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AMINO ACID SEQUENCE HOMOLOGIES IN ALFA-SARCIN, RESTRICTOCIN AND MITOGILLIN

R. RODRIGUEZ*, C. LOPEZ-OTIN+, D. BARBER+, J.L. FERNANDEZ-LUNA+
G. GONZALEZ*, and E. MENDEZ+1

+Servicio de Endocrinologia, Centro Ramon y Cajal
Carretera de Colmenar Km 9, 100, Madrid, Spain

*Departamento de Bioquimica, Facultad de Ciencias
Universidad Complutense, Madrid, Spain

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The NH_2-terminal amino acid sequence of the three anti-tumor proteins, alfa-sarcin, mitogillin and restrictocine, has been determined for 20 cycles by automated sequencing procedure. A high degree of sequence homology was observed in this region of the molecule. In addition, extensive sequence homology, ranging from 65 to 100% was found in three other carboxymethylcysteine-containing peptides isolated and sequenced from each molecule.

INTRODUCTION

Alfa-sarcin, restrictocin and mitogillin are three antitumor agents produced by two different Aspergillus strains (1-3). These three molecules have been characterized as basic polypeptide chains with molecular weights of about 16,000 (4-8). They inactivate the eukaryotic 60 S ribosomal subunit by cleavage of the large RNA (4-6). These proteins are also powerful inhibitors "in vivo" of protein synthesis in picornavirus infected cells, since they are able to penetrate the cell only when the latter is first infected with picornavirus (8).

The antibodies for alfa-sarcin can prevent the action of the toxin on the ribosomes. Alfa-sarcin antiserum cross-reacts with the other two toxins from Aspergillus and is also able to prevent their effects on the ribosome (6).

We now report partial structural characterization of these polypeptides and show that they exhibit extensive sequence homologies in the peptides around the disulfide bonds.

1. To whom correspondence should be addressed.

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MATERIALS AND METHODS

Materials. Alpha-sarcin, restrictocin and mitogillin were kindly given to us by Dr. D. Vazquez (Centro de Biologia Molecular, Madrid, Spain) and Dr. D.M. Schuurmans (Department of Public Health, Lansing, Michigan). TPCK-treated trypsin, cellulose thin-layer plastic sheets and other reagents not specified were from Merck (Darmstadt, F.G.R.). o-phthaladehyde was from Sigma (St. Louis, MO, USA). Reagents for automatic sequence determination were from Beckman (Palo Alto, CA, USA).

Reduction and alkylation. 50 mg of protein (35 mg/ml) in 1 M Tris-HCl buffer, pH 8.5, containing 0.002 M EDTA and 6M guanidinium hydrochloride was incubated with 0.1 M DTT for 100 min at 37°C. Radioactivity-labeling was achieved by adding 150 µCi (^{14}C)-iodoacetic acid (54 Ci/mol) and incubating the mixture for 15 min at room temperature in the absence of light. Unlabeled iodoacetic acid was then added to a final concentration of 0.2 M and excess of reagents was removed by gel filtration on Sephadex G-25 column.

Tryptic digestion. 25 mg of reduced and carboxymethylated proteins were digested with TPCK-trypsin at an enzyme/substrate ratio of 1:100 (w/w) in 0.2 M N-methylmorpholine acetate buffer, pH 8.2, for two hours at 37°C.

Ion exchange chromatography. Tryptic peptides from the reduced and ^{14}C-carboxymethylated proteins were fractionated on Dowex M-71 column (0.3 x 20 cm) equilibrated in 0.01 M pyridine acetate buffer, pH 2.1. The column was developed at 50°C at a flow rate of 6 ml/h as follows: 0.01 M pyridine acetate pH 2.1 (30 ml), 0.05 pyridine acetate pH 2.1 (25 ml) and 0.1 M pyridine acetate pH 2.8 (25 ml); finally the following pyridine acetate gradients were used successively: (a) 55 ml each of 0.1 M pH 2.8 and 0.7 M pH 3.7 (b) 45 ml each of 0.7 M pH 3.7 and 2.0 M pH 4.8. Fractions of 0.6 ml were collected. Aliquots of 20 µl every second fraction were used for detecting peptides with o-phthaladehyde after alkaline hydrolysis (9) and for radioactivity measurements.

Amino acid analysis. Peptides were hydrolyzed with 0.15-0.20 ml of 5.7 N HCl containing 0.05% (v/v) 2-mercaptoethanol at 110°C for 20 hours. The analyses were performed in a Beckman 121 M amino acid analyzer.

Amino acid sequences. Automatic Edman degradations were performed with a Beckman Sequencer model 890 B. Edman manual procedure for step-wise degradation (10) was used for some of the cysteine containing peptides. The anilinothiazolinone (ATZ) amino acid obtained in each step was analyzed as free amino acid after regeneration with 5.7 M HCl/0.1% $SnCl_2$ at 150°C for four hours (11). An aliquot was converted to the phenylthiohydantoin (PTH) amino acid and analyzed by thin layer chromatography (TLC) either by two dimensional chromatography on polyamide sheets (12) or on silica gel plates using previously described solvents (13). Cysteine residues were located by measuring the radioactivity from each degradation step.

RESULTS

Isolation of tryptic peptides containing cysteine residues from alfa-sarcin, mitogillin and restrictocin. The proteins labeled with ^{14}C-iodoacetic acid at each of their cysteine residues were digested with trypsin as described under Experimental Procedures. The digested material was chromatographed on Dowex M-71 ion exchange resin. The pattern of

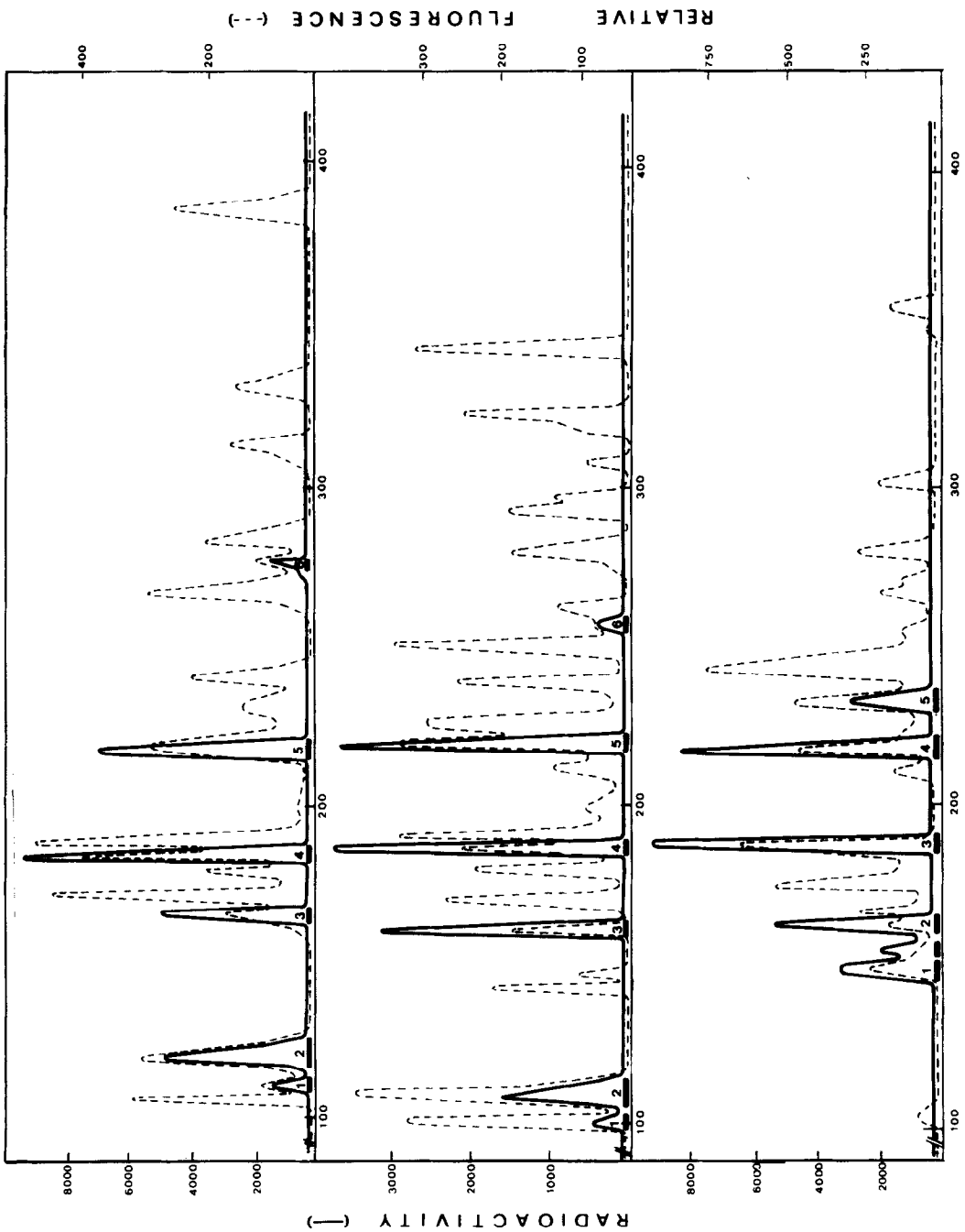

Figure 1. Elution profile of the ion exchange chromatography for tryptic hydrolyzates from Restrictocin, Mitogillin and alfa-Sarcin (from the top to the bottom).

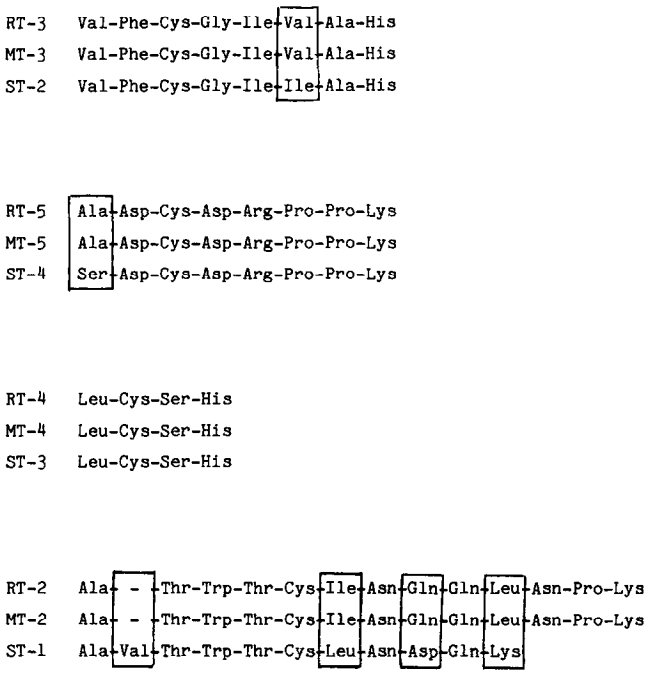

Figure 2. Comparison of the amino acid sequence of the four carboxymethylcysteine-containing tryptic peptides from Restrictocin, Mitogillin and alfa-Sarcin. Differences are indicated in boxes. The dash indicates the presence of a gap in the sequence.

distribution of peptides from the three proteins is shown in Fig. 1. Although most of the peptides indicated by bars were pure, they were further purified by cellulose thin layer chromatography. A total of six different radioactive peptides were isolated from each molecule; two were present in a low yield. The amino acid compositions of the four major carboxymethylcysteine-containing peptides from each protein are shown in Table 1. The amino acid sequences obtained by Edman degradation are given in Figure 2. Peptide St-5 (Table 1) is the same peptide as St-2 (Fig. 2) with additional Thr-Lys at the COOH-terminus.

NH_2-terminal sequence analyses. The NH_2-terminal sequences of the intact ^{14}C-carboxymethylated proteins alfa-sarcin, mitogillin and restrictocin were determined by automatic degradation. The sequence of the first 19 amino acid residues of mitogillin and restrictocin as well as the first 20 amino acid residues from alfa-sarcine are given in Figure 3. This analysis established the location of the carboxymethylcysteine-containing

TABLE I. Amino acid composition of the cysteine containing tryptic peptides[a] of Alpha Sarcin, Restrictocin and Mitogillin.

	RT-2	RT-3	RT-4	RT-5	MT-2	MT-3	MT-4	MT-5	ST-1	ST-2	ST-3	ST-4	ST-5
Lys	1.0			0.9	0.9			0.9	1.0			1.0	1.0
His		0.9	1.0			0.9	0.9			0.9	1.0		0.9
Arg				1.0				1.0				1.0	
CM-Cys	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.1	1.0
Asx	1.9			2.2	1.9			2.3	2.6			2.6	
Thr	1.9				1.9				1.9				1.0
Ser			0.8				0.8				0.9	0.8	
Glx	2.2				2.0				1.0				
Pro	0.9			1.8	0.9			1.9				2.0	
Gly		1.0				1.0				0.9			1.0
Ala	1.1	0.9		0.9	0.9	1.0		1.0	0.9	0.9			0.9
Val[c]		2.0				2.0			0.8	0.8			0.8
Ile[c]	0.8	0.6			1.0	0.7				1.6			1.8
Leu	0.8		0.9		1.0		0.9				1.0		
Phe		0.9				0.9				0.9			0.9
Trp[b]	+				+				+				
Total Residues	13	8	4	8	13	8	4	8	11	8	4	9	10
Yield (nmol)	222	248	629	617	75	185	270	265	240	310	523	360	195

[a] Residues per mole of peptide.

[b] Determined separately as described (14).

[c] 72 Hours hydrolysis value only.

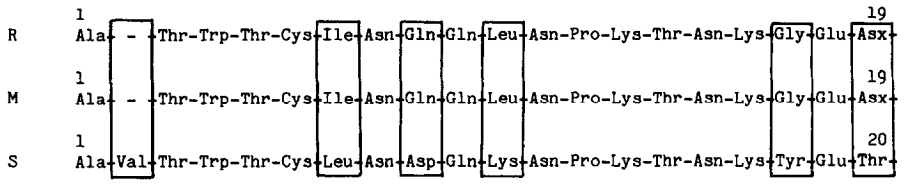

Figure 3. Comparison of the N-terminal sequence of Restrictocin (R), Mitogillin (M) and alfa-Sarcin (S). Differences are indicated in boxes. The dash indicates the presence of a gap in the sequence.

tryptic peptides, RT-2, MT-2 and ST-1 (Fig. 2) at the NH_2-terminus of each molecule. Peptide RT-2 and MT-2 correspond to residues 1-13 of restrictocin and mitogillin respectively and peptide ST-1 correspond to residues 1-11 of alfa-sarcin (Fig.3). The state of amidation of aspartic acid residues located at position 19 in the mitogillin and restrictocin molecules was not determined.

DISCUSSION

The amino acid sequences around the disulfide bridges in mitogillin and restrictocin are identical, and MT-2 and RT-2 are located at the NH_2-terminal region of mitogillin and restrictocin respectively as automatic sequence studies indicate. This identity extends through 19 residues from the NH_2-terminal amino acid for both proteins. The peptide segments sequenced for these two molecules represent about 30% of the total sequence and are distributed throughout the peptide chain, suggesting a nearly complete sequencial homology between the two proteins. Recently, we have also obtained evidence for such structural homology by comparison of tryptic fingerprints (Gavilanes et al., unpublished observations).

Eight substitutions are observed in the sequence of alfa-sarcin in comparison with that from mitogillin and restrictocin (Fig. 2 and 3). These differences represent 15% of the sequenced regions. The NH_2-terminal amino acid sequence analysis of alfa-sarcin reveals that it is identical in 14 out of 20 positions with restrictocin and mitogillin (identity 70%) (Fig. 3). The extent of homology in the carboxymethylcysteine-containing tryptic peptides extends from 85-100% (Fig. 2). Thus, the antitumor effects observed for mitogillin, restrictocin and alfa-sarcine are probably related

to the presence of an identical active site containing the homologous sequences. Moreover, the high degree of amino acid sequences homology in the peptides described in this paper and the presence of many other common tryptic peptides in the three molecules (Gavilanes et al., unpublished observations) may also explain the existence of common antigenic determinants - the cross-reactivity of the alfa-sarcin antiserum with restrictocin and mitogillin (6).

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