Effect of Divalent Metal Ion on the Structure, Stability and Function of *Klebsiella pneumoniae* Nicotinate-NucleotideAdenylyltransferase: Empirical and Computational Studies

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The continuous threat of drug-resistant *Klebsiella pneumoniae* justifies identifying novel targets and developing effective antibacterial agents. A potential target is nicotinate nucleotide adenylyltransferase (NNAT), an indispensable enzyme in the biosynthesis of the cell- dependent metabolite, NAD^+. NNAT catalyses the adenylation of nicotinamide/nicotinate mononucleotide (NMN/NaMN), using ATP to form nicotinamide/nicotinate adenine dinucleotide (NAD^+/NaAD). In addition, it employs divalent cations for co-substrate binding and catalysis and has a preference for different divalent cations [1]. In this study, we described the biophysical structure of NNAT from *K. pneumoniae* (KpNNAT) and the impact of divalent cations on its activity, conformational stability and substrate-binding using experimental and computational approaches. The experimental study was executed using an enzyme-coupled assay, far-UV circular dichroism, and thermal shift assays, alongside molecular dynamic simulation. KpNNAT showed maximum activity and minimal conformational changes with Mg^{2+} as a cofactor compared to Zn^{2+}, Cu^{2+} and Ni^{2+}. The binding of the substrate ATP affects KpNNAT dynamics, while the dynamics of ATP binding depend on the presence and type of divalent cation. The data presented in this study facilitates a better understanding of how divalent cations can impact the mechanism of NNAT, and hence their preferences for specific divalent cations. It also answers the proposition that variation in divalent cation specificity disturbs NNAT structure and contributes to their functioning. The information obtained from this study would serve as a basis for further evaluation towards designing structure-based inhibitors with therapeutic potential.

Reference

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