CHEMISTRY JOURNAL OF MOLDOVA. General, Industrial and Ecological Chemistry. *2018, 13(1), 111-116*
ISSN (p) 1857-1727 ISSN (e) 2345-1688
http://cjm.asm.md
http://dx.doi.org/10.19261/cjm.2018.482

STRUCTURAL ASPECTS OF LACTOFERRIN AND SERUM TRANSFERRIN OBSERVED BY FTIR SPECTROSCOPY

Gheorghe Duca [a], Lilia Anghel [a*], Raul Victor Erhan [b,c]

[a]*Institute of Chemistry of Academy of Sciences of Moldova, 3, Academiei str., Chisinau MD-2028, Republic of Moldova*
[b]*Institute for Energy Technology, 18, Instituttveien str., Kjeller 2027, Norway*
[c]*Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, 30, Reactorului str., Bucharest - Magurele MG-6, Romania*
[*]*e-mail: lilia.anghel@chem.asm.md*

Abstract. In this work, Fourier transform infrared spectroscopy was used to highlight the structural differences between the human lactoferrin and human serum transferrin. The results clearly show the structural differences of human lactoferrin and human serum transferrin. The second derivative analysis of the FTIR spectra allows the direct identification of secondary structure components of the human lactoferrin and human serum transferrin.

Keywords: human lactoferrin, human serum transferrin, FTIR spectroscopy, amino acid, secondary structure.

Received: 29 March 2018/ Revised final: 02 May 2018/ Accepted: 02 May 2018

Introduction

Fourier transform infrared spectroscopy (FTIR) is a powerful physicochemical analytical technique classically used for structure determination of small molecules. Each molecule is determined by its unique structure and therefore it will exhibit a unique FTIR spectrum, containing the vibrations of its structural bonds. FTIR analytical applications have allowed the structural characterization of biological material such as proteins [1]. There is a considerable amount of information that can be deduced from the spectral parameters such as band position, bandwidth and absorption coefficient, about the structure of amino acids residues, bound ligands or cofactors and protein secondary structures [2]. This aspect confirms that FTIR spectroscopy can be regarded as a valuable tool for the investigation of proteins.

FTIR spectra of proteins are highly complex and therefore can be difficult to interpret. There are two approaches for FTIR spectra interpretation. The first approach is the identification of amino acids side-chains absorption bands. Side-chains provide valuable information on the molecular reaction mechanism. This kind of research will help to identify the catalytically important amino acids residues, their protonation state, coordination of metal ions, and will give some insights into the molecular mechanisms [3]. The second approach of the FTIR spectroscopic investigations is the analysis of secondary structures of the proteins. FTIR spectra of proteins also contain nine characteristic IR absorption bands attributed to: amide A and B (~3300 and ~3070 cm^{-1}); amide I (1690-1600 cm^{-1}); amide II (~1575-1480 cm^{-1}); amide III (1301-1229 cm^{-1}); amide IV (767-625 cm^{-1}); amide V (800-640 cm^{-1}); amide VI (606-537 cm^{-1}); amide VII (200) [1,4]. Of these, the most prominent vibrational bands are amide I and amide II. The secondary structures vibration bands are predominately in the region of the amide I: α-helix (1654 cm^{-1}); β-sheets (1633, 1684 cm^{-1}); β-turns (1672 cm^{-1}); disordered (1654 cm^{-1}) [5]. Therefore, mostly the amide I region is used for the analysis of the secondary structures. This is done using mathematical data analysis methods such as Fourier self-deconvolution (FSD)-curve fitting [6], derivative and second derivative analysis [7].

The family of transferrins comprises monomeric glycoproteins with an approximate molecular mass of 80 kDa that share common biological functions such as iron binding and transportation. This family of proteins is divided into five classes: serum transferrin, ovotransferrin, melanotransferrin, lactoferrin and inhibitor of carbonic anhydrase [8]. X-ray crystallographic data available on Protein Data Bank [9] show that all transferrins consist of a single polypeptide chain of 600-700 amino acids, which are mostly organized in α-helix and β-sheets structures. All

transferrins share similar architecture comprising two ellipsoidal lobes (*N* and *C*) connected *via* a short linker region (Figure 1*(a)* and *(b)*). Each lobe is able to bind one Fe(III) ion [10]. The metal ion is coordinated by four amino acid side-chains: a carboxylate from one aspartate residue, two phenolate oxygen atoms from two tyrosine residues and one imidazole from one histidine residue. The coordination is completed by two oxygen atoms of the carbonate ion (Figure 1*(c)*). The most prominent structural difference between the proteins from the family of transferrins is the linker region between the two lobes (Figure 1*(a)* and *(b)*). For example, lactoferrin linker is an uncompleted *α*-helix structure and the serum transferrin linker is represented by an unstructured residue conformation (Figure 1*(b)*). This structural difference influences the internal flexibility of protein. Human serum transferrin and lactoferrin share approximately 42% structural identity [11]. Although these proteins present overall structural similarities, their properties slightly differ, *e.g.* human serum transferrin releases iron at pH around 5.5 whilst lactoferrin retains iron until pH 3.0 [10].

The purpose of this work is to show the application of FTIR technique for the identification of structural differences between human lactoferrin and human serum transferrin, members of the protein family of transferrins.

Experimental

Iron loaded human lactoferrin and human serum transferrin were purchased from Sigma-Aldrich.

The FTIR spectra were recorded in the 400-4000 cm^{-1} range at room temperature using a Perkin Elmer IR spectrometer (Institute of Chemistry of Academy of Sciences of Moldova).

Each sample consisted of a quantity of 20 mg of dry powder of lyophilized proteins that was used for IR measurements without any further chemical treatment.

A dedicated software Spectrum version 6.2.0.0055 (2007) was used for spectral data treatment and baseline correction against the air. Further, mathematical analysis was performed using OriginPro 8 data analysis and graphing software.

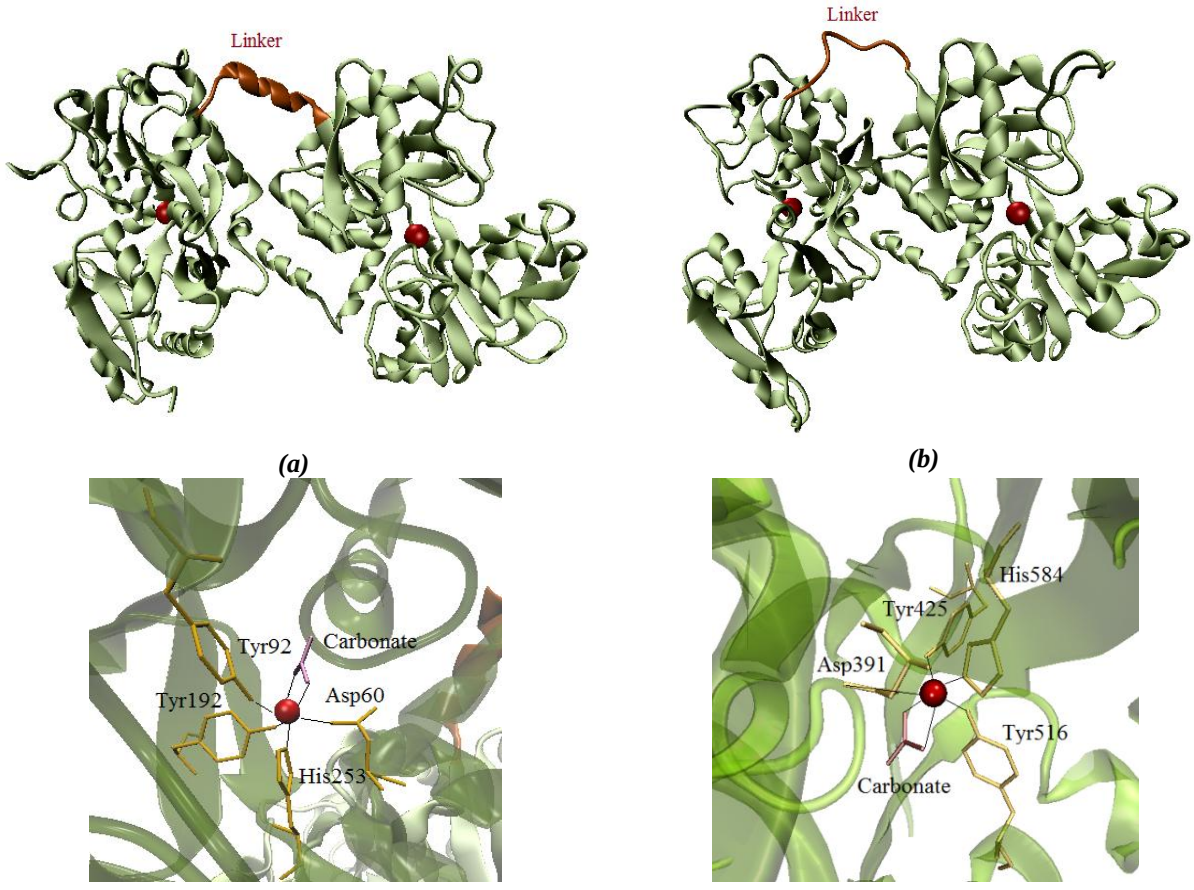
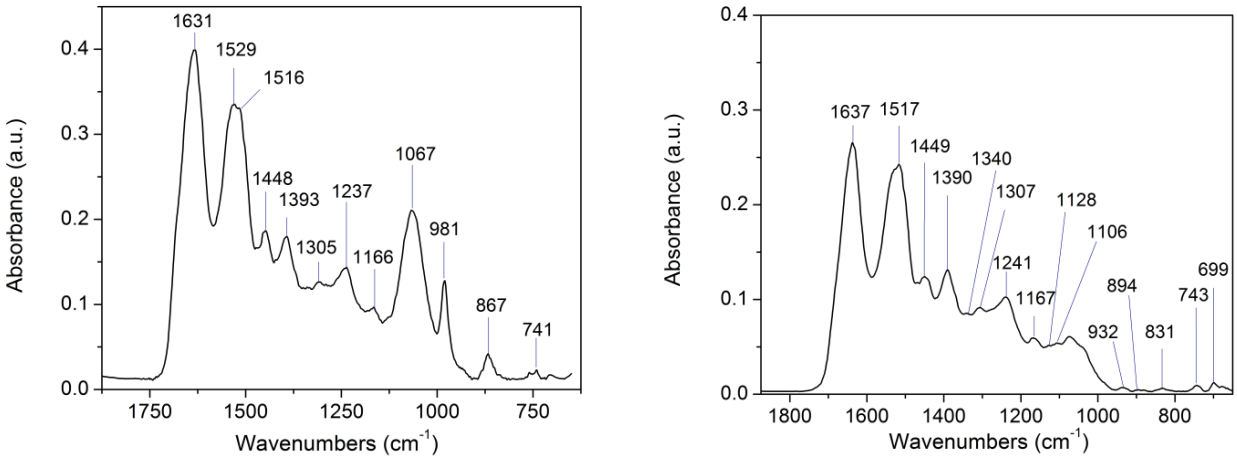


Figure 1. Graphical representation of the studied proteins. The overall structure of human lactoferrin *(a)* (PDB entry 1B0L [12]) and human serum transferrin (PDB entry 3QYT [13]) *(b)* molecules showing an overall similar conformation. The close-up view shows the iron-binding sites in the *N*-lobe of human lactoferrin *(c)* and *C*-lobe of human serum transferrin *(d)*.

Results and discussion

FTIR spectroscopy is an experimental method used for structural characterization of peptides and proteins in solutions and in crystalline phase. This technique is used for the investigation of secondary structures components of proteins (α-helix, β-sheets, *etc*.) and for the identification of specific amino acid side chains that influence the biological role of proteins. Thus, FTIR spectroscopy was applied for structural characterization of iron loaded human lactoferrin and human serum transferrin, the obtained spectra are presented in Figure 2.



(a) ***(b)***

Figure 2. FTIR spectra of human lactoferrin *(a)* and human serum transferrin *(b)*.

Amino acids side chains analysis

Biological properties of lactoferrin and transferrin derive from their structural properties and their main function of iron uptake. Even though both proteins are members of the same family, their biological functions slightly differ, *e.g.* the primary biological role of human serum transferrin is the uptake and transport of iron whilst lactoferrin lacks the transportation role [14]. The high resolution crystallographic data of lactoferrin and serum transferrin available on Protein Data Bank show that these proteins share the same metal and anion binding sites (Figure 1*(c)* and *(d)*), but the second coordination shell of the iron binding site presents differences. It was found that the metal ion is released from the binding pocket of the *N*-lobe of serum transferrin through a "dilysine trigger mechanism" with the participation of Lys206 and Lys296 from the second coordination shell [15]. The binding pocket of the *N*-lobe of lactoferrin contains two arginine molecules (Arg201 and Arg210) instead of the two lysine molecules of serum transferrin. This particular structural difference influences the iron binding/release properties of lactoferrin and transferrin. Thus, FTIR spectroscopy was applied to the investigation of amino acids side chains that influence the biological properties of human lactoferrin and human serum transferrin. The main absorption peaks of the amino acids side chains were identified according to literature data [2,5,16].

A comparison of the FTIR spectra of lactoferrin and serum transferrin showed absorption bands associated with histidine, lysine, phenylalanine, tyrosine, aspartic acid, tryptophan, serine and threonine. All other amino acids adsorption bands overlap with the absorption bands of other amino acids or polypeptide backbone.

The strong absorption bands observed at 1631 cm^{-1} (Figure 2*(a)*) and 1637 cm^{-1} (Figure 2*(b)*) that overlap with the absorption band of amide I were assigned to the stretch vibration of histidine [2,5].

Aspartic acid has a strong absorption band near 1402 cm^{-1}. The band position shits with +60/-90 cm^{-1} in the presence of the metal ion. FTIR spectrum of human lactoferrin contains an absorption band at 1393 cm^{-1}, presenting a downshift with 9 cm^{-1} from the value reported in the literature [2,5], whilst the spectrum of human serum transferrin has a noticeable adsorption band at 1390 cm^{-1} and a downshifting with 12 cm^{-1}. These observations indicate the involvement of the carboxyl group of aspartic acid in the formation of the ionic bond to ferric ion.

Three signals of tyrosine were registered at 1516 cm^{-1}, 1236 cm^{-1}, 1166 cm^{-1} in the FTIR

spectrum of human lactoferrin, and 1517 cm^{-1}, 1241 cm^{-1}, 1167 cm^{-1}, in the FTIR spectrum of human serum transferrin [2,5].

A strong absorption arises at 1067 cm^{-1} in the FTIR spectrum of human lactoferrin, which was assigned to N≡C or C=C stretch and C-H deformation vibrations of tryptophan. The side chains of tryptophan are known for their strong influence on the antimicrobial activity of proteins [16]. This experimental observation confirms that the antimicrobial activity of human lactoferrin is linked to the tryptophan presence in its polypeptide structure. This structural aspect differentiates the biological activity of human lactoferrin in comparison to human serum transferrin.

Another spectral difference consists in the presence of a prominent signal at 981 cm^{-1} and at 741 cm^{-1} in the FTIR spectrum of human lactoferrin (Figure 2*(a)*) that were assigned to serine and threonine, respectively [2,5]. These signals are absent in the case of human serum transferrin (Figure 2*(b)*). This structural difference explains the proteolitic activity of human lactoferrin [17] when comparing to human serum transferin.

Secondary structures analysis

The amide I region is almost exclusively used for the secondary structure analysis of proteins, although the amide II and amide III regions could offer additional information. To extract the main components, the broad band between 1680 and 1600 cm^{-1} assigned to amide I (Figures 2*(a)* and *(b)*) was further analyzed using derivative and second derivative technique, the resulted spectra are presented in Figure 3.

The main absorption in the amide I region is given by the α-helices between 1648 and 1657 cm^{-1} [4,5]. This sequence of derivative spectra reveals that human lactoferrin contains more α-helix components in comparison to human serum transferrin (Figure 3*(a)* and *(b)*).

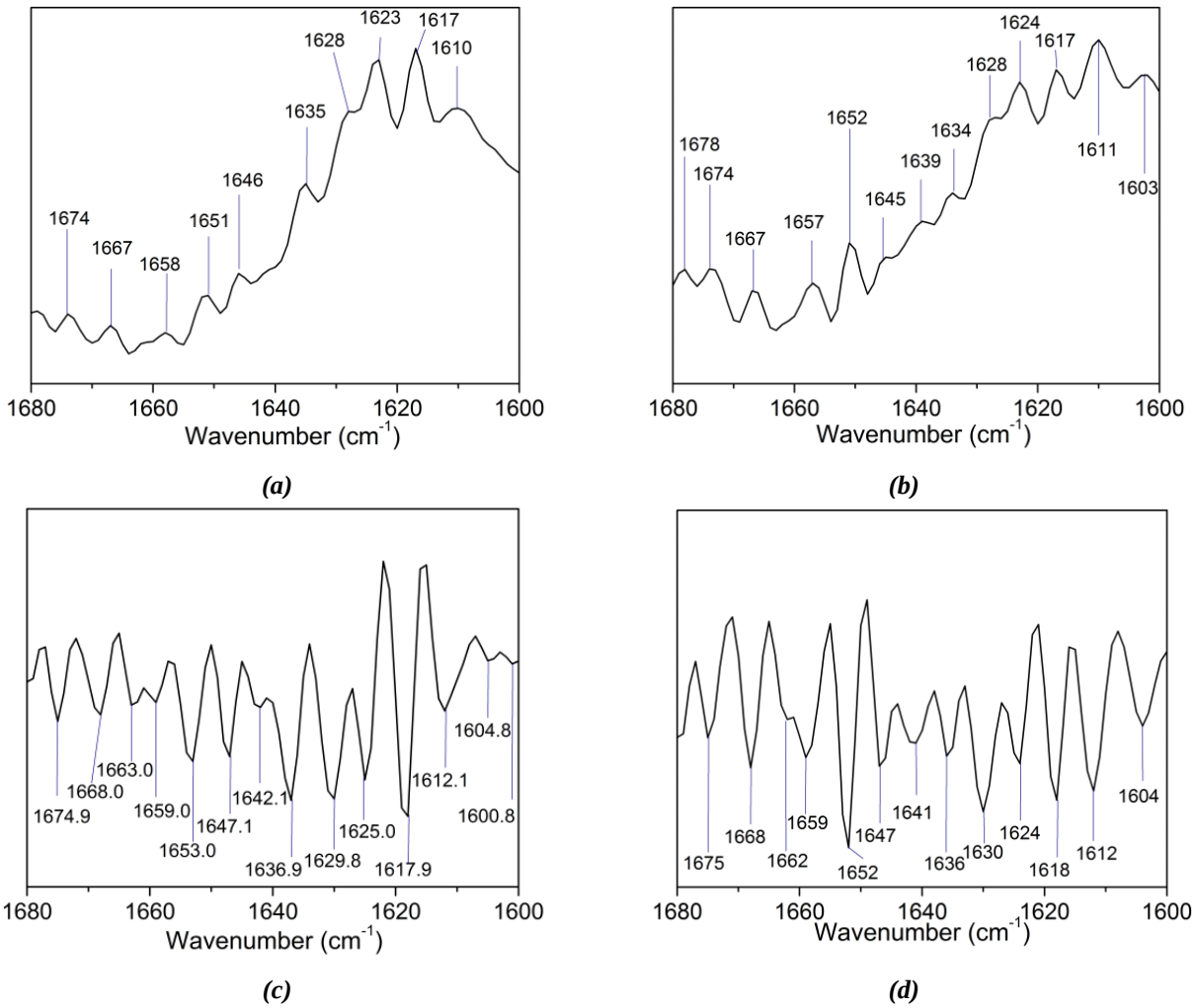


Figure 3. Mathematical analysis of the amide I region from the FTIR spectra. Derivative of *(a)* human serum transferrin and *(b)* human lactoferrin. Second derivative of *(c)* human serum transferrin and *(d)* human lactoferrin.

This observation is confirmed by the second derivative spectra (Figure 3*(c)* and *(d)*), the prominent peak at 1652 cm^{-1} observed in the spectrum of human lactoferrin and a small peak at 1653 cm^{-1} observed in the spectrum of human serum transferrin.

The bands between 1620 cm^{-1} and 1640 cm^{-1} are assigned to β-components [1,4,5]. Figure 3*(c)* shows that human serum transferrin contains predominantly β-sheets structures, with absorption bands observed at 1637 cm^{-1}, 1630 cm^{-1} and 1625 cm^{-1}. The second derivative FTIR spectra of human lactoferrin contains two prominent bands observed at 1620 cm^{-1} and 1604 cm^{-1} corresponding to a higher content of aggregated strands in comparison to human serum transferrin (1612 cm^{-1} and 1605 cm^{-1}) [5]. This important result offers additional explanation regarding the mobility and hence the reactivity of human lactoferrin and human serum transferrin. The results show that FTIR spectroscopy can be successfully used for the identification of structural differences in proteins with similar architecture and biological functions.

Conclusions

FTIR spectroscopy was successfully used for studying the composition and secondary structural conformation of proteins. The results derived from the FTIR spectra highlight the differences between human lactoferrin and human serum transferrin.

The strong absorption band at 1067 cm^{-1} identified in the FTIR spectrum of human lactoferrin was attributed to vibrations of tryptophan side-chains that are linked with the antimicrobial activity of the protein. This structural aspect differentiates the biological activity of lactoferrin in comparison to human transferrin. Other spectral differences were observed at 981 cm^{-1} and 741 cm^{-1} in the FTIR spectrum of human lactoferrin that were assigned to serine and threonine, respectively. These signals are absent in the case of human serum transferrin. This structural difference is related to the proteolitic activity of human lactoferrin, a biological function that human serum transferrin lacks.

The derivative and second derivative analysis of the amide I band clearly indicates that human lactoferrin contains predominantly α-helix components, whilst the human serum transferrin contains more β-components. This important result offers additional information with regard to the mobility and hence the reactivity of human lactoferrin and human serum transferrin.

Acknowledgements

This research work is part of a grant framework of the Romanian Plenipotentiary at JINR-Dubna, No. 95/15.02.2016-48 and No. 219/10.04.2017-46, within the JINR Theme No. 04-4-1121-2015/2017.

References

1. Jung, Ch. Insight into protein structure and protein–ligand recognition by Fourier transform infrared spectroscopy. Journal of Molecular Recognition, 2000, 13(6), pp. 325-351. DOI: https://doi.org/10.1002/1099-1352(200011/12)13:6<325::AID-JMR507>3.0.CO;2-C
2. Barth, A. Infrared spectroscopy of proteins: Review. Biochimica et Biophysica Acta (BBA) - Bioenergetics, 2007, 1767(9), pp. 1073-1101. DOI: https://doi.org/10.1016/j.bbabio.2007.06.004
3. Martínez-Felipe, A.; Imrie, C.T.; Ribes-Greus, A. Study of structure formation in side-chain liquid crystal copolymers by variable temperature Fourier Transform infrared spectroscopy. Industrial and Engineering Chemistry Research, 2013, 52 (26), pp. 8714-8721. DOI: 10.1021/ie303130e
4. Kong, J.; Yu, S. Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta Biochimica et Biophysica Sinica 2007, 39(8), pp. 549-559. DOI: https://doi.org/10.1111/j.1745-7270.2007.00320.x
5. Barth, A.; Zscherp, Ch. What vibrations tell us about proteins? Quarterly Reviews of Biophysics, 2002, 35(4), pp. 369-430. DOI: https://doi.org/10.1017/S0033583502003815
6. Yang, H.; Yang, S.; Kong, J.; Dong, A.; Yu, S. Obtaining information about protein secondary structures in aqueous solution using Fourier transform IR spectroscopy. Nature protocols, 2015, 10, pp. 382-396. DOI: 10.1038/nprot.2015.024
7. Dong, A.; Caughey, B.; Caughey, W.S.; Bhat, K.S.; Coeg, J.E. Secondary structure of the pentraxin female protein in water determined by infrared spectroscopy: effects of calcium and phosphorylcholine. Biochemistry, 1992, 31(39), pp. 9364-9370. DOI: 10.1021/bi00154a006
8. InterPro: protein sequence analysis & classification https://www.ebi.ac.uk/interpro/entry/IPR018195.
9. Bernstein, F.C.; Koetzle, T.F.; Williams, G.J.B.; Meyer Jr., E.F.; Brice, M.D.; Rodgers, J.R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. The protein data bank: A computer-based archival file for macromolecular structures. Journal of Molecular Biology, 1977, 112, pp. 535-542. DOI: https://doi.org/10.1111/j.1432-1033.1977.tb11885.x
10. Anghel, L. Physico-chemical aspects of the process of microbiological synthesis of iron nanoparticles. Ph.D. Thesis, Institute of Chemistry of Academy of Sciences of Moldova, Chisinau, Republic of Moldova, 2016. http://www.cnaa.md/en/thesis/24880/

11. Anghel, L. Lactoferrin: analysis of the structure profile. Chemistry Journal of Moldova, 2014, 9(2), pp. 99-106. DOI: dx.doi.org/10.19261/cjm.2014.09(2).14
12. Sun, X.-L.; Baker, H.M.; Shewry, S.C.; Jameson, G.B.; Baker, E.N. Structure of recombinant human lactoferrin expressed in *Aspergillus awamori*. Acta Crystallographica Section D, 1999, D55, pp. 403-407. DOI: https://doi.org/10.1107/S0907444998011226
13. Yang, N.; Zhang, H.; Wang, M.; Hao, Q.; Sun, H. Iron and bismuth bound human serum transferrin reveals a partially-opened conformation in the N-lobe. Nature Scientific Reports, 2012, 2, pp. 1-7. DOI: 10.1038/srep00999
14. Brooks, C.L.; Arutyunova, E.; Lemieux, M.J. The structure of lactoferrin-binding protein B from *Neisseria meningitidis* suggests roles in iron acquisition and neutralization of host defences. Acta Crystallographica Section F, 2014, F70, pp. 1312-1317. DOI: https://doi.org/10.1107/S2053230X14019372
15. Dewan, J.C.; Mikami, B.; Hirose, M.; Sacchettini, J.C. Structural evidence for a pH-sensitive dilysine trigger in the hen ovotransferrin N-lobe: Implications for transferrin iron release. Biochemistry, 1993, 32(45), pp. 11963-11968. DOI: 10.1021/bi00096a004
16. Chan, D.I.; Prenner, E.J.; Vogel, H.J. Tryptophan- and arginine-rich antimicrobial peptides: Structures and mechanisms of action. Biochimica et Biophysica Acta (BBA) – Biomembranes, 2006, 1758 (9), pp. 1184-1202. DOI: https://doi.org/10.1016/j.bbamem.2006.04.006
17. Hendrixson, D.R.; Qiu, J.; Shewry, S.C.; Fink, D.L.; Petty, S.; Baker, E.N.; Plaut, A.G.; St. Geme, J.W. 3rd. Human milk lactoferrin is a serine protease that cleaves *Haemophilus* surface proteins at arginine-rich sites. Molecular Microbiology, 2003, 47(3), pp. 607-617. DOI: https://doi.org/10.1046/j.1365-2958.2003.03327.x