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AMINO TERMINAL AMINO ACID SEQUENCE OF MACROMOMYCIN, A PROTEIN ANTITUMOR ANTIBIOTIC

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SUMMARY

Forty-five amino acids at the amino terminus of macromomycin, a protein antitumor antibiotic, have the following sequence:
Ala-Pro-Gly-Val-Thr-Val-Thr-Pro-Ala-Thr-Gly-Leu-Ser-
Asn-Gly-Glu-Thr-Val-Thr-Val-Ser-Ala-Thr-Gly-Leu-Thr-
Pro-Gly-Thr-Val-Tyr-His-Gly-Glu-Ser-Ala-Val-Ala-Glu-
Pro-Gly-Val-Ile-Gly-Pro- .....
Of the first 31 residues in this sequence 16 are homologous with the amino terminal sequence in neocarzinostatin, a protein antitumor antibiotic which also degrades DNA. Because of this conservation of structure, this region of the molecule may be involved in the mechanism of action of these proteins.

INTRODUCTION

Macromomycin (MCR)*, a protein antitumor antibiotic isolated from Streptomyces macromomyceticus, has a molecular weight of 12,500 daltons. It was shown by Chimura and co-workers to increase the lifespan of mice bearing sarcoma 180 and L-1210 leukemia by 150%[1]. Subsequent studies showed MCR to be active against L-1210 and P-388 leukemias, B-16 melanomas, Lewis lung carcinoma, and TA3Ha mammary adenocarcinoma ascites cells [2-4]. MCR and neocarzinostatin (NCS)* belong to a group of antitumor antibiotics distinguished by their protein structure and their ability to degrade DNA.

* The abbreviations used are: MCR-macromomycin; NCS-neocarzinostatin; SDS-sodium dodecyl sulfate; GC-gas chromatography; PTH-phenylthiohydantoin.

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Although NCS has been studied extensively [5], little is known of the mechanism of action and structure of MCR.

Recently, it has been suggested that DNA degradation may be a primary mechanism of MCR action since it causes breakage of isolated and intracellular DNA at concentrations and time points comparable with those necessary for cytotoxicity [6-9]. Mechanisms such as interactions with the cell membrane have also been proposed [2]. To define the molecular mechanism of action of MCR it is important to determine the structures within the MCR molecule that interact at the cellular and molecular levels. This report presents the sequence of the amino terminal 45 residues of MCR and compares it to the analogous sequence in NCS.

MATERIALS AND METHODS

Beckman reagents (Beckman Instruments, Palo Alto, Calif.) were used in the Beckman 890B sequencer and 121MB amino acid analyzer. Glass distilled ethyl acetate was obtained from Burdick and Jackson (Muskegon, Mich.). Hydroiodic acid (58%) was purchased from Fisher Scientific (Houston, Tx.). All other reagents were analytical grade. Deionized and distilled H_2O was used in all operations.

MCR was purified from a lyophilized bacterial culture filtrate obtained from Bristol Laboratories (lot #W322G230). MCR activity was detected using the PM2 DNA breakage assay [9]. Crude material was dissolved in and dialyzed against buffer A (10 mM TRIS, pH 8.0) and applied to a DEAE CL Sepharose 4B column equilibrated with buffer A. Bound MCR was eluted with a linear 0-50 mM NaCl gradient. Fractions containing MCR were pooled and dialyzed against Buffer A. The DEAE step was then repeated and the pool of MCR-containing fractions was concentrated and dialyzed against buffer A. This fraction was passed through a Sephadex G50 gel filtration column equilibrated in buffer A. MCR-containing fractions were pooled, concentrated, and dialyzed against water. SDS* polyacrylamide gel electrophoresis [10] of 100 µg purified protein yielded only one commassie blue-stained band indicating >99.9% purity. Amino terminal analysis yielded only alanine as was found previously [9,11].

Samples of protein (4.5 - 12.5 mg) were treated in a Beckman 890B protein sequencer using a program similar to that of Brauer *et al* [12], except 0.5 M quadrol was used. Resulting phenylthiazolinone derivatives were converted to the PTH amino acid by the treatment with 0.1 M HCl for 10 minutes at 80° under N_2. Ethyl acetate-extracted PTH derivatives were analyzed directly by GC [13]. Amino acid analysis of free amino acids was performed after hydrolysis of the PTH derivative with 58% HI for 18 hours at 130° *in vacuo*.

TABLE I
IDENTIFICATION OF PTH AMINO ACIDS

Cycle Number	GC[1]	AAA[1,2]	(% Yield)[3]	Residue
1	Ala	Ala	(47)	Ala
2	Pro, Thr	Pro	(24)	Pro
3	Gly	Gly	(60)	Gly
4	Val	Val	(20)	Val
5	Pro, Thr	αAB[2]	(16)	Thr
6	Val	Val	(16)	Val
7	Pro, Thr	αAB	(16)	Thr
8	Pro, Thr	Pro	(16)	Pro
9	Ala	Ala	(29)	Ala
10	Pro, Thr	αAB	(12)	Thr
11	Gly	Gly	(17)	Gly
12	Ile, Leu	Leu	(20)	Leu
13	Ser	Ala	(18)	Ser
14	Asn	Asp	(6)	Asn
15	Gly	Gly	(14)	Gly
16	N/C[2]	Glu	(10)	Glu
17	Pro, Thr	αAB	(10)	Thr
18	Val	Val	(10)	Val
19	Pro, Thr	αAB	(10)	Thr
20	Val	Val	(11)	Val
21	Ser	Ala	(11)	Ser
22	Ala	Ala	(18)	Ala
23	Pro, Thr	αAB	(10)	Thr
24	Gly	Gly	(5)	Gly
25	Ile, Leu	Leu	(6)	Leu
26	Pro, Thr	αAB	(5)	Thr
27	Pro, Thr	Pro	(4)	Pro
28	Gly	Gly	(5)	Gly
29	Pro, Thr	Thr	(6)	Thr
30	Val	Val	(4)	Val
31	Tyr, Val	Tyr	(28)	Tyr
32	N/C	His	(4)	His
33	Gly	Gly	(5)	Gly
34	N/C	Glu	(3)	Glu
35	Ser	Ala	(5)	Ser
36	Ala, Ser	Ala	(7)	Ala
37	Val	Val	(3)	Val
38	Val, Ala	Ala	(5)	Ala
39	N/C	Val, Glu	(2)	Glu
40	Pro, Thr	Pro	(1)	Pro
41	Gly, Pro, Thr	Gly	(2)	Gly
42	Ala, Val	Val	(4)	Val
43	Ile, Leu	Ile	(0.3)	Ile
44	Gly	Gly	(3)	Gly
45	Pro	Pro	(0.2)	Pro

1. Selection of amino acid in GC analysis was by determining the peak with the most prominent change relative to the previous step. Choice of amino acid by amino acid analysis was the same as with GC but was corroborated by calculating quantities of all amino acids.
2. Abbreviations: AAA-amino acid analysis; N/C-no prominent changes detected; αAB-alpha amino butyric acid.
3. % yield is based on nmol of amino acid obtained by amino acid analysis and on total nmol protein in the sequencer.

Ala-Pro-Gly-Val-Thr-Val-Thr-Pro-Ala-Thr-
1 5 10

Gly-Leu-Ser-Asn-Gly-Glu-Thr-Val-Thr-Val-
11 15 20

Ser-Ala-Thr-Gly-Leu-Thr-Pro-Gly-Thr-Val-
21 25 30

Tyr-His-Gly-Glu-Ser-Ala-Val-Ala-Glu-Pro-
31 35 40

Gly-Val-Ile-Gly-Pro-
41 45

Figure 1. Sequence of amino terminal 45 amino acids of MCR indicating repeated sequences.

RESULTS AND DISCUSSION

The results of 45 cycles of Edman degradation are shown in Table I. Most residues were identified by both GC and amino acid analysis. Asparagine, glutamine, and serine were distinguished only by GC but their identification was corroborated by the appearance of aspartic acid, glutamic acid, or alanine, respectively, upon amino acid analysis.

A number of features of this sequence are noteworthy (Fig. 1). Of the 5-6 proline residues in MCR [2,14], four (residues 8, 27, 40, 45) are located in this portion of the molecule. Thus, this part of the molecule has little opportunity to form extensive ordered structure such as α-helix or β-pleated sheet. In addition, near the amino terminus are two sets of tetrapeptides with similar or identical sequences. Residues 9-12 and 22-25 have identical sequences while residues 4-7 and 18-21 differ by only one residue (threonine instead of serine).

NCS, the only other protein antitumor antibiotic whose complete amino acid sequence was recently determined [17], has been shown to degrade DNA, and has been reported to be active against human leukemias and hepatomas [5]. As shown in Figure 2, there are several similarities in the amino termini of MCR and

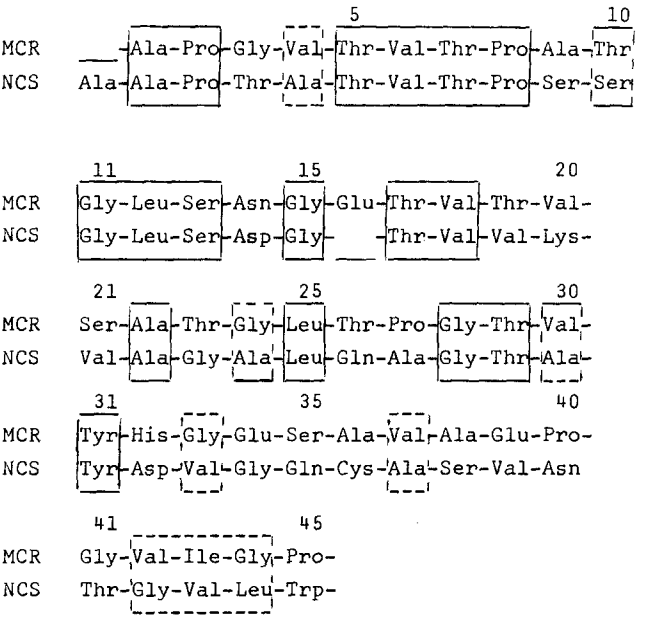

Figure 2. Comparison of amino terminal structures of MCR and NCS. Solid boxes indicate exact homology; dashed boxes indicate similar types of amino acid in same position.

NCS. Considering the indicated deletions, 16 of the first 31 residues in NCS (residues 1-2, 5-8, 11-13, 15, 17-18, 22, 28-29, 31) have the same sequences as in MCR. Five other residues in NCS (residues 4, 10, 24, 25, 30) are of the same type as in MCR. Of three tetrapeptides, residues 5-8 are identical while residues 10-13 and 28-31 differ by only one amino acid. Inasmuch as NCS has only one tyrosine [17], and MCR has only 2 or 3 tyrosine residues [11,14] the homology at residue 31 is particularly noteworthy. Since these two antibiotics both appear to degrade DNA [6-9, 15, 16] conservation of these sequences suggest that this region of the molecule may be involved in their mechanism of action.

REFERENCES

1. Chimura, H., Ishizuka, M., Hamada, M., Hori, S., Kimura, K., Iwanage, J., Takeuchi, T. and Umezawa, H. (1968) J. Antibiot. (Tokyo) 21: 44-49.

2. Kunimoto, T., Hori, M., and Umezawa, H. (1972) Cancer Res. 32: 1251-1256.

3. Lippman, M.M. (1974) Cancer Chemother. Rep. 58: 181-187.

4. Lippman, M.M., Laster, W.R., Abbott, B.J., Venditti, J., and Baratta, M. (1975) Cancer Res. 35: 939-945.

5. Legha, S.S., VonHoff, D.D., Slavik, M. and Muggia, F.M. (1976) Oncology 33: 265-270.

6. Sawyer, T.H., Crooke, S.T. and Prestayko, A.W. (1978) Proc. Amer. Assoc. Cancer Res. 19: 38.

7. Beerman, T.A. (1978) Biochem. Biophys. Res. Comm. 83: 908-914.

8. Suzuki, H., Nishimura, T., Moto, K. and Tanaka, N. (1978) J. Antibiot. (Tokyo) 31: 875-883.

9. Sawyer, T.H., Prestayko, A.W. and Crooke, S.T. (1979) Cancer Res. 39(4), in press.

10. Anderson, C.W., Baum, P.R. and Gesteland, R.R. (1973) J. Virol. 12: 241-252.

11. Yamashita, T., Naoi, N., Watanabe, K., Takeuchi, T. and Umezawa, H. (1976) J. Antibiot. (Tokyo) 29: 415-423.

12. Brauer, A.W., Margolies, M.M. and Haber, E. (1975) Biochem. 14: 3029-3035.

13. Pisano, J.J. and Bronzert, T.J. (1969) J. Biol. Chem. 24: 5597-5607.

14. Im, W.B., Chiang, C-K, and Montgomery, R. (1978) J.Biol. Chem. 253: 3259-3264.

15. Beerman, T.A. and Goldberg, I.H. (1977) Biochim. Biophys. Acta. 475: 281-293.

16. Beerman, T.A., Poon, R. and Goldberg, I.H. (1977) Biochim. Biophys. Acta 475: 294-306.

17. Maeda, H., Glaser, C.B., Kuromizu, K. and Meienhofer, J. (1974) Arch. Biochem. Biophys. 164: 379-385.