Structural characterization of *Trypanossoma brucei* spliceosomal protein U5-15K

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INTRODUCTION: The parasites from *trypanosomatidae* family show highly conserved biochemical processes among them, such as the particular process of messenger RNA processing called trans-splicing, where the regions of two separated transcripts (instead of a single pre-mRNA) are bounded. All mature messengers are initialized by the same sequence (40 nucleotides) called splice leader and require ribonucleoproteins (snRNPs – U1, U2, U4/U6 e U5). U5-15k, U5 specific protein, is essential to mRNA processing as well as cell viability. **OBJECTIVE:** This work refers to the optimization of *Trypanosoma brucei* spliceosomal protein U5-15k expression and purification, as well as trials tracking crystallization conditions and self-cleavage tests. **MATERIALS AND METHODS:** The expression of U5-15k was accomplished using the construction U5-15k/pETSUMO in *Escherichia coli* (BL21 pLysS). The purification was based on affinity chromatography and the pure protein was used to perform tests in order to verify the self-cleavage as well as its inhibition, CD (circular dichroism) comparing the secondary structure composition of U5-15k before and after the cleavage, and DSF (differential scanning fluorimetry) to optimize the purification buffer. **RESULTS AND DISCUSSION:** Purified U5-15k incubated at room temperature for a period of five days showed a loss of approximately 46 amino acids. The same process was repeated using five potential inhibitors among which DTT was the most efficient. The CD experiments showed a secondary structure composition in agreement with the result predicted by Psipred. The same couldn't be done with the cleaved protein due to problems with the sample. The DSF experiments showed that U5-15k have more stability at low concentrations of salt and pH between 6,8 and 7,5. **CONCLUSION:** So far it's possible to affirm that *T. brucei*'s U5-15k presents self-cleavage activity which is inhibited by DTT. More experiments are required to identify the cleavage site such as mass spectrometry and the CD experiments need to be repeated.

Key words: U5-15k, trans-splicing, Trypanosoma brucei
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