Protein Disulphide Isomerase Purification From Stallion Epididymal Spermatozoa

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Protein disulphide isomerase is an important member of the synthesis route and structure-function maintenance of proteins in eukaryotes, by reduction, isomerization and oxidation of thiol groups (SH). Our research group has postulated that PDI would control the oxidation of -SH groups in sperm proteins during the epididymal maturation process. The aim of this work is to asses a protocol to purify protein disulphide isomerase from equine spermatozoa, through adaptation and standardization of the protocol established by Hilson *et al*, 1984. The epididymidis were removed by surgical castration, carefully dissected, and the luminal content obtained by incising and squeezing the tissue in PBS. The sperm samples were washed by centrifugation (760 x g for 10min / each) and sonicated for 30s (10 times) to obtain the cellular protein extract. Protein samples underwent heat treatment followed by ammonium sulfate fractionation to clean it from undesirable proteins. The next purification step consisted of an anion exchange chromatography that uses 20 mM sodium phosphate buffer pH 6.3. PDI whose isoelectric point is between 4,2-4,8 remains retained in the column and is eluted by a salt gradient. Every step of the purification process was monitored by SDS-PAGE. Electrophoretic analysis showed a relative increase of a 53 kDa band concentration. Our preliminary results has achieved a PDI enrichment of about 7-fold in comparison with samples without any treatment. After finished the purification process, our purpose is to assess the enzyme activity in immature and mature sperm cells obtained from the proximal caput and cauda epididymal regions.

Key words: protein disulfide isomerase, purification, spermatozoa.
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