

L-ARGININE AND L-GLUTAMIC ACID INCREASE THE CONTENT OF PROTEIN C IN THE EARLY STAGES OF ISOLATION FROM DONOR PLASMA

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Current large-scale production of blood-derived pharmacological preparations is aimed at expanding the list of products and deeper extraction of target proteins especially at the prepurification stage. In particular, this problem becomes critical for the isolation of proteins like protein C (PC), which is present in plasma in trace amounts.

Aim. We aimed to improve the buffer composition to minimize the interaction of PC with other proteins and lipids that are inevitably present in the stock material.

Methods. The content of protein C in plasma and its derivatives was assessed by the amidolytic activity to the chromogenic substrate S2366. A decrease in homologous impurities and plasma enrichment with protein C was provided by selective bulk adsorption on DEAE-cellulose.

Results. Here we describe that an equimolar mixture of two amino acids (L-arginine and L-glutamic acid) essentially increased the content of protein C at the stage of cryo-depleted plasma prepurification, including initial dilution and subsequent enrichment of plasma with protein C due to selective bulk adsorption on DEAE-cellulose. Additionally, it was revealed that solutions of these amino acids, when combined, inhibit the induced amidolytic activity of protein C and increase its solubility (in contrast to other plasma proteases).

Conclusion. Pre-adding of a mixture of L-arginine and L-glutamic acid to cryo-depleted plasma as part of the working buffer significantly optimizes the prepurification of protein C, providing a 5-fold increase in its yield after elution from DEAE-cellulose.

Key words: protein C; donor plasma; fractionation; L-arginine; L-glutamic acid.

Protein C (PC) is a proenzyme of activated protein C (APC) (EC 3.4.21.69), a serine protease from the group of vitamin K-dependent enzymes. APC is a key natural anticoagulant in blood plasma, which, by inhibiting the activity of coagulation factors Va and VIIIa, regulates the reactions of the coagulation cascade and the process of thrombus formation in the vascular bed. Recent studies have also revealed coagulation-independent cytoprotective function of APC [1], which includes anti-apoptotic, neuroprotective, and endothelial barrier protective actions [2–6]. Recently, APC has been shown to inhibit the formation of extracellular neutrophil traps and the

release of cytokines by leukocytes [5–8]. In connection with epidemics of infectious diseases, especially with respect to COVID-19, the anti-inflammatory properties of APC are of particular importance [9–11]. Considering this, the improvement of the industrial production of protein C together with recombinant biotechnology remains a prerequisite for its wider availability for practical biomedicine.

In large-scale production, purified PC is usually obtained from human blood plasma by combining traditional techniques of Cohn (alcohol) fractionation with modern methods of ion exchange and affinity chromatography. The most attractive industrial techniques are

those that make it possible to separate PC at the early stages of complex processing of blood plasma without disrupting the purification scheme for albumin and immunoglobulins. However, unlike proteins, which are traditionally purified from blood plasma, PC belongs to the so-called minor components, the content of which does not exceed 1 mg per 100 ml of plasma. This feature determines more stringent requirements for the efficiency of PC purification in order to increase its final yield.

As evidenced in practice, the most significant PC losses occur at the initial stages of stock material processing, including the separation of the cryo-poor supernatant, its preliminary coarse purification from ballast impurities, and the concentrating of the target protein. Cryo-poor supernatant is a very complex mixture of proteins capable of aggregation, spontaneous activation, chemical or enzymatic denaturation and degradation.

Most of the existing commercial Cohn fractionation products typically contain protein aggregates up to 25% of the total protein concentration. This is facilitated by non-physiological conditions for the extraction of proteins with organic solvents (in particular, ethyl alcohol), which cause significant denaturation of enzymes and proenzymes [12]. Conformational changes in the structure of denatured proteins are accompanied by the exposure of an additional number of reactive sites to the periphery of the protein globule. In particular, the degree of hydrophobicity of the protein surface increases, which enhances adhesion to other macromolecules and colloidal particles.

Plasma colloids are known to be predominantly lipid-containing particles, such as lipid-albumin associates, lipid complexes with apolipoproteins (very low, low and high density lipoproteins), chylomicrons, as well as complex aggregates of free fatty acids, triglycerides, and phospholipids. So, blood plasma is an extremely favourable environment for hydrophobic immobilization of denatured proteins.

This state of the starting material, as a rule, does not allow overcoming 16–36% of the final yield of the purified product, regardless of the PC purification scheme, even if it includes the stage of immune-affinity chromatography [13, 14]. A decrease in the yield of the product at individual stages of purification appears to be the result of multiple interactions such as 1) retention of PC on the surface of proteins and phospholipids due to Ca^{2+} -dependent binding; 2) aggregation of PC

with other macromolecules due to hydrophobic interactions; 3) spontaneous activation of PC and subsequent complexation with substrate-like and inhibitor-like compounds; 4) its proteolysis and autodegradation.

From these properties of PC in concentrated protein solutions, it seems promising to use an equimolar mixture of two amino acids (L-arginine and L-glutamic acid) as an additive to increase its solubility. As described, such [15, 16] and other [17, 18] mixtures are used successfully in technologies for the isolation of recombinant proteins, which usually tend to aggregate and precipitate. So, the aim of this work was to test a buffer composition designed with L-Arg and L-Glu to minimize the interaction of PC with other proteins and lipids that are inevitably present in the stock material.

The presence of PC in protein mixtures is routinely monitored by determining the amidolytic activity of PC after its thrombin-independent activation (PCa). So, first of all, we checked how an equimolar mixture of L-Arg and L-Glu affects the amidolytic activity of PCa, induced by a specific non-physiological activator. Second, we wanted to study the effect of an equimolar mixture of L-Arg and L-Glu on the solubility of PC in cryo-plasma diluted with phosphate buffer in a 1:2 ratio. Third, we aimed to find out whether the presence of amino acid (AA) additives affects the ability of PC to adsorb on DEAE-cellulose, the anion-exchange sorbent.

Materials and Methods

Evaluation of the induced amidolytic activity of protein C. Crude protein C in human plasma and products of chromatography was activated by an activator from snake (s. *Agkistrodon Contortrix Contortrix*) venom in a ratio of 1:8, respectively. In this way, the available amount of the proenzyme PC was converted into an active state, PCa, which was detected by the hydrolysis reaction of the chromogenic substrate S2366. Since the reactivity of S2366 with PCa could interfere with other plasma proteases, the total rate of substrate hydrolysis was measured in two aliquots of plasma, adding the protein C activator to only one of them. Thus, the PCa-independent, total amidolytic activity of plasma proteases, P (without the addition of the PCa activator) and the total PCa-dependent activity, Pact (with the addition of the activator) were determined. Hence, the induced amidolytic activity of PCa was

determined as the difference between Pact and P ($PCa = Pact - P$).

In general, the amidolytic activity of P or Pact was determined in samples prepared in the wells of a microtiter plate by mixing 10 μ l of plasma and 80 μ l of 0.05 M Tris-HCl buffer (pH 8.1) or 80 μ l of snake venom activator diluted with 0.05 M Tris-HCl buffer (pH 8.1), respectively. Then, after 5 min of incubation, 40 μ l of chromogenic substrate S2366, pyroGlu-Pro-Arg-pNA * HCl (0.3 mM, final concentration) was added to the wells. An increase in colour due to the release of para-nitroaniline was recorded at 405 nm every five minutes for 20 min. The results were expressed in relative units of optical density (D_{405}) or in dD_{405}/min .

Plasma protein C solubility assay. The frozen pooled donor plasma was rapidly thawed at + 4 °C with constant stirring. After complete thawing, the plasma was centrifuged at 3000 g, 20 min, and then the sediment was discarded using cryo-poor plasma for further analysis. Namely, half of the plasma was diluted with buffer 1 (0.05 M sodium phosphate, pH 6.25, containing 0.15 M NaCl, 0.05 M L-Arg, 0.05 M L-Glu, 1mM benzamidine hydrochloride); the second half was diluted with buffer 2 (0.05 M sodium phosphate, pH 6.25, containing 0.15 M NaCl and 1 mM benzamidine hydrochloride). For a more complete dissolution of PC, plasma samples after dilution were left on a magnetic stirrer at + 4 °C for two hours. The solubility of PC (in%) was assessed by comparing the rates of hydrolysis of the chromogenic substrate S2366 in plasma samples before and after dilution, after induction of PCa activity with a snake (s. *Akistrodon Contortrix Contortrix*) venom activator.

DEAE adsorption. Fresh-frozen plasma were thawed at 2–4 °C and centrifuged at 3000 g for 30 min to isolate the cryoprecipitate. The supernatant (cryo-poor plasma) was diluted to protein concentration of 2.5–3.0 mg/ml with 0.1 M Na-phosphate buffer, pH 6.0, containing 0.1 M NaCl and 1 mM benzamidine HCl with or without adding mixture of the 0.05 M L-Arg and 0.05 M L-Glu. DEAE-Cellulose "Servacell" was equilibrated with the same working buffers and mixed with diluted cryo-poor plasma in 1:2 (v/v) ratios. After washing the sorbent with the working buffers by 3 cycles of centrifugation, protein C was eluted with the same buffers containing 0.3 M NaCl. To establish complete equilibrium, the adsorbent with elution buffer was placed in a refrigerator overnight. The eluate was separated by centrifugation at 3000 g for 20 min. Protein

concentration was determined by the Bradford method [19].

Western blot analysis. Plasma or eluate samples were diluted with denaturing 5 \times Laemmli electrophoretic buffer, containing β -mercaptoethanol in a final concentration 10% (v/v). After fractionation by electrophoresis in gradient 7–15% polyacrylamide gel, proteins were transferred onto the nitrocellulose membrane by the electroblot. Then, the membrane was blocked in 5% solution of skim milk and probed with rabbit primary anti-protein C antibodies (Sigma, product # P4680) (1:800 dilution) at 4 °C overnight. After thorough washing in phosphate buffered saline containing 0.05% Triton X-100 (PBST), the membrane was incubated with corresponding HRP-conjugated secondary antibodies for 90 min at 37 °C. Then, non-specifically bound antibodies were removed by 5-times washes in PBST for 5 min each. Specific immunostaining was developed by incubation of membranes in 0.05% 3,3'-diaminobenzidine in 50 mM Tris-HCl (pH 7.4), containing 0.01% hydrogen peroxide. Immunoreactive polypeptide bands of various molecular weights were identified by extrapolation to relative mobility of pre-stained proteins with known molecular weight (PageRuler Prestained Protein Ladder, Fermentas, Lithuania, cat. no. 26616).

Results and Discussion

A mixture of L-arginine and L-glutamic acid reduces the amidolytic activity of PCa in undiluted plasma. The catalytic domain of APC, like other serine proteases, contains an electron transfer system within the catalytic triad His211, Asp257 and Ser360. In addition, positively charged exosites are exposed around the perimeter of the active site [20, 21]. Therefore, it can be expected that a mixture of oppositely charged L-Arg and L-Glu will somehow modulate the catalytic activity of PCa.

To test this assumption, a mixture of L-Arg and L-Glu in 0.05 M Tris-HCl buffer (pH 8.1) together with a chromogenic substrate was added to the PCa-containing plasma samples, and then the rate of S2366 hydrolysis was compared with the control (reaction mixture without amino acids). As shown in the Fig. 1, the presence of AA decreased the optical density depending on the concentration. So, at the 10th min, the optical density of the solution was lower than the control by 20%, 22.3% and 42.6%, if the solution included L-Arg and L-Glu at an equimolar concentration of 3.8, 15.4 and 30.8 mM, respectively.

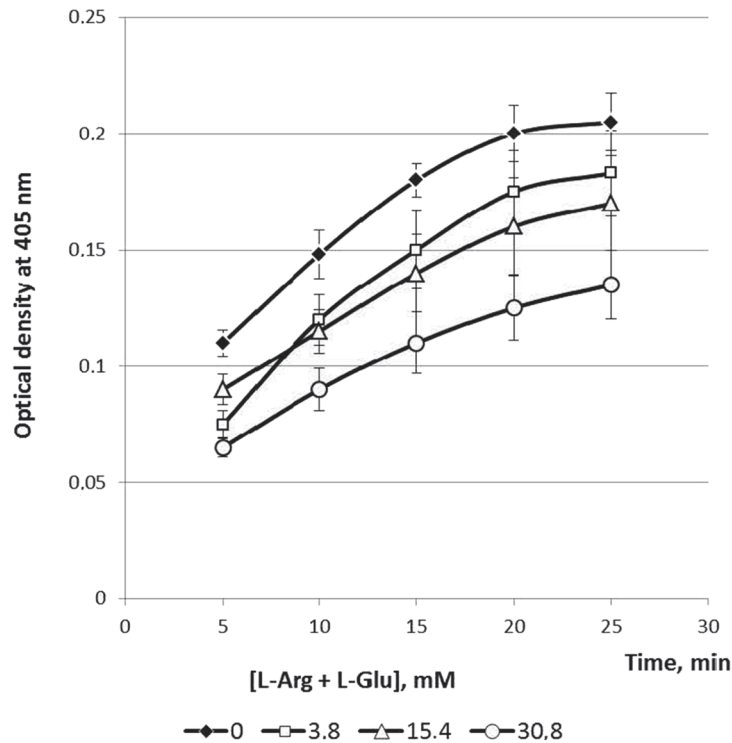


Fig. 1. Equimolar mixture of L-Arg and L-Glu suppresses the hydrolysis of S2366 by plasma proteases in a concentration-dependent manner

In a separate experiment, it was found that the inhibitory effect develops over time, reaching a maximum value after 20–40 min, and then does not change. Apparently, amino acids can reversibly block the active site of the enzyme due to electrostatic interactions with anion binding and aryl binding subsites.

A mixture of L-arginine and L-glutamic acid increases the solubility of crude PC after plasma dilution. Dilution of cryo-poor plasma in buffer 1 (with added AA) and buffer 2 (without AA) provided an increase in the total PCa-dependent activity, P_{act} (taking into account dilution factor 3) by 74% and 33%, respectively (Fig. 2). These data indicate a corresponding increase in the solubility of PC in diluted plasma.

At the same time, cleavage of para-nitroaniline from the chromogenic substrate S2366 was also found in cryo-poor plasma samples not pre-incubated with an activator from snake venom. This PCa-independent hydrolysis of S2366 appears to be catalysed by other plasma proteases capable of recognizing the amino acid sequence in the p-Glu-Pro-Arg tripeptide. Several plasma enzymes are known that can interfere with the analysis of PCa activity, since C2366 is also sensitive for factor XIa, kallikrein, plasmin, and thrombin

with K_m 1.2, 0.60, 0.40, and 0.15 mM, respectively [22]. The total amidolytic activity, P of these enzymes in plasma we were found to increase by 120% and 105% after dilution with buffers 1 and 2, respectively, indicating a corresponding increase in their solubility (Fig. 2).

It should be noted that after dilution with buffer 2, the plasma proteins did not respond to the action of the PC activator from the snake venom, which was manifested in the absence of a corresponding increase in amidolytic activity, confirmed by the fact that $P \approx P_{act}$ (Fig. 2). However, the plasma diluted with buffer 1 retained the ability of the crude PC to be activated. This is evidenced by the increase in amidolytic activity induced by the protein C activator, in addition to the total protease activity ($P_{act} > P$) (Fig. 2). Whereas in the presence of L-Arg and L-Glu, it was possible to detect the activity of PCa in diluted samples, which indicates an increase in PC solubility provided by amino acids.

Concentrating of crude protein C by sorption on DEAE-Cellulose. The process for the large-scale production of highly purified plasma-derived protein C is recommended to include a prepurification step to enrich the PC content. For this purpose, inexpensive

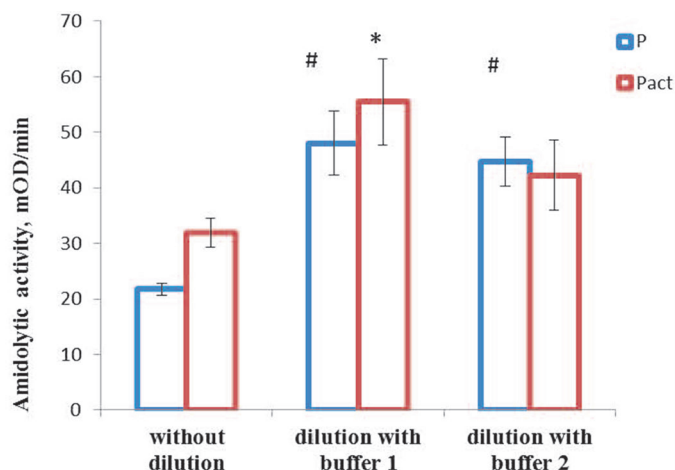


Fig. 2. Dilution and addition of L-Arg and L-Glu to cryo-poor plasma increases the amidolytic activity of plasma proteases

— $P < 0.05$ for P activity versus control; * — $P < 0.05$ for PCa activity versus control

ion-exchange sorbents, like DEAE-cellulose, suitable for bulk adsorption are usually used. Optimization of conditions for ion-exchange chromatography should, as is known, affect the ratio of the concentrations of the target and ballast proteins, their agreeability and solubility, as well as hydrophobic or hydrophilic properties [19]. The use of equimolar mixture of L-Arg and L-Glu to optimize these parameters for PC purification seems promising, although it is unclear how the presence of charged amino acids will affect the adsorption capacity of the sorbent.

So, we carried out chromatographic pre-purification of protein C from plasma diluted with 0.15 M Na-phosphate buffer, pH 6.0, with and without added amino acids (data are given in the table). It was found that the addition of AA reduces the total protein-binding capacity of DEAE-cellulose, as evidenced by an increase in the amount of unbound protein by 27.6% relative to the variant without the addition of AA. Despite this, a high specific activity of PCa was found in the eluate in the first variant (with pre-added AA) while in the second (without AA) it was not detected at all.

A comparison of the specific activity of protein C in the eluate and in cryo-poor plasma shows that the use of AA significantly optimizes the prepurification stage, which provides a 5-fold increase in the yield of protein C. Obviously, this is due to the more efficient separation of ballast proteins remaining in the unbound fraction, since the presence of AA was unfavorable for them. This result is also confirmed by the data of immunoblot analysis (Fig. 3).

It can be seen that, in samples without AA, protein C (or its fragments) aggregates with many other plasma proteins, while in the presence of AA, such protein interactions are insignificant.

The very low concentration of protein C in human plasma (~4 mg/L) causes the technical difficulties that limit the use of conventional chromatographic techniques for separating it from other vitamin K-dependent enzymes with similar physico-chemical properties. The recovery of proteins present in trace quantities in human plasma is considered an expensive, time-consuming and labour-intensive practice.

Taking into account the properties of an equimolar mixture of L-Arg and L-Glu described in [16], we used them to enrich the crude protein C in the processed cryo-poor plasma. This approach, as expected, was effective for the pre-treatment of PC-containing protein concentrates, since it was guided by the peculiarities of the surface topography of the PC.

Apparently, the observed suppression of amidolytic activity under the synergistic action of AA is associated with the structure of the catalytic domain of protein C. As is known, electrostatic interactions in the domain are provided by a cluster of acidic AA residues (as part of the Ca-binding loop), two clusters of basic AA residues at the entrance to the catalytic pocket and at its bottom, as well as an extensive hydrophobic zone [20]. These structural elements are responsible for the recognition of substrates, inhibitors and cellular receptors by protein C. Thus, the synergistic action of AA appears to block the catalytic center of the (pro)enzyme,

An equimolar mixture of L-Arg and L-Glu affects the amidolytic activity and protein content in the fractionation products of cryo-poor plasma on DEAE-cellulose

Fraction	Activity, OD*min ⁻¹		Amount of protein, mg	Specific activity, OD*min ⁻¹ *mg ⁻¹		
	P	Pact		P	Pact	PCa
PBS						
plasma	0.219±0.026	0.295±0.031	2.23±0.15	0.098	0.132	0.034
non-binding	0.076±0.017	0.106±0.008	0.98±0.23	0.078	0.108	0.031
washing	0.060±0.010	0.075±0.012	0.43±0.17	0.139	0.174	0.036
eluate	0.099±0.014	0.084±0.022	0.27±0.11	0.367	0.311	–
PBS+ L-Glu+L-Arg						
plasma	0.185±0.022	0.274±0.034	2.60±0.28	0.071	0.105	0.034
non-binding	0.105±0.026*	0.171±0.031*	1.25±0.28*	0.084	0.137	0.053
washing	0.063±0.012	0.098±0.023	0.41±0.22	0.154	0.238	0.085
eluate	0.094±0.018	0.146±0.018*	0.28±0.11	0.336	0.521	0.186

Note: * — $P < 0.05$ for plasma and corresponding chromatographic fractions pretreated with PBS+ L-Glu+L-Arg vs PBS.

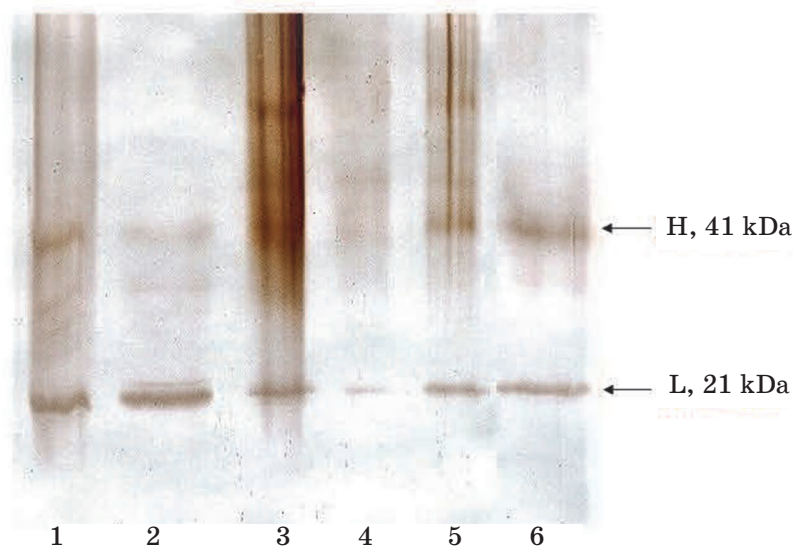


Fig. 3. Immunoblotting of protein C in cryo-poor plasma and eluate fraction:

line 1 — plasma 1 (diluted with 0.1 M PBS+AA mixture); line 2 — eluate from plasma 1; line 3 — plasma 2 (diluted with 0.1 M PBS); line 4 — eluate from plasma 2; line 5 — 2-fold concentrated eluate from plasma 2; line 6 — standard of protein C, 1 mcg (H, heavy chain; L, light chain). Samples were reduced with 10% β-mercaptoethanol

protecting against irreversible inhibition and nonspecific high molecular weight interactions.

As we have shown, the simultaneous addition of L-arginine and L-glutamic acid can increase the solubility of plasma proteins unevenly. Namely, in our study, the solubility of proteases with interfering activity increased by 15%, and the solubility of PC — by 41%. We believe that this is due to the specific features of the surface of the PC molecule. Two large oppositely charged

clusters of amino acids at different poles of the protein C globule [20] can form strong ionic contacts with any charged components of the solution (both positively and negatively charged). Simultaneous neutralization of clusters with a mixture of oppositely charged L-Arg and L-Glu can weaken these contacts without decreasing the PC solubility. In addition, L-glutamic acid affects the barrier to protein unfolding, i.e. prevents their denaturation; and together with

L-arginine tends to mask all protein-exposed hydrophobic spots, weakening the interaction of PC with other proteins [15].

As it turned out, the synergism of AA can also be effectively used at the stage of rough purification of PC from cryo-poor plasma using DEAE chromatography step. Apparently, the equimolar mixture of L-Arg and L-Glu differently screen ionic and polar groups on the surface of separated plasma proteins, which affect their adsorption by the sorbent. When protein comes into contact with an adsorbent, the protein can interact with two, three, or more binding sites, which leads to a polydispersity of the interaction forces. With the dominance of anion exchange, DEAE-cellulose can retain the ability to hydrophobic interactions [19]. Apparently, the AA mixture can correct (to some extent) the inhomogeneity of the adsorbent. Interestingly, arginine is known to prevent hydrophobic interactions by binding to non-polar amino acid residues presented on the surface of the protein globule [15, 16]. Together with inhibition of the active centre of PC and a decrease in its protein-protein interactions, this can contribute to an increase in the concentration of PC in the eluate.

Finally, it should be noted that, in addition to amino acids, the degree of dilution of protein concentrates directly affects protein-protein interactions. As follows from our results, after threefold dilution of cryo-poor plasma, the amidolytic activity of PCa and other proteases did not decrease, but, on the contrary, increased. Interestingly, a similar unexpected result was obtained in a study [23], where the possibility of enhanced clotting at moderate degrees of plasma dilution was revealed. The authors showed that coagulation is more sensitive to the decrease in plasma concentration of inhibitors than procoagulant factors, which is due to the different kinetics of their reactivity.

Another explanation is based on the dipole interactions of water molecules with the protein surface, which is highly heterogeneous due to local distributions of polar and nonpolar domains on the protein surface. The ratio of negatively charged, positively charged and charge neutral polar and nonpolar amino acids determines the orientation and dynamics of water molecules on the surface domains of proteins, thereby

contributing to their solubility. Negatively charged amino acids are considered most beneficial for protein solubility, followed by positively charged amino acids, and finally neutrally charged amino acids [18].

According to immunoblot analysis, samples pre-treated with AA show less PC captured by other proteins. Apparently, the AA mixture suppresses the formation of protein C derivatives, which aggregate with some plasma proteins. It has long been known, for example, that plasmin, spontaneously produced in plasma, causes nonspecific activation or degradation of protein C [24]. Cleavage of the heavy chain of protein C with plasmin yields fragments with a molecular weight of less than 20,000. This is followed by a slower degradation of the light chain of protein C [24].

Conclusions

The modern industry for the production of pharmacological blood preparations tends to development of capture technologies for plasma proteins from crude plasma fractions. An advantage of this approach is considered to be the ability to extract fragile or trace plasma proteins, such as PC or factor VIII, directly from whole plasma. This avoids the use of preliminary extraction steps such as salt precipitation or filtration, which are known for low recovery and potential denaturing effects.

We believe that the use of an equimolar mixture of L-Arg and L-Glu can be successfully combined with both traditional methods of plasma pre-treatment and modern methods of capturing PC directly from blood plasma. By reducing protein aggregation, AAs also make it possible to use discarded side fractions containing target minor proteins. In addition, the approach we propose can be easily integrated into the scheme of batch extraction of the prothrombin complex (including PC) without significantly affecting it.

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The authors declare no conflict of interests.

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L-АРГІНІН І L-ГЛУТАМІНОВА КИСЛОТА ЗБІЛЬШУЮТЬ ВМІСТ ПРОТЕЇНУ С НА РАННІХ СТАДІЯХ ВИДІЛЕННЯ З ДОНОРСЬКОЇ ПЛАЗМИ

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Сучасне великомасштабне виробництво фармакологічних препаратів крові орієнтоване на розширення номенклатури продуктів і глибшу екстракцію цільових протеїнів, особливо на стадії їх попереднього очищення. Зокрема, ця проблема стає критичною для виділення протеїнів, подібних до протеїну С (РС), який присутній у плазмі в невеликій кількості.

Мета. Метою роботи було поліпшити склад буферу так, щоб мінімізувати взаємодію РС з іншими протеїнами і ліпідами, які обов'язково присутні у вихідному матеріалі.

Методи. Вміст протеїну С у плазмі та її похідних оцінювали за амідолітичною активністю щодо хромогенного субстрату S2366. Зменшення кількості гомологічних домішок і збагачення плазми протеїном С забезпечували селективною об'ємною адсорбцією на DEAE-целюлозі.

Результати. Показано, що додавання еквімолярної суміші двох амінокислот (L-аргінін і L-глутамінова кислота) істотно збільшувало вміст протеїну С на етапі попереднього очищення криозбідненої плазми, який включає початкове розведення і подальше збагачення плазми протеїном С селективною об'ємною адсорбцією на DEAE-целюлозі. Додатково виявлено, що розчини цих амінокислот за спільної дії інгібують індуквану амідолітичну активність протеїну С і підвищують його розчинність (на відміну від інших протеаз плазми).

Висновок. Внесення суміші L-аргінину і L-глутамінової кислоти в криозбіднену плазму в складі робочого буферу значно оптимізує стадію попереднього очищення протеїну С, забезпечуючи 5-кратне збільшення його виходу після елюції з DEAE-целюлози.

Ключові слова: протеїн С; донорська плазма; фракціонування; L-аргінін; L-глутамінова кислота.

L-АРГИНИН И L-ГЛУТАМИНОВАЯ КИСЛОТА УВЕЛИЧИВАЮТ СОДЕРЖАНИЕ ПРОТЕИНА С НА РАННИХ СТАДИЯХ ВЫДЕЛЕНИЯ ИЗ ДОНОРСКОЙ ПЛАЗМЫ

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Современное крупномасштабное производство фармакологических препаратов крови ориентировано на расширение номенклатуры продуктов и более глубокую экстракцию целевых протеинов, особенно на стадии их предварительной очистки. В частности, эта проблема становится критической для выделения протеинов, подобных протеину С (РС), который присутствует в плазме в следовых количествах.

Цель. Целью работы было улучшить состав буфера так, чтобы минимизировать взаимодействие РС с другими протеинами и липидами, которые неизбежно присутствуют в исходном материале.

Методы. Содержание протеина С в плазме и ее производных оценивали по амидолитической активности к хромогенному субстрату S2366. Уменьшение количества гомологичных примесей и обогащение плазмы протеином С обеспечивали селективной объемной адсорбцией на DEAE-целлюлозе.

Результаты. Показано, что эквимоллярная смесь двух аминокислот (L-аргинин и L-глутаминовая кислота) существенно увеличивала содержание протеина С на этапе предварительной очистки криообедненной плазмы, включающем начальное разбавление и последующее обогащение плазмы протеином С за счет селективной объемной адсорбции на DEAE-целлюлозе. Дополнительно выявлено, что растворы этих аминокислот при совместном действии ингибируют индуцированную амидолитическую активность протеина С и повышают его растворимость (в отличие от других протеаз плазмы).

Вывод. Внесение смеси L-аргинина и L-глутаминовой кислоты в криообедненную плазму в составе рабочего буфера значительно оптимизирует стадию предварительной очистки протеина С, обеспечивая 5-кратное увеличение его выхода после элюции с DEAE-целлюлозы.

Ключевые слова: протеин С; донорская плазма; фракционирование; L-аргинин; L-глутаминовая кислота.