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Research paper

Inhibition of 7,8-diaminopelargonic acid aminotransferase from *Mycobacterium tuberculosis* by chiral and achiral anologs of its substrate: Biological implications

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ABSTRACT

7,8-Diaminopelargonic acid aminotransferase (DAPA AT), a potential drug target in *Mycobacterium tuberculosis*, transforms 8-amino-7-oxononanoic acid (KAPA) into DAPA. We have designed an analytical method to measure the enantiomeric excess of KAPA, based on the derivatization of its amine function, by *ortho*-phtalaldehyde and *N*-acetyl-L-cysteine, followed by high pressure liquid chromatography separation. Using this methodology and enantiopure samples of KAPA it appeared that racemization of KAPA occurs rapidly (half-lives from 1 to 8 h) not only in 4 M HCl but more importantly in the usual pH range, from 7 to 9. Furthermore, we showed that racemic KAPA, and not enantiopure KAPA, was used in all previous studies. The only valid enantioselective synthesis of KAPA is that reported by Lucet et al. (1996) *Tetrahedron: Asymmetry* 7, 985–988. KAPA is produced as a pure (*S*)-enantiomer by KAPA synthase and by microbial production and DAPA AT only uses (*S*)-KAPA as substrate. However, (*R*)-KAPA is an inhibitor of this enzyme. It binds to the pyridoxal 5′-phosphate form (K_{i1} = 5.9 ± 0.2 μM) and to the pyridoxamine 5′-phosphate form (K_{i2} = 1.7 ± 0.2 μM) of *M. tuberculosis* DAPA AT. Molecular modeling showed that (*R*)-KAPA forms specific hydrogen bonds with T309 and the phosphate group of the cofactor of DAPA AT. Desmethyl-KAPA (8-amino-7-oxooctanoic acid), an achiral analog of KAPA, is also a potent inhibitor of *M. tuberculosis* DAPA AT. This molecule binds to the enzyme in a similar way than (*R*)-KAPA with the following constants: K_{i1} = 4.2 ± 0.2 μM, and K_{i2} = 0.9 ± 0.2 μM. These findings pave the way to the design of new antimycobacterial drugs.

1. Introduction

The quest for new antibiotics directed against resistant strains of pathogenic bacteria is, at present, of crucial importance [1]. This is particularly true in the case of *Mycobacterium tuberculosis*, the causative agent of tuberculosis, a major infectious disease. According to the World Health Organization (WHO) this disease still causes 2 million deaths per year, worldwide [2]. Furthermore, the emergence of multi-drug resistant strains might compromise the eradication of this old disease.

The biosynthesis of biotin, a cofactor for carboxylases, has been identified as a possible target for antibiotics directed against mycobacteria. This cofactor is biosynthesized from pimeloyl-CoA in four steps as depicted in Fig. 1 and an inhibitor of any of these four steps can potentially be an antibiotic. The biosynthetic enzymes of this pathway, from *Escherichia coli* and *Bacillus sphaericus*, have been well studied [3] and, more recently, the diaminopelargonic acid aminotransferase (DAPA AT) [4–6], and the 8-amino-7-oxononanoic acid (KAPA) synthase [7] from *M. tuberculosis*, have been purified and characterized. A recent report has validated, by gene inactivation in *Mycobacterium smegmatis*, the step catalyzed by DAPA AT as a potential therapeutic target [8] and we have shown that the DAPA AT from *M. tuberculosis* is inactivated by amiclenomycin, a natural antibiotic, and by synthetic analogs. One of the analogs completely inhibited the growth of *M. smegmatis* cells at 100 μg mL^{-1} suggesting that these DAPA AT inhibitors were potentially new leads to antibiotics against mycobacteria [5].

While DAPA AT has been studied by different groups [4–6,9–17], there is still some confusion, in the literature, regarding the enantiomeric purity of the substrate KAPA and its configuration.

Abbreviations: AdoMet, *S*-adenosyl-L-methionine; AdoMTOB, *S*-adenosyl-(4-methylthio-2-oxobutanoic acid); DAPA, 7,8-diaminopelargonic acid (7,8-diaminononanoic acid); DAPA AT, 7,8-diaminopelargonic acid aminotransferase; DTB, dethiobiotin; ee, enantiomeric excess; EPPS, (*N*-(2-hydroxyethyl)piperazine-*N*′-(2-propanesulfonic acid); KAPA, 8-amino-7-oxononanoic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; OPA-NAC, *ortho*-phtaladehyde-*N*-acetyl-L-cysteine reagent; PDP, Protein Data Bank; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate.

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Enzymes

EC 2.6.1.62	S-adenosyl-L-methionine:8-amino-7-oxononanoate aminotransferase
EC 2.3.1.47	8-Amino-7-oxopelargonate synthase
EC 6.3.3.3	7,8-diaminononanoate:carbon-dioxide cyclo-ligase (ADP-forming), dethiobiotin synthase

Furthermore, this pyridoxal 5′-phosphate (PLP)-dependent enzyme is subjected to strong substrate inhibition by KAPA [5,10] but this inhibition has not been studied in details. We thus decided to study the effect of KAPA enantiomers and of achiral analogs of the substrate KAPA on the activity of this aminotransferase.

We report here an assay for the determination of the enantiomeric excess (ee) of KAPA samples. Using this simple method we confirm that natural KAPA is of (*S*)-configuration and that it is produced enzymatically and by microbial production as a pure enantiomer. We also show that KAPA racemizes easily in different conditions. Moreover, we report here that (*R*)-KAPA and desmethyl-KAPA, an achiral analog, strongly inhibit DAPA AT. The binding site for (*R*)-KAPA on *M. tuberculosis* DAPA AT has been delineated by molecular modeling. These interesting findings pave the way to the development of analogs of (*R*)-KAPA and desmethyl-KAPA as new inhibitors of DAPA AT for the design of antibiotics directed against mycobacteria.

2. Material and methods

2.1. Materials

UV–visible spectra were obtained on an Uvikon-930 Kontron (Bioserv, Thiais, France) spectrophotometer or a Lambda-40 Perkin Elmer (Norwalk, CT, USA) apparatus. Centrifugations were run on a Sorval RF5plus centrifuge (Kendro, Courtaboeuf, France) or on an Eppendorf Centrifuge 5415D (Eppendorf, Le Pecq, France). HPLC separations were achieved on a Waters 1525 system equipped with a Waters 2487 detector (Waters, Saint Quentin-en-Yvelines, France). NMR spectra were recorded on a Brücker Avance 400 spectrometer and mass spectrometry analyses were performed on a Thermo Scientific ITQ1100 apparatus with direct exposure probe. Melting points were measured with a Kofller hot bench.

2.2. Chemicals and biochemicals

(*R*)-HCl–KAPA (mp = 108 °C, $[\alpha]_D^{23}$ −46.1 (*c* 1.0, MeOH)) and (*S*)-HCl–KAPA (mp = 108 °C, $[\alpha]_D^{22}$ + 48.1 (*c* 1.0, MeOH)) were synthesized according to Lucet et al. [18] and were a kind gift of C. Mioskowski (CEA, Saclay, France). *Rac*-HCl–KAPA (mp = 139–140 °C) was obtained as already described [19,20]. (7*R*, 8*S*)-DAPA was a kind gift of J. Crouzet (Sanofi-Aventis, Vitry sur Seine, France). Pimeloyl-CoA was prepared as already described [19,20]. All other chemicals were purchased from Sigma–Aldrich (St Louis, MO, USA) and were of the highest purity available. Culture medium components were purchased from Difco Laboratories (Detroit, MI, USA).

2.3. Syntheses

8-amino-7-oxooctanoic acid hydrochloride, **3**, and 8-amino-8-methyl-7-oxononanoic acid hydrochloride, **6**, were synthesized according to the method already described [21] but with slight modifications (see Fig. S1).

2.3.1. Ethyl 4-[(tert-*butoxycarbonyl)amino]-3-oxobutanoate* ***1***

To a solution of Boc-glycine (9 g, 51.4 mmol) in dry tetrahydrofuran (300 mL) under argon and stirring, a solution of 1,1′-carbodiimidazole (10 g, 61.7 mmol) in dry tetrahydrofuran (30 mL) was added dropwise at room temperature. The mixture was stirred

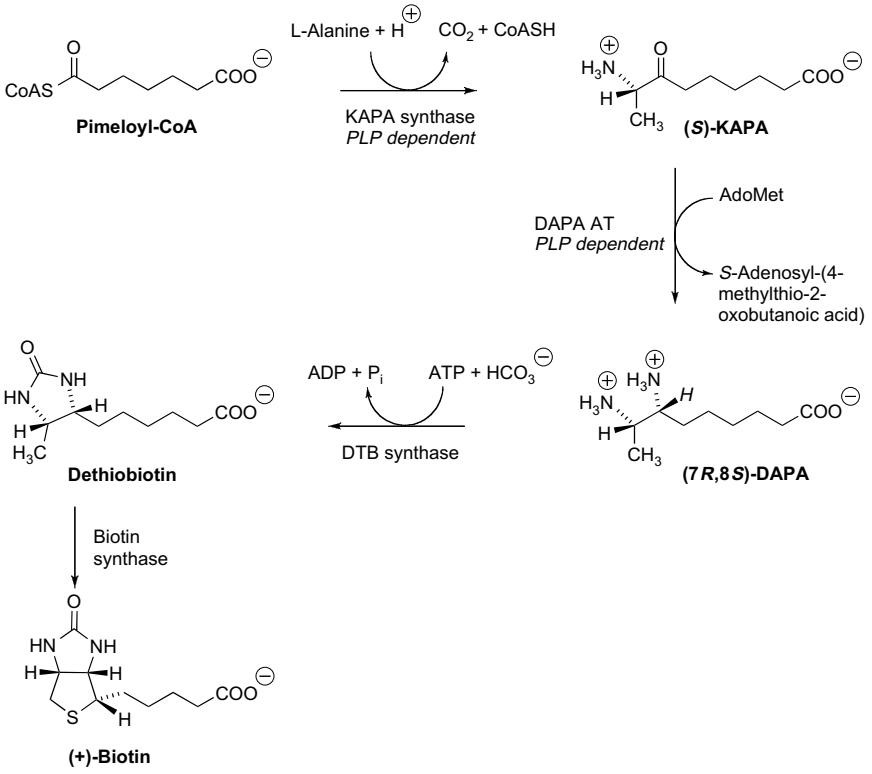

Fig. 1. Biosynthetic pathway to biotin. The structures were drawn to emphasize on the stereochemical outcome of the reactions.