Abstract

This bachelor thesis deals with isolation and partial characterisation of cow protease follicular fluids. At first, the follicular fluid was isolated according to molecular weights by means of gel chromatography on Sephadex G-100. As another method of separation there was selected the ion exchange chromatography on Sephadex DEAE. For the characterization of resulting fractions SDS electrophoresis, assessment of proteolytic activity of the proteolytic substrate azakasein, zymography and differential SDS electrophoresis were used. The collected fractions of the follicular fluid defined by the gel chromatography method had the relative molecular weights raging 114 000 – 131 000 in fraction pattern I, 44 000 – 51 000 in fraction pattern II, 151 000 – 204 000 in fraction pattern III, 57 000 – 99 in fraction pattern IV, 14 000 – 38 000 in fraction pattern V and 180 – 500 in fraction pattern VI.

From the results of the proteolytic activity I decided to work further only with fraction patterns III, IV and V collected from the gel chromatography and with the pattern of the delayed fraction PBS II collected from the ion exchange chromatography. These patterns reported the highest specific activity.

Inhibitors were used to determine which type of protease occurs in thee chosen fractions. In the fraction pattern III collected from the gel chromatography occurred aspartate proteases, metalloproteases and serine proteases; while the fraction IV contains serine proteases; fraction V contains aspartate and serine proteases. In the pattern of the delayed fraction PBS II collected from the ion exchange chromatography aspartate proteases, metalloproteases and serine proteases occurred.

By the means of the differential SDS electrophoresis there was observed the cow follicular fluid protein autolysis. The results show that the cow follicular fluid is subjected to autolysis, but autolysid needs a longer time to produce visible pattern.