Mass spectrometry as tool for single-domain antibody development against leishmaniasis

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Single-domain antibodies derived from camelids (VHH) have been shown to be promising for the generation of new biopharmaceuticals for the treatment of Neglected Tropical Diseases (NTD), including leishmaniasis. Adverse effects and the increasing parasite resistance to drugs instigate the search for safe and selective therapies. Given trypanothione reductase (TR) is a promising target for new antileishmanial agents, as well as biotechnological versatility of single-domain antibodies (VHH), this work aimed to use a peptide mapping approach to identify and to characterize VHH capable of inhibiting recombinant TR from *Leishmania braziliensis* (rTRLb) by LC-MS. For this, two variants of the VHH were incubated with RapiGest SF, reduced by adding dithiothreitol and alkylation with iodoacetamide. Tryptic digestion was performed at 37 °C for 16h. Peptides were analyzed by nanoLC-MS coupled to a Q Exactive™ Plus Biopharma mass spectrometer, a hybrid quadrupole-orbitrap analyzer and electrospray ionization source. Peptides were separated by reverse-phase chromatography, using an analytical column EASY-Spray™ PepMap RSLC (C18, 2 µm, 100A°, 75 µm x 50 cm). 1 uL samples were injected with a constant flow of 400 nL.min-1 and elution gradient was performed within the range 5-98 % of solvent B (ACN+0.1% AF) in solvent A (0.1% AF). Precursor mass was measured by an Orbitrap at 70,000 resolution, mass range 375-1500 m/z, AGC target $3x10^6$, in the positive ion mode with a capillary voltage of 2.0 kV. A data dependent acquisition analysis was performed with the 5 most abundant precursor ions selected for a high energy collision-induced dissociation fragmentation with a resolution of 17,500, AGC target $1x10^5$, injection time of 50 ms and dynamic exclusion of 10s. Data analyses were performed in PatternLab 5.0.0.141 and Biopharma Finder 5.1 software. Carbamidomethylation was defined as fixed modification, and protein abundance was classified by Normalized Spectral Abundance Factor (NSAF). The results from the MS study showed that we were able to confidently detect a significant number of peptides across two different anti-TRLb VHH variants with high confidence (FDR <1%). The accurate and reproducible confirmation of the sequence was achieved with 99% and 80% sequence coverage for clones 3RT01 and 3RT09, respectively. For 3RT01, we detected an average of 1058 peptides, including 173 unique ones. The NSAF was calculated to be 79.5%, and the molecular weight of these peptides was found to be approximately 15,921.7 Da. Additionally, the correct disulfide bond linkages at positions Cys24-97 were verified. In the case of the 3RT09, was discovered roughly 1490 peptides, comprising 132 unique ones. This sample had a higher NSAF value of 65.7%, indicating greater abundance compared to the previous sample. Its molecular weight was calculated to be approximately 16,163.8 Da, and only one instance of carbamidomethylation was found on cysteine 97. Mass spectrometry analysis provided detailed and reliable peptide identification of the anti-TRLb VHH antibody variants, with high sequence coverage for both clones, corroborating an important step towards the development of a promising therapeutic alternative for leishmaniasis.

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