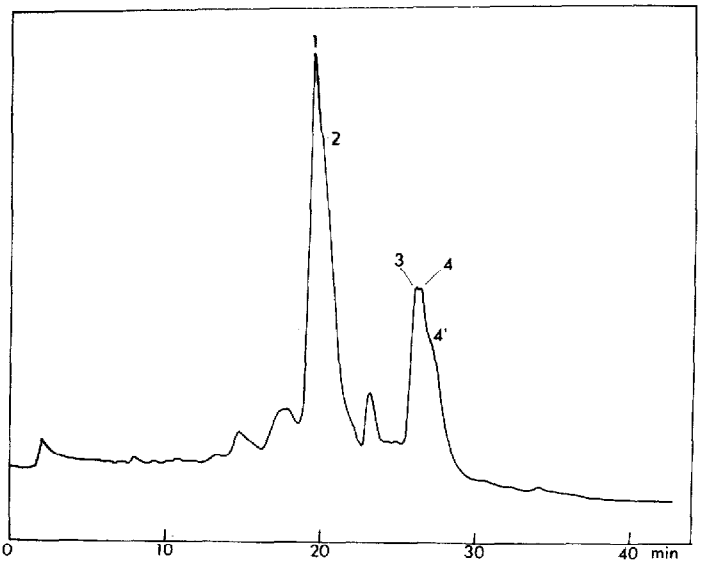


Fig. 4. Separation of the BrCN peptides from yeast aspartyl-tRNA synthetase contained in the fractions 80–91 eluted from a Sephadex G 50 column, by reversed-phase HPLC. Conditions as in Methods. Column: Waters. Solvents: A, 0.05% TFA; B, acetonitrile. The gradient was linear from 0 to 80% B in 43 min; flow-rate 2 ml/min, at room temperature. Monitoring at 220 nm, detector scale 0.2. Sample: 18 nmol of BrCN peptides were dissolved in 100 µl 0.05% TFA (with addition of 10 µl 100% TFA) and injected. Each peak was manually collected and an aliquot (1/10th) was used for amino acid analysis: 1 = ascending part and top of the peak; 2 = descending shoulder; 3 = ascending part of the peak; 4 and 4' = descending part and shoulder. Peptides corresponding to some of these peaks (1 and 2) have been analysed and sequenced (Table III).

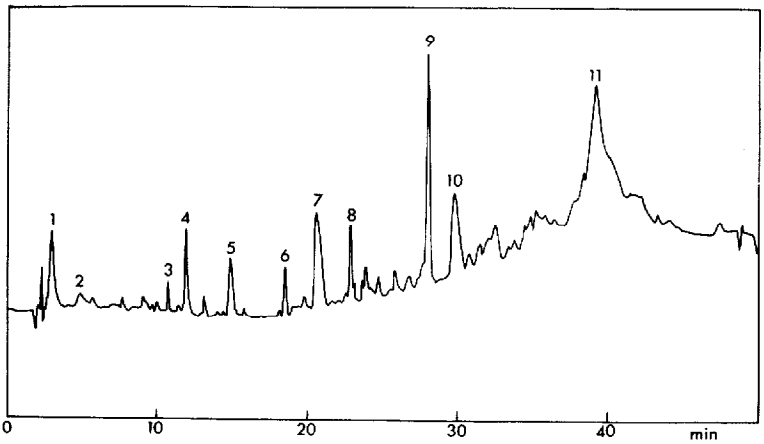


Fig. 5. Separation of the tryptic peptides of peptide BrCN-1 (see Fig. 4) on a Altex RP-18 column (as in Methods). Solvents: A, 0.05% TFA; B, methanol. The gradient was linear from 0 to 80% B in 43 min; flow-rate 1.5 ml/min at room temperature. Detection conditions as in Fig. 4. Sample: 30 nmol of tryptic digest of BrCN-1 were dissolved in 100 $\mu$ of 0.05% TFA and injected. Each peak was manually collected and an aliquot (1/10th) was used for amino acid analysis (data not shown).

4 are identical) which is rarely the case for the smaller ones (Figs. 5 and 6). The recovery of the large peptides (Fig. 4) is relatively high, about 50%, as estimated from amino acid analyses of aliquots. Some of the corresponding sequences are listed in Table III: the manual sequential degradation method[4] enabled us to sequence eight residues of the N-terminal part of peptide BrCN-1. Similarly, we have sequenced almost all of the tryptic peptides purified as shown in Fig. 5. The peptides were eluted in a high yield, 50–95%. These peptides can therefore be aligned and overlapped in the larger BrCN-1 fragment. The high number of peptides in Fig. 5 is due to the chymotryptic-like activity of the trypsin used.

As shown in Table III, some peptides are hydrophobic. Worth noticing are the

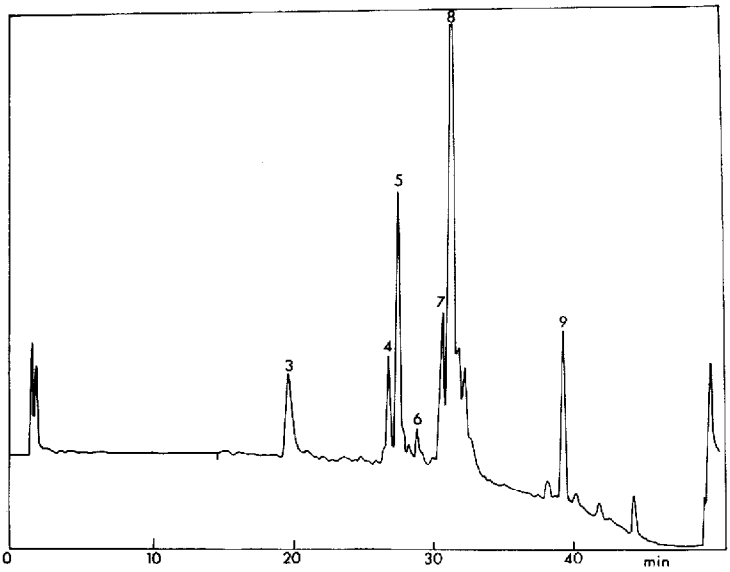


Fig. 6. Separation of the BrCN peptides (about 10 nmol) from yeast aspartyl-tRNA synthetase contained in the fractions 126–139 eluted from a Sephadex G 50 column, by reversed-phase HPLC. Conditions as in Fig. 4, except that the gradient was exponential (curve 8 of the gradient delivery system) (see Table III).

TABLE III

SEQUENCES OF BrCN-PEPTIDES OF ASPARTYL-tRNA SYNTHETASE

*Peptide BrCN-1 (Fig. 4, peaks 1 and 2)*
Leu-Arg-Ala-Ala-Gly-Lys-Glu-Ile-Gly-Asp-Phe-Glu-Asp-Leu-Ser-Thr-Glu-Asn-Glu-Cys-Lys-Phe-Leu-Gly-Lys-Leu-Val-Arg-Asp-Lys-Tyr-Asp-Thr-Asp-Phe-Tyr-Leu-Ile-Asp-Lys-Phe-Pro-Leu-Val-Glu-Asp-Gly-Leu-Arg-(Thr, Glx, Gly, Ile, Phe)-Gly-Ala-Leu-Asp-Ser-Ser-Lys-Asp-Phe-Glu-Arg-Leu-Ile-Pro-Phe-Tyr-Thr-Met

*BrCN peptides of aspartyl-tRNA synthetase (Fig. 6)*
Peak 7: Pro-Asp-Pro-Ala-Asn-Pro-Lys-Tyr-Phe-Asn-Ser-Ser-Asp-Phe-Met
Peaks 4 and 5: Val-Arg-Leu-Thr-Tyr-Lys-Gln-Gly-Ile-Glu-Met
Peak 3: Pro-Lys-Arg-Leu-Arg-Pro

*Other BrCN peptides of aspartyl-tRNA synthetase*
Phe-Tyr-Leu-Asp-Leu-Lys-Asn-Ile-Arg-Arg-Ala-Ser-Leu-Phe-Pro-Arg-Asp-(Ser, Glx, Pro, Gly 2)-Met

Arg-Gly-Glu-Glu-Ile-Leu-Ser-Gly-Ala-Gln-Arg-Ile-His-Asp-Ala-Arg-Leu-Glu-Gln-Leu-Met

proline-rich sequences found in two peptides (Fig. 5, peaks 7 and 3, the latter corresponding to the C-terminal part of the enzyme).

In brief, all these results suggest the presence of two distinct domains in aspartyl-tRNA synthetase, one hydrophilic and one hydrophobic.

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