Determination of lactoferrin in human prostate carcinoma cells

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Introduction

Lactoferrin is 80 kDa iron-binding glycoprotein with isoelectric point (pI) within the range from 8 to 8.5 [1]. This protein occurs in body fluids such as milk, saliva, tears, sweat, blood, urine [2], where it is involved in protection of the organism against bacterial infection [3]. Lactoferrin was also found in seminal vesicles in prostatic cancer pacients [4]. Its increased level is used as a marker of inflammation [5]. Nevertheless it was also studied as possible marker of some types of cancer. In addition it was suggested its antitumor activity in some cases [6]. Aim of this work was determination of lactoferrin from cultivated prostate cancer cells.

Results

We used ion exchange chromatography with monolithic column (IEC) (Fig. 1) and offline photometric detection [7]. Optimized condition for IELC was 25 mM Tris-HCl, pH = 7 as a mobile phase A (MFA) and 2 M NaCl in MFA as a mobile phase B (MFB) and flow rate 4 ml·min^{-1} (Fig. 2A) Separation was carried out under gradient elution. Isolated fraction of lactoferrin was analyzed on automated spectrophotometer using Bradford´s method. Purity of fraction was checked using SDS-PAGE gel electrophoresis (Fig. 2B). This method enabled us to determine very low levels of lactoferrin in samples. Observed limit of detection was 1 μg·ml^{-1} (Fig. 2C). Our results were correlated with ELISA method for lactoferrine analysis (Fig. 3) The corellation coefficient R^2 = 0.8446 was estimated.

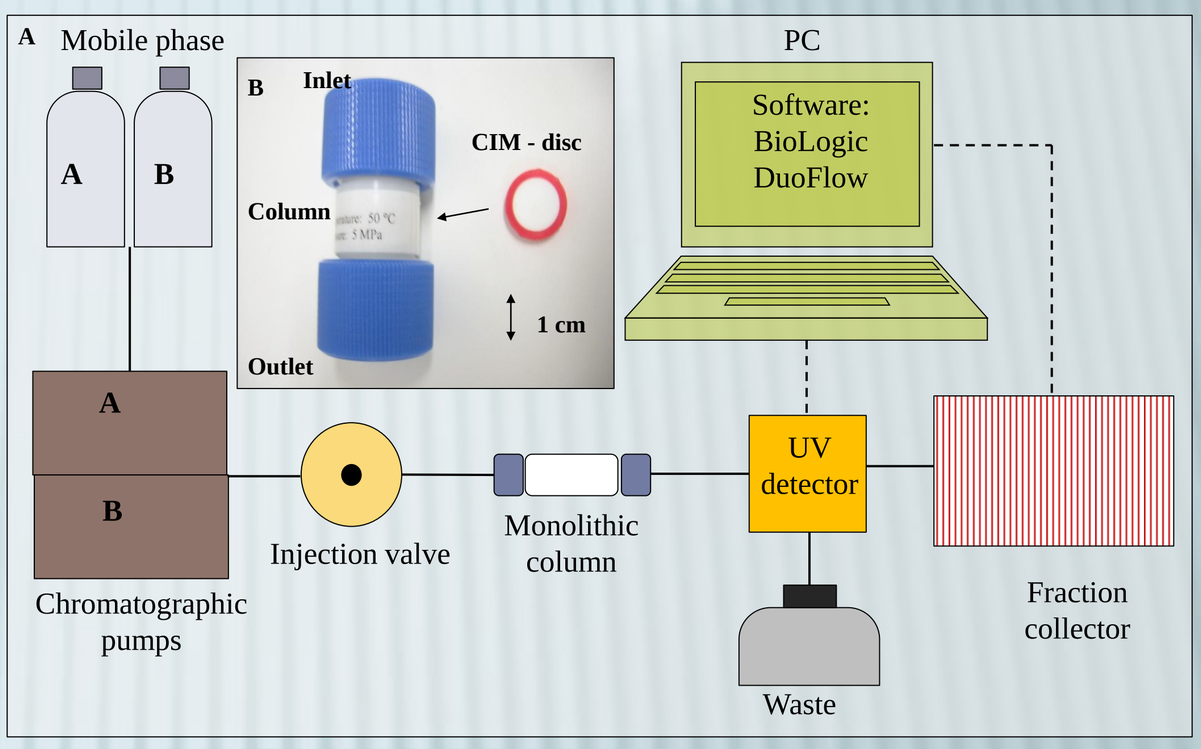

Fig. 1: A) Scheme of IEC. B) Monolithic column

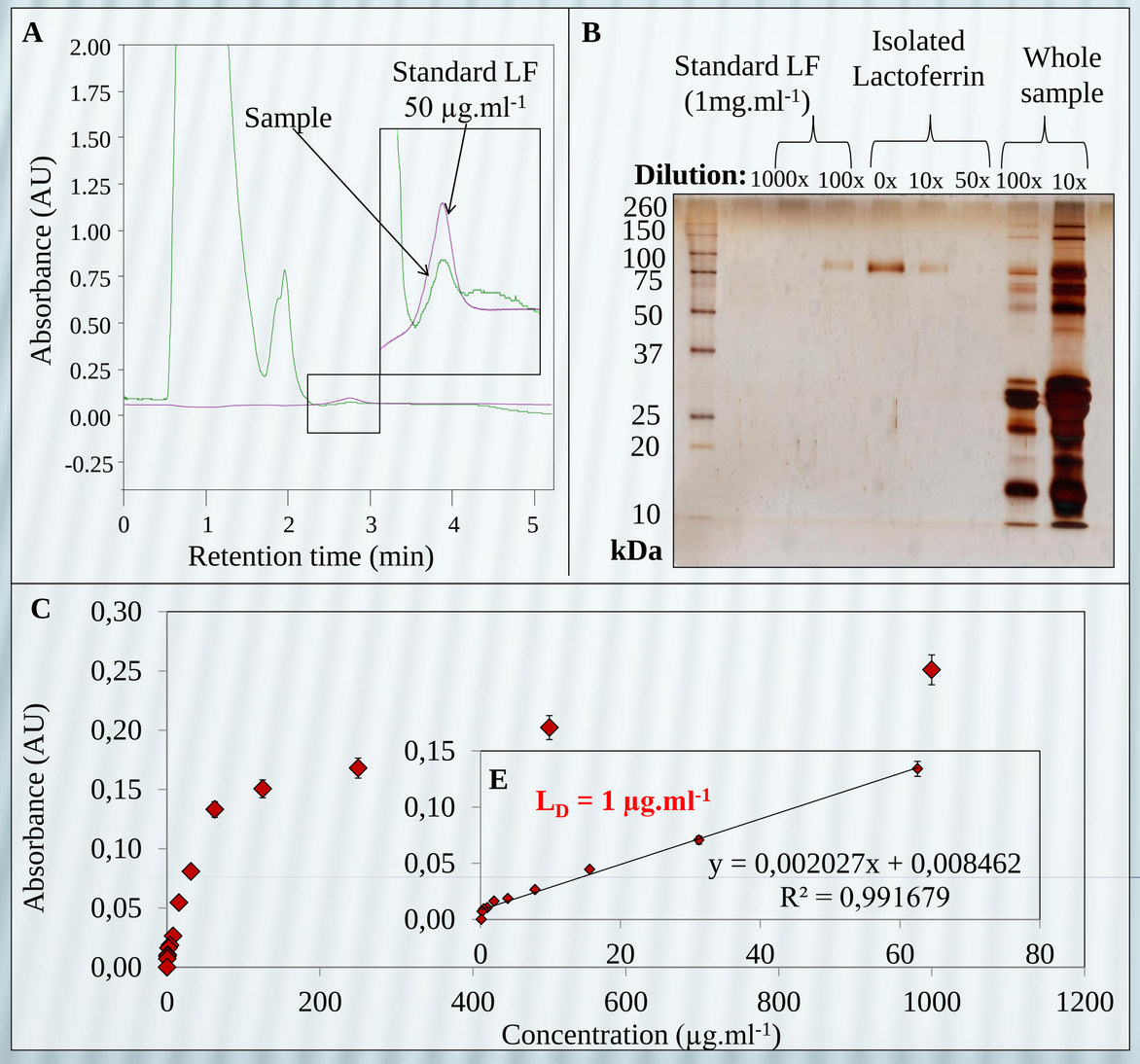

Fig. 2: **A**) Chromatogram of sample of prostatic cells and standard of LF. **B**) Electophoreogram of studied samples of prostatic cells. **C**) Obtained calibration curve of the method. The limit of detection 1 μg.ml^{-1} was estimated.

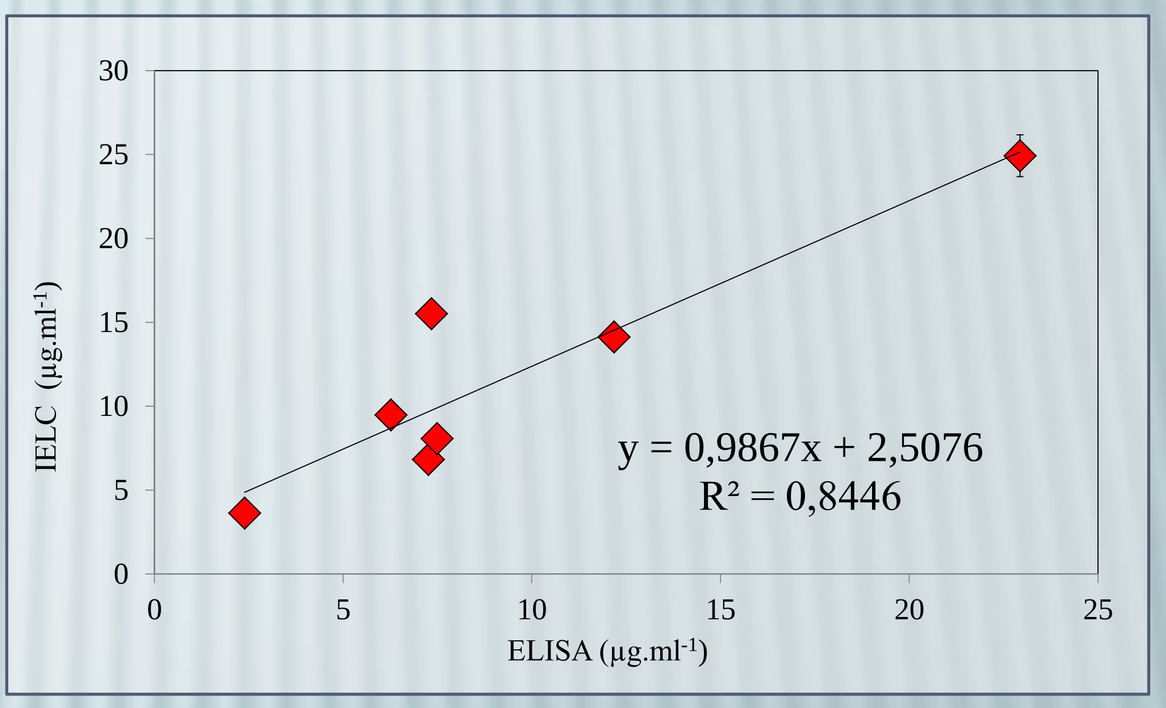

Fig. 3: Correlation curve of determined lactoferrin by ELISA and ion exchange liquid chromatography IEC method (n = 3)

Conclusions

We optimised method for lactoferrine determination using ion exchange liquid chromatography with off-line photometric detection. The purity of isolated lactoferrine was by SDS-PAGE verified. Limit of detection 1 μg.ml^{-1} was obtained.

Acknowledgement

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