ORT_18 - Standardization of the PEGylation reaction of aptamers for MultiDrug Resistant (MDR) Bacteria

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Introduction: MDR bacteria causes nosocomial and community-acquired infections associated with immunosuppressed patients. As few antibiotics are effective to treat these infections, the development of new therapies becomes important. Aptamers are molecules that bind to a specific target molecule with potential therapeutic use to control infections. Surface proteins of MDR bacteria are a possible target for aptamers that should be conjugated with polyethylene glycol (PEGylation) to increase their plasma half-life and clinical efficacy.

Objective: The objective of this project is standardizing the PEGylation reaction of a DNA aptamer which binds a surface protein from a MDR bacteria, developed in Bio-Manguinhos and the in-process analytical methods.

Methodology: The DNA aptamer (20KDa) was synthetized by SELEX method and reacted (200mM, PBS pH8.0) for 1h with 5 molar excess of PEG-Succinimidyl carbonate 12KDa (PEG-SCC). The resulting product was analyzed by analytical methodologies like agarose and polyacrylamide gel electrophoresis (12%) stained with Gel Red, iodine or imidazole plus zinc sulfate to identify the conjugate. Capillary electrophoresis (CZE) and Ion-exchange chromatography (IEX; Hitrap Q) also were used to analyze all molecules.

Results: Agarose gel stained with Gel Red detected a band with molecular weight higher than 20 KDa and consequently less electrophoretic mobility than the free aptamer. The same result was observed in gel stained with imidazole plus zinc sulfate. However, it was possible identify PEG-SCC only in gel stained with iodine, showing the limitation of these analytical techniques to discriminate all molecules involved in the reaction. Electropherograms obtained by CZE for PEG-SCC and aptamer showed peaks with good resolution. Unfortunately, a peak correspondent to the conjugate was not detected using the conditions studied. On the other hand, IEX technique showed good performance in resolve peaks corresponding to aptamer PEGylated, free aptamer and PEG-SCC.

Conclusion: The analytical methodologies suggested the success of the PEGylation reaction. CZE and IEX are promising techniques to monitor the conjugation reaction. The preparative IEX could be used to isolate aptamer PEGylated in a larger scale process.

Keywords: Multidrug resistant bacteria; Aptamer; PEGylation