Mycobacterium tuberculosis PROTEIN TYROSINE KINASE A (PTKA) AUTOPHOSPHORYLATION IS INHIBITED BY S-NITROSYLATION

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Introduction: Tuberculosis continues to be one of the most notorious infectious diseases caused by Mycobacterium tuberculosis and yet remains as a serious public threat. PtkA, an odd kinase from this parasite, is able to phosphorylate PtpA and increase its activity, thus favouring the establishment of the parasite inside the human macrophage. NO radicals are produced by macrophage in response to the infection and are likely to interfere with the infection processes. In this picture, PtkA emerges as a potential target of NO and its derivatives. Material and Methods: PtkA and its mutant were cloned expressed, purified, and exposed to GSNO in vitro assays. Its kinase and autophosphorylation activity as well as its structural integrity were assessed by mass spectrometry, circular dichroism and other biochemical tools. Results and Discussion: At first, a mass shift of about 80 Da was observed in the MS spectra of PtkA incubated with ATP, indicating the addition of a phosphate molecule to its structure. The addition of this molecule also caused a structural change in PtkA folding, as depicted by circular dichroism. Interestingly, after incubation with GSNO this mass addition was prevented and its effects on secondary structure partially resumed. Our results also pointed out that PtkA was a subject of S-nitrosylation, which indicates that the autophosphorylation activity of PtkA is inhibited by the previous S-nitrosylation of its unique Cys61 residue. Further experiments are still required to proper address this preliminary conclusion. However, its implications are wide, since PtkA inhibition could impact PtpA phosphatase activity, whose disruption may lead to parasite death by macrophage digestion.

Keywords
S-nitrosylation, autophosphorylation, protein tyrosine kinase A

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