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GENERAL BIOLOGY

Analysis of a Regulatory Protein Isolated from the Bovine Prostate

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One of the main functions of the prostate is secretion. It is known that the reproductive status of the body is largely determined by the properties of the prostate secretion [1]. The secretion contains low-molecular-weight compounds (ions, citric acid, etc.) and high-molecular-weight proteins, including Zn-α–glycoprotein (MM = 34 kDa) [2], proteolytic enzymes, prostaglandins, prostate-specific antigen (PSA) (MM = 29 kDa) [2], prostate secretory protein (PSP94) (MM = 13 kDa) [3], and specific vesicles referred to as prostasomes [4, 5]. the functions of many secretion components are unknown. Therefore, it is important to study novel biologically active components of the prostate secretion. Earlier, we have showed that different tissues of animals and plants contain regulatory proteins exhibiting biological activity at very low concentrations (10^{-8} to 10^{-12} mg protein/ml). These proteins can influence important cellular processes, such as cell adhesion and migration, proliferation and differentiation, and protein synthesis [6–10].

In this work, we identified a protein that is active at very low concentrations in the mammalian prostate tissue. The protein studied was isolated from the bovine prostate by extraction of the prostate tissue under certain conditions and purified according to the method developed earlier. This purification method includes double salting-out of the tissue extract and isoelectrofocusing in a density gradient of sucrose [6, 7]. The fraction of the regulatory protein was studied in the pH range 4.38–5.05 [6]. The protein identification was performed by the adhesiometric method of biological testing developed for the identification of proteins from the group studied. We showed that this fraction had a membrane-tropic affect at very low concentrations *in vitro* [9]. The fraction was analyzed by electrophoresis in polyacrylamide gel in the presence of sodium dodecylsulphate or in the absence of the detergent [11].

The results shown in Figs. 1 and 2 demonstrate that the main component of the fraction studied was a protein with an Rf about 0.90 ± 0.05. The low-molecular-weight protein was isolated after electrophoresis in the absence of sodium dodecylsulphate and studied by biological testing [6, 7]. We found that the protein studied affected *in vitro* the plasmamembrane properties of mammalian cells at a concentration of 10^{-14} mg/ml. The data obtained suggest that the bovine prostate contains a protein possessing the physicochemical properties and biological activity that were very similar to the properties of the regulatory proteins isolated earlier from other tissues that had biological activity at very low concentrations [6–10]. The location of the regulatory protein was studied in the prostate tissue by immunohistochemistry [12]. The fraction of the regulatory protein obtained by isoelectrofocusing (pH 4.38–5.05) was used as an antigen to obtain rabbit

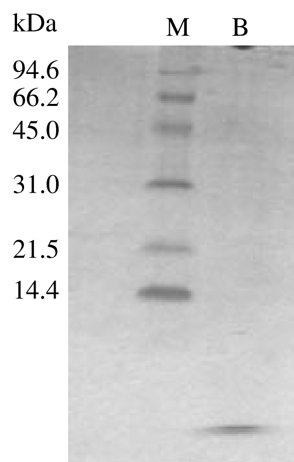

Fig. 1. The electrophoretogram of proteins separated in 15% polyacrylamide gel under denaturing conditions. M, marker proteins (Chelicon, Russia) with the following molecular weights (in kilodaltons): cellulase, 94.6; bovine serum albumin, 66.2; ovalbumin, 45.0; carbonic anhydrase, 31.0; trypsin inhibitor, 21.5; lysozyme, 14.4. B, the protein from the fraction with pH 4.38 – 5.05.

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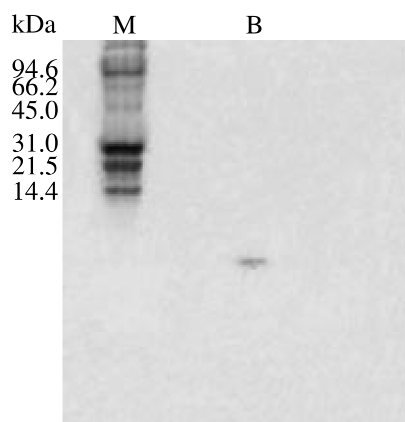

Fig. 2. The electrophoretogram of proteins separated in 7.5% polyacrylamide gel under native conditions. M, marker proteins (Chelicon, Russia) with the following molecular weights (in kilodaltons): cellulase, 94.6; bovine serum albumin, 66.2; ovalbumin, 45.0; carbonic anhydrase, 31.0; trypsin inhibitor, 21.5; lysozyme, 14.4. B, the protein from the fraction with pH 4.38–5.05.

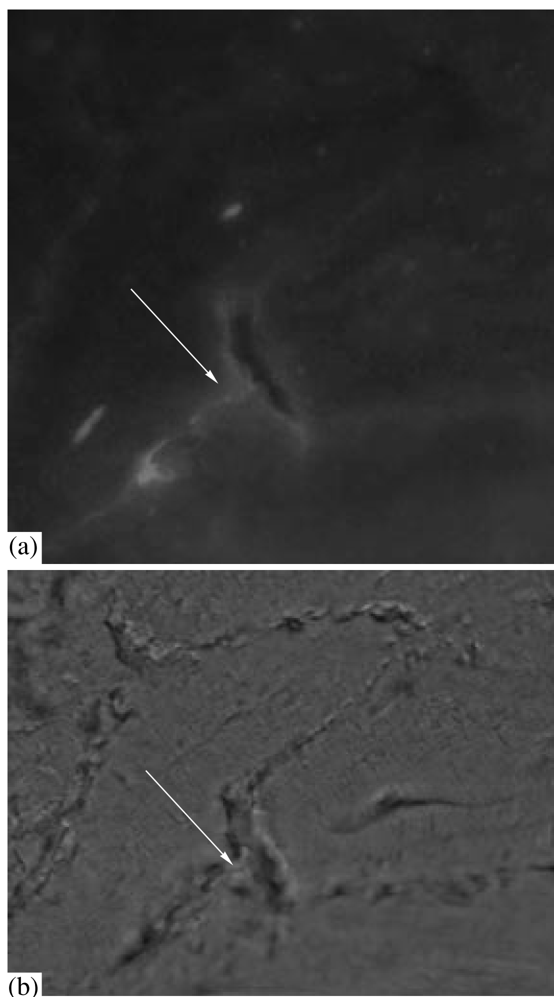

Fig. 3. The location of the protein from the fraction with pH 4.38–5.05 on the apical surface of the secretory epithelium of the mouse prostate (magnification, about ×10; lens, ×40). (a) The immunohistochemical reaction with the use of secondary FITC-labeled antibodies on the histological slice; (b) the histological slice, phase contrast.

serum containing polyclonal antibodies [13]. The presence of specific antibodies was determined by ELISA.

Earlier, it was found that the biological activity of regulatory proteins from the same group studied was characterized by tissue specificity but not species specificity [9]. Therefore, the location of the protein isolated from the bovine prostate was studied in the mouse prostate tissue by means of an immunohistochemical reaction using the obtained primary antibodies and commercial FITC-labeled secondary antibodies. The data shown in Fig. 3 suggest that the immunohistochemical reaction detected the location of the regulatory protein studied on the apical surface of the secretory epithelium that forms the walls of the prostate duct. Possibly, the protein identified was located on the outer surface of the plasma membrane of epithelial cells, and the secretion contained it. Taking into account the data obtained in the previous studies, which suggest that regulatory proteins of this group influence the physical and chemical properties of the body fluids [14], we may hypothesize that the regulatory protein isolated from the prostate may modulate the properties of the secretion.

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