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NMR identification of ligands of aminoglycoside resistance enzymes

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Abstract

Bacterial resistance to aminoglycosides is mainly the result of enzyme-catalyzed chemical modifications of these antibiotics, which prevents their binding to their target. In order to circumvent this mechanism, an attractive possibility would be to block these enzymes, using selective inhibitors. This work describes a rational strategy aimed at isolating specific ligands of these enzymes, using NMR spectroscopy. Using magnetization transfer techniques, the identification of contacts between elementary pharmacophores and the protein target allows the guidance of hit improvement from a very early stage. ***To cite this article: F. Maurice et al., C. R. Chimie 9 (2006)***.

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Résumé

La résistance bactérienne aux aminoglycosides est principalement la conséquence de l'action d'enzymes qui modifient chimiquement ces antibiotiques et les empêchent ainsi de se lier à leur cible. Pour contourner ce mécanisme, une possibilité intéressante consisterait à bloquer l'action de ces enzymes au moyen d'inhibiteurs sélectifs. Nous décrivons une approche rationnelle utilisant la spectroscopie RMN pour isoler des ligands spécifiques de ces enzymes. En utilisant des techniques de transfert d'aimantation, l'identification de contacts entre des pharmacophores élémentaires et la cible protéique permet de guider de manière précoce le processus d'amélioration des touches obtenues. ***Pour citer cet article : F. Maurice et al., C. R. Chimie 9 (2006)***.

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1. Introduction

Aminoglycosides are broad-spectrum antibiotics which act by binding to the decoding site of ribosomal 16S RNA [1,2], they are used with β-lactams in polytherapies against severe infections caused by gram-negative bacteria and staphylococci, mostly of nosocomial origin. Resistance to these antibiotics arise mainly through the action of aminoglycoside-modifying enzymes which catalyze the covalent addition of acetyl, phosphate or nucleotidyl groups onto amino or hydroxyl functions [3]. Among the modifying enzymes found in clinical strains, *N*-6′ aminoglycoside acetyl transferases (AAC(6′)) are the most prevalent ones, accounting for 50–75% of the resistance phenotypes (their mechanism is shown in Fig. 1A). These have been classified in subfamilies (AAC(6′)-I, AAC(6′)-II, AAC(6′)-III and AAC(6′)-IV, see [2]), based on their specificity profile vs. the various clinically used aminoglycosides (Gentamicin, Amikacin, Isepamicin...). This picture has recently been getting more complicated, as very broad-spectrum variants have begun to emerge [4], which confer resistance to almost all aminoglycosides and are thus a serious threat to current therapies.

In order to circumvent this problem, it would be desirable to either isolate new aminoglycosides, which escape the current resistance mechanisms or to design specific inhibitors of their modifying enzymes. Up to now, however, progress in these two directions has been hampered by the difficulties of aminoglycoside chemistry. These result from the combination of two factors: the large number of functional groups and chiral centers in these molecules and their intrinsic symmetry (see for instance kanamycin, Fig. 1B) and in particular that of the central ring, 2-deoxystreptamine (2-DOS), a *meso* compound. Synthetic routes to this compound are known but are still quite involved (reviewed in [5]) and attempts to replace it with alternate, simpler scaffolds have so far only been moderately successful [6]. There is thus a need for an efficient 2-DOS mimic, as a building block for aminoglycoside resistance enzyme inhibitors. The present work describes the identification of such a compound and of an NMR-based strategy to characterize derivatives of this molecule with improved affinities to one of the new extended-spectrum, clinical forms of AAC(6′), AA(6′)-Ib_{11} [4].

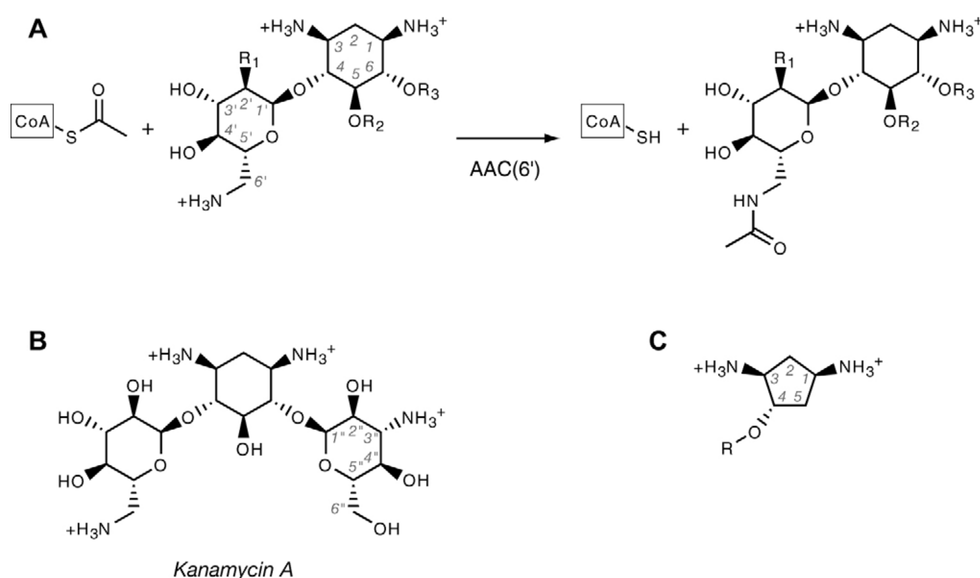

Fig. 1. (**A**) Aminoglycoside acetylation reaction catalysed by AAC(6′) enzymes. Shown is the common core structure, which corresponds to neamine. Amines are shown in protonated form, as they usually are at physiological pH. CoA stands for Coenzyme A. R1 is either –OH or –NH_2. Aminoglycosides are either substituted at R2 or R3, yielding the neomycin and kanamycin family, respectively. (**B**) Structure of kanamycin A, showing the quasi-symmetry about the median plane of deoxystreptamine ring. (**C**) Generic structure of the deoxystreptamine analogues used in this study.

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