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Purification and characterization of aminoglycoside phosphotransferase APH(6)-Id, a streptomycin-inactivating enzyme

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Abstract As part of an overall project to characterize the streptomycin phosphotransferase enzyme APH(6)-Id, which confers bacterial resistance to streptomycin, we cloned, expressed, purified, and characterized the enzyme. When expressed in *Escherichia coli*, the recombinant enzyme increased by up to 70-fold the minimum inhibitory concentration needed to inhibit cell growth. Size-exclusion chromatography gave a molecular mass of 31.4 ± 1.3 kDa for the enzyme, showing that it functions as a monomer. Activity was assayed using three methods: (1) an HPLC-based method that measures the consumption of streptomycin over time; (2) a spectrophotometric method that utilizes a coupled assay; and (3) a radioenzymatic method that detects production of ^{32}P-labeled streptomycin phosphate. Altogether, the three methods demonstrated that streptomycin was consumed in the APH(6)-Id-catalyzed reaction, ATP was hydrolyzed, and streptomycin phosphate was produced in a substrate-dependent manner, demonstrating that APH(6)-Id is a streptomycin phosphotransferase. Steady-state kinetic analysis gave the following results: K_m(streptomycin) of 0.38 ± 0.13 mM, K_m(ATP) of 1.03 ± 0.1 mM, V_{max} of 3.2 ± 1.1 μmol/min/mg, and k_{cat} of 1.7 ± 0.6 s^{-1}. Our study demonstrates that APH(6)-Id is a bona fide streptomycin phosphotransferase, functions as a monomer, and confers resistance to streptomycin.

Keywords Streptomycin · Antibiotic resistance · Aminoglycoside phosphotransferase · APH(6)-Id

Introduction

The aminoglycoside antibiotic streptomycin (Sm), discovered by Waksman and coworkers in the 1940s [1], has been used clinically in combination with other drugs for the treatment of pulmonary tuberculosis caused by *Mycobacterium tuberculosis* infection. Although Sm is still used in some developing countries, its use has diminished in recent years due to its toxicity and the fact that many bacteria have become resistant to it. Sm acts by binding to the bacterial 16S ribosomal RNA (rRNA), thereby interfering with both the binding of transfer RNA (tRNA) to the ribosomal A-site and the proofreading step of protein translation [2]. The disruption of protein biosynthesis, in turn, causes permeabilization of the cell membrane, which is responsible for much of streptomycin's bactericidal effects [3]. Resistance can arise through mutations in protein or RNA components of the ribosome, but more commonly resistance to Sm (and other aminoglycoside antibiotics) occurs due to modification of the antibiotic through phosphorylation, acetylation, or adenylation mediated by a specific kinase (aminoglycoside *O*-phosphotransferase, APH), *N*-acetyltransferase (AAC), or *O*-nucleotidyltransferase (ANT), respectively, encoded by genes present in the resistant bacteria [4].

Sm is produced by the soil-dwelling Gram-positive bacterium *Streptomyces griseus*, which also contains the gene for an enzyme, APH(6)-Ia, that protects the organism against the toxic effects of its own antibiotic [5, 6]. The recombinant APH(6)-Ia protein has been expressed in *Escherichia coli* [7] and the native enzyme has been

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