Nucleoside diphosphate kinase b (NDKb) is an enzyme responsible for the production of nucleoside triphosphates and is involved in many cellular processes. This protein is secreted and considered a pathogenic factor of intracellular pathogens including *Leishmania*. This work aims to perform site-directed mutations on the coding sequence of the *Leishmania major* NDKb (LmNDK) and investigate the effect of these mutations in the spectroscopic and functional properties. The recombinant native NDKb and mutants R17A, E28A, P95S, P100S-$\Delta$5 and $\Delta$5 were cloned in pET28a and purified by affinity chromatography from *E. coli* extracts. The solution characterization was carried out with the recombinant LmNDK and the P95S, P100S-$\Delta$5 and $\Delta$5-Cterm mutants. Circular dichroism (CD) and intrinsic fluorescence of tryptophan emission experiments showed LmNDK is more stable than described NDKs and that mutations, especially in the *Kpn* loop (P95S, P100S-$\Delta$5), decreased the structure stability against pH and guanidine solutions. The ANS binding showed, unlike other NDKs, LmNDK does not adopt a molten globule state during denaturation in contrast with P95S mutant in guanidine. The catalytic activity analysis of all enzymes showed the P95S had a similar LmNDK activity, but other mutants exhibited a decreased activity. All proteins presented a cytotoxic effect in the ATP presence, indicating that other functional activity can be performed by LmNDK, independent of the phosphate acceptor. In the donor and phosphate acceptor presence, the enzymes reduced the cytotoxic effect, especially those presenting an increased phosphotransfer activity.

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