ISOLATION AND CHARACTERIZATION OF PREDOMINANT PROTEINS IN HUMAN SEMINAL COAGULUM

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ABSTRACT

By using two-dimensional gel electrophoresis, human seminal coagulum revealed two major protein bands of 55 and 72 Kd whichwere heterogeneous and had a wide range in pI. Isolation of these two proteins was carried out by gel filtration. Gel filtration on Sephadex G-200 and Sepharose 4B in the presence of 5M urea provided better resolution than gel filtration on Sephadex G-100 in the presence of 5M urea or 4M guanidine HCl. The two major proteins were electrophoretically eluted from SDS-gels, a single band of each polypeptide was obtained when reanalysed on SDS-PAGE. The sialic acid contents were determined by using MBTH (3-methyl-2-benzothiazolinone hydrozone HCl), an average of 12.2 and 14.7 sialic acid residues per moles of 55 and 72 Kd polypeptides respectively were found. About 40% of bound sialic acid were released from seminal coagulum and two polypeptides (55 and 72 Kd) by neuraminidase treatment. Prior treatment of the seminal coagulum with neuraminidase before two dimensional gel analysis was able to reduce the heterogeneity in pI. When amino acid composition of seminal coagulum and two polypeptides of 55 and 72 Kd were analysed, it was found that they were similar. Asx, glx lys and Ser were the predominant amino acids.

When human seminal coagulum was digested with trypsin, it caused the degredative products similar to the pattern of seminal plasma protein at 10 minutes after ejaculation. Proteolytic degredative products of tryptic digestion of two polypeptides showed substantial similarity on two dimensional gel electrophoretic pattern. Chymotryptic digestion also showed the similarity of the two polypeptides.