

Human gastric juice contains mainly aspartic proteases - pepsin A and pepsin C. Both pepsins are produced by gastric mucosa as inactive pepsinogens (pepsinogen A and pepsinogen C) that differ in their physico-chemical and immunological properties. Both pepsinogens consist of molecular variants, isozymogens. Pepsinogens are activated to the corresponding pepsins in the acidic environment of the gastric lumen. (...) A subject of this Ph.D. thesis is a part of a long-term investigation that

focuses on the elaboration of methods for the separation of gastric aspartic proteases that would be suitable for monitoring of their changes in mentioned diseases. This thesis was mainly focused on preparation of affinity sorbents suitable for separation of pepsins and pepsinogens. The choice of ligands was based on the substrate N-acetyl-L-phenylalanyl-3,5-diiodo-L-tyrosine that is used to differentiate pepsin A and pepsin C. The following three affinity sorbents were prepared: iodinated L-tyrosine-Sepharose, 3,5-diiodo-L-tyrosine-Sepharose, and N-acetyl-L-phenylalanine-Sepharose. The basic characteristics of the prepared affinity sorbents were determined using the model enzyme (porcine pepsin A). The comparison of the chromatographic behavior of porcine pepsin A and its complex with pepstatine A showed that the enzyme active site was not involved in the enzyme interaction with ligands. In addition, participation of a phosphate group of the pepsin molecule was proved in the interaction of porcine pepsin A with N-acetyl-L-phenylalanine-Sepharose. All prepared sorbents were used for the separation of human pepsin A and pepsin C from acidified extract of gastric mucosa of patients with various types of diseases, eventually from gastric juice of healthy subjects. Out of the prepared sorbents, N-acetyl-L-phenylalanine-Sepharose was found to be the most suitable for this purpose.