Russian Journal of Bioorganic Chemistry, Vol. 26, No. 7, 2000, pp. 461–465. Translated from Bioorganicheskaya Khimiya, Vol. 26, No. 7, 2000, pp. 516–521.

Analytical Biotechnology of Recombinant Peptides and Proteins: II.[1] A Confirmation of the Primary Structure of Fusion Protein Containing Human Proinsulin and Optimization of Its Proteolysis by Trypsin

N. V. Sergeev, N. S. Glukhova, I. V. Nazimov,[2] V. A. Gulyaev, I. A. Donetskii, and A. I. Miroshnikov*

Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, GSP-7 Moscow, 117871 Russia

Received January 13, 2000; in final form, March 13, 2000

Abstract—The kinetics of trypsin proteolysis of the fusion protein (FP) containing human proinsulin was studied by a set of analytical micromethods. These were the microcolumn reversed-phase HPLC and the qualitative identification by MALDI-TOF mass spectrometry and amino acid sequencing. The first stage of the proteolysis was shown to be the cleavage of FP into the leader fragment and proinsulin. The subsequent splitting off of *C*-peptide from proinsulin results in the formation of Arg^{B31}-Arg^{B32}-insulin. The effect of temperature on the formation of de-Thr^{B30}-insulin, a by-product, was also studied. The structure of FP was confirmed by the peptide mapping technique, and the leader fragment was shown to contain no *N*-terminal Met residue.

Key words: analytical biotechnology; fusion protein, analysis; MALDI-TOF mass spectrometry; peptide mapping; recombinant proteins, analysis; reversed-phase HPLC