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Purification of prothrombin complex proteins from human plasma in anion exchange resin using pseudoaffinity chromatography

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Background

Prothrombin complex contains the vitamin K dependent coagulation factors II, VII, IX, X, protein C, and protein S. It has been used for the treatment of congenital coagulation disorders and is recommended for reversing oral anticoagulation [1]. Prothrombin complex proteins require Ca^{2+} to express their activities. The conformational change induced by Ca^{2+} finds a practical application in the purification processes by modifying the affinity of these proteins to chromatographic resins. The elution of proteins by variation of calcium concentration is called chromatography of pseudoaffinity [2]. In this study we exploit this property of the vitamin dependent coagulation factors to develop a new method for purification of prothrombin complex proteins from human plasma using an anion exchange resin.

Methods

Plasma was directly applied to the ANX Sepharose FF column, previously equilibrated with citrate buffer 25 mM containing NaCl 85 mM and $CaCl_2$, pH 6. The unbound proteins were washed out with the same buffer. After a washing with citrate buffer containing NaCl 200 mM, elution was carried out in the same buffer with a linear calcium gradient from 2.5 mM to 25 mM. Three different buffers were tested: citrate, Bis-Tris and MES. Finally, column was washed with citrate buffer containing 500 mM NaCl. Chromatographic fractions were analyzed by: activity of Protein C using the chromogenic method as representative of the prothrombin complex proteins, protein content by the Bradford method, and SDS-PAGE.

Results and discussion

Protein C eluted within the $CaCl_2$ concentration range studied (2.5 to 25 mM). Using NaCl, this protein eluted only with a much higher salt concentration (> 250 mM), confirming that the mechanisms of elution with these 2 salts are different. It was also observed that a wash with 200 mM NaCl improved the purification. Therefore, the method combines conventional anion exchange with pseudoaffinity chromatography. Chromatograms of the experiments presented different profiles: citrate buffer presented 2 peaks, while Bis-Tris and MES presented only one, indicating that citrate buffer led to a better separation of the proteins. The SDS-PAGE gels showed that contaminant proteins coeluted with the prothrombin complex proteins, but in comparison to purifications with NaCl gradient, a much better purification was achieved with the $CaCl_2$ gradient. Further experiments will be performed to identify the $CaCl_2$ eluted proteins.

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