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STUDY ON THE DEPENDENCE OF ALPHA-FETOPROTEIN CONTENT IN HUMAN CORD BLOOD EXTRACTS ON EXTRACTION CONDITIONS

Key words: *cord blood, alpha-fetoprotein, cryodestruction, biologically active substances, cryoextract.*

Alpha-fetoprotein (AFP) is a multifunctional transport protein with selective biological activity, characteristic of human cord blood and valuable in terms of its therapeutic potential [5, 6, 10]. Research findings have shown that AFP can modulate immune responses, stimulate tissue regeneration, and suppress inflammatory processes [5, 9]. This protein is used for treatment of autoimmune disorders (for example, rheumatoid arthritis and multiple sclerosis), liver failure, as well as for accelerating wound healing and nervous tissue regeneration [3, 7]. In addition, AFP exhibits antitumor activity, making it a promising candidate for the treatment of oncological diseases [1, 5]. Considering these properties, the development of AFP-based therapeutics is an important objective of modern biotechnology. The use of not only recombinant AFP but also extracts obtained from natural sources such as cord blood or placenta makes it possible to preserve the native structure of the protein and its biological activity.

Known approaches for isolating AFP from cord blood or abortive blood include fractionation with subsequent removal of cellular components. However, the reported data indicate that AFP exists both in free and bound forms, specifically, it associates with receptor complexes on cells of leukocytic origin and on hematopoietic stem cells, is detected in the cytoplasm as part of protein complexes, and also forms extracellular complexes with various ligands [2, 6]. In addition, it has been demonstrated that certain biologically active regions of the native AFP molecule are hidden within the globule and become accessible only after conformational modification induced by physicochemical factors such as temperature, pH, and the ionic composition of the medium [6, 8]. From this perspective, investigating the efficiency of source material disruption under different conditions appears promising, as this may potentially influence the final yield of the target product.

The aim of this study was to evaluate the efficiency of low-temperature modes of cord blood disruption and to assess the dependence of AFP yield in the extracts on the salt composition and pH of the extraction medium.

Human cord blood was collected during physiologically normal deliveries at 38—40 weeks of gestation with informed consent from the donors. Whole cord blood (n = 3) was mixed with ex-

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traction media of varying salt composition and pH in a 1 : 4 ratio and incubated for 30 min at 18—20 °C. The following extraction media were used: 150 mM NaCl (pH 7.4 and 5.0), 150 mM KCl (pH 7.4 and 5.0), and 10 mM Tris-HCl (pH 7.4).

The obtained samples were subjected to cryodestruction by combining rapid — slow freezing modes followed by rapid — slow thawing in 5-mL containers. Rapid freezing was performed at a rate of 30 °C/min to a final temperature of –196 °C, and rapid thawing was carried out using a water bath at 38 °C. Slow freezing was performed at a rate of 1 °C/min in liquid nitrogen vapor, whereas slow thawing was conducted at room temperature (18—20 °C).

As controls, commonly used methods of cell disruption were applied: hypotonic lysis (10 mM Tris-HCl, pH 7.4, was added to the samples) and high-temperature disruption (samples were incubated for 30 min at 70 °C). The samples were then centrifuged for 10 min at 11,000 g. Supernatants were collected and used to determine AFP content by a two-site chemiluminescent immunometric assay using the "IMMULITE 2000 AFP" reagent kit (Siemens Healthineers, USA).

Data are presented as mean ± standard deviation (mean ± SD, $n = 3$ donors per group). For each donor, the value was averaged from three technical replicates. Statistical comparisons between groups were performed using the nonparametric Mann-Whitney U test.

The results demonstrated that both low-temperature modes markedly increased the AFP content in the extracts compared not only with thermodestruction but also with hypotonic lysis. As shown in the Table, the AFP content in extracts obtained by hypotonic lysis was (47.7 ± 7.052) IU/mg of protein, which was at least twofold lower than after applying each of the 8 variants of low-temperature exposure. Following thermodestruction, AFP remained in the extracts only in residual amounts, as expected.

Differences in the parameter under study were observed when applying the rapid-freezing/rapid-thawing and slow-freezing/slow-thawing modes after treatment with NaCl solution at pH 7.4. Specifically, the amount of AFP in the extract obtained after slow freezing and slow thawing was 32.2% higher than in the extract produced by rapid freezing followed by rapid thawing ($p \leq 0.01$).

Given the well-known sensitivity of AFP to conformational changes [6, 8], it is plausible that the applied temperature regimens and different pH conditions may have contributed to modifications of the protein's tertiary structure, thereby influencing its biological activity. In this study, the functional activity of AFP was not evaluated; however, the reported data indicate that conformational alterations can either activate hidden biologically active regions of the molecule or lead to partial inactivation, depending on the physicochemical conditions of the environment [6, 8]. Therefore, optimization of extraction parameters affects not only the quantitative yield of the protein but may also be associated with modulation of its functional properties, which warrants further investigation.

AFP content in human cord blood extracts under different destruction conditions and varying salt and pH of the extraction medium

Extracting solution	AFP content, IU/mg protein		
	Rapid freezing — rapid thawing	Slow freezing — slow thawing	Thermal destruction (70 °C)
NaCl			
pH 7.4	109.12 ± 19.9 *#	160.85 ± 22.5 *# **	10.98 ± 8.3 #
pH 5.0	148.26 ± 21.5 *#	124.16 ± 51.5 *#	30.39 ± 3.0 ×
KCl			
pH 7.4	126.36 ± 40.6 *#	123.34 ± 27.0 *#	36.31 ± 3.3
pH 5.0	136.49 ± 25.2 *#	177.75 ± 32.4 *#	13.67 ± 8.5 #
Hypotonic lysis	47.7 ± 7.05		

Notes: * significant differences *versus* thermal destruction ($p \leq 0.01$); # significant differences *versus* hypotonic lysis ($p \leq 0.01$); × significant differences *versus* hypotonic lysis ($p \leq 0.05$); ** significant differences *versus* rapid freezing-thawing in the corresponding extraction solution ($p \leq 0.05$).

Thus, the obtained data confirm that the release of AFP from cord blood depends on the applied cooling — thawing regimens, as well as on the salt composition and pH of the extraction medium. Furthermore, the results of this study are consistent with the findings of G.M. Fahy *et al.* [4], indicating that the most extensive disruption of cellular components occurs during slow cooling of biological material within the water crystallization temperature range (particularly at the eutectic point of the solution) and recrystallization. These conclusions emphasize the importance of controlling both temperature and chemical parameters to optimize disruption and extraction processes, which is a key factor for further improvement of methods for preserving biological materials and obtaining high quality products for medical and biotechnological applications.

REFERENCES

1. Bei R, Mizejewski GJ. Alpha-fetoprotein is more than a hepatocellular cancer biomarker: from spontaneous immune response in cancer patients to the development of an AFP-based cancer vaccine. Curr Mol Med. 2011; 11(7): 564—81.
2. Bogdanov AYu, Bogdanova TM, Ilin AI. Endocytic pathway of alpha-fetoprotein in mice bone marrow hematopoietic stem cells: molecular characterization and role in biological activity modification. Cytol Genet. 2014; 48: 21—32.
3. Dudich E. MM-093, a recombinant human alpha-fetoprotein for the potential treatment of rheumatoid arthritis and other autoimmune diseases. Curr Opin Mol Ther. 2007; 9(6): 603—10.
4. Fahy GM, Wowk B. Principles of ice-free cryopreservation by vitrification. Methods Mol Biol. 2021; 2180: 27—97.
5. Głowska-Ciemny J, Szymański M, Kuszerska A, et al. The role of alpha-fetoprotein (AFP) in contemporary oncology: the path from a diagnostic biomarker to an anticancer drug. Int J MolSci [Internet]. 2023 Jan 28 [cited 2025 Feb 04]; 24(3):2539. Available from: https://www.mdpi.com/1422-0067/24/3/2539
6. Gulevskyy AK, Akhatova YuS. Current concept to the structural and functional properties of alfa-fetoprotein and the possibilities of its clinical application. Biotechnologia Acta. 2021;14(1): 25—37.
7. Lin B, Liu K, Wang W, et al. Expression and bioactivity of human α-fetoprotein in a Bac-to-Bac system. Biosci Rep [Internet]. 2017 Jan 17 [cited 2025 Feb 06]; 37(1): BSR20160161. Available from: https://portlandpress.com/bioscirep/article/37/1/BSR20160161/56527/Expression-and-bioactivity-of-human-fetoprotein-in
8. Mizejewski GJ. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes, and conformational variants. Exp Biol Med. 2001; 226: 377—408.
9. Mizejewski GJ. Alpha-fetoprotein: Immunomodulation in autoimmune diseases during pregnancy and puerperium stages. GSC Biol Pharm Sci. 2022; 20(02): 102—13.
10. Munson PV, Adamik J, Butterfield LH. Immunomodulatory impact of α-fetoprotein. Trends Immunol. 2022; 43(6): 438—48.

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ДОСЛІДЖЕННЯ ЗАЛЕЖНОСТІ ВМІСТУ АЛЬФА-ФЕТОПРОТЕЇНУ
В ЕКСТРАКТАХ ПУПОВИННОЇ КРОВІ ЛЮДИНИ ВІД УМОВ ЕКСТРАГУВАННЯ

Ключові слова: кордова кров, альфа-фетопротеїн, кріодеструкція, біологічно активні речовини, кріоекстракт.