NH_2-TERMINAL SEQUENCE OF THE AMINOGLYCOSIDE ACETYLTRANSFERASE (3)-I MEDIATED BY PLASMID RIP 135

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1. Introduction

The therapeutic use of aminoglycoside antibiotics is frequently hampered by the occurrence of resistant bacteria which contain plasmid-mediated inactivating enzymes [1–3]. Among clinical isolates of bacteria resistant to gentamicin, acetylation of the 3-NH_2 group of the deoxystreptamine moiety is a widespread type of modification [4,5]. Aminoglycoside acetyltransferases (3) isolated from various sources form distinct groups characterized by differences in substrate range and physical properties [6–8]. The enzyme mediated by plasmid RIP 135 [9], for example, can modify gentamicin but not tobramycin or neomycin, whereas the enzyme from *Pseudomonas aeruginosa* PST can inactivate all these antibiotics. It is not known whether enzymes with different substrate specificities have evolved from one enzyme by mutational alteration, if they are derived from a common bacterial origin, or if they are the products of unrelated genes.

We have been investigating the evolutionary origin and extent of transfer of aminoglycoside modifying enzymes. Unfortunately, the difficulties encountered in purifying the acetyltransferases, in particular their instability, make it improbable that the substantial quantities of homogeneous enzymes necessary for complete amino acid sequence determinations will be obtained. However, the gene coding for the aminoglycoside acetyltransferase(3)-I mediated by plasmid RIP 135 has recently been cloned onto the colicin E1 plasmid (Haas, M. J., unpublished), and it is now feasible to determine its DNA sequence. We report here the NH_2-terminal sequence of AAC(3)-I in order to (1) facilitate locating the origin of the nucleic acid sequence which codes for its structural gene, and (2) probe its relationship to the acetyltransferases which modify the 6′-NH_2 and 2′-NH_2 groups of aminoglycosides [3,11–13].

Abbreviations: AAC(3)-I, aminoglycoside acetyltransferase(3)-I; PTH, phenylthiohydantoin, RCM, reduced and carboxymethylated

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2. Materials and methods

AAC(3)-I, a tetrameric protein of 63 000 molecular weight [10], isolated from *E. coli* RIP 135/C600 was a gift of J. W. Williams, D. B. Northrop and M. J. Haas.

Twelve milligrams dry weight of purified enzyme were reduced and carboxymethylated as described [14], except guanidine hydrochloride (5 M) was used instead of 8 M urea. Dansyl amino terminal identification was carried out as described [15].

Amino acid sequence analysis was performed using a Beckman automatic sequencer 890 C and the Beckman DMAA peptide program in the laboratory of R. D. Cole. Identification of PTH-amino acids was carried out by (1) gas-liquid chromatography [16] on a Varian gas chromatograph 1840 A; (2) high-pressure liquid chromatography [17] on a Waters Associates liquid chromatograph with a μBondapak C_{18} column; (3) amino acid analysis after HI hydrolysis [18] on a Beckman 121 automatic amino acid analyzer.

3. Results and discussion

The amino acid composition of RCM-AAC(3)-I agreed with published values [10]. Dansyl N-terminal analysis of the preparation gave a spot corresponding to methionine sulfone.

One hundred and thirteen nanomoles of RCM-AAC(3)-I were sequenced through 40 cycles in a single run using the automatic sequencer. The sequence of the NH_2-terminal 21 residues is shown in fig.1. The yield of leucine at the nineteenth cycle was approximately 14 nmol. Residue 14 could not be identified by any of the three methods used; we could not eliminate the possibility that it was methionine or tryptophan.

The partial sequence reported here is the first sequence determination of an aminoglycoside modifying enzyme. Comparison of the AAC(3)-I sequence with NH_2-terminal published data on several plasmid-mediated chloramphenicol acetyltransferases [19] and carbohydrate binding proteins [20] did not uncover evidence of any sequence resemblance.

Immunological studies using antiserum to AAC(3)-I indicated that it shares some homology with other plasmid-mediated 3-*N*-acetyltransferases of narrow substrate specificity (e.g. R1033 from *P. aeruginosa* [21]) and with the 2′-NH_2 aminoglycoside acetyltransferases (White, T. J., unpublished; [3]). However, no immunological cross-reactivity could be detected between AAC(3)-I and either: the AAC(3) enzymes having a broader substrate specificity or the 6′-NH_2 acetyltransferases (Haas, M. J. and White, T. J., unpublished). Additional sequence studies at the protein and nucleic acid level will be necessary to understand the evolutionary relationships among these diverse inactivating enzymes.

1 5
Met - Leu - Arg - Ser - Ser -
6 10
Asn - Asp - Val - Thr - Gln -
11 15
Gln - Gly - Ala - X - Pro -
16 20
Lys - Thr - Lys - Leu - Gly - Gly -

Fig.1. NH_2-terminal sequence of aminoglycoside acetyltransferase(3)-I mediated by plasmid RIP 135.

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