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COMPARATIVE ANALYSIS OF α-HEMOLYSIN AND β-HEMOLYSIN BY HPLC AND FT-IR

Biological Science

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ABSTRACT

Hemolysins or haemolysins are lipids and proteins that cause lysis of red blood cells by destroying their cell membrane. Most hemolysins are protein compounds, but others are lipids biosurfactants. α-hemolysin (α-toxin, Hla) is the prototype for the class of small β-barrel pore-forming cytotoxins (PFTs). α-toxin is secreted as a water soluble monomer, capable of binding and oligomerization into a heptameric structure on the host cell membrane. β-Hemolysin is produced by specialized microorganisms and most readily identified by the characteristic zone of darkening or discoloration that it produces on sheep blood-agar plates. HPLC has revolutionized the efficiency and speed of separation of molecules in general and peptides in particular. An FTIR spectrometer simultaneously gathers high-spectral-resolution data over a wide spectral range. This confers a substantial benefit over dispersive spectrometer techniques. In the present study HPLC chromatograms and FT-IR spectra of both the toxins were compared for better enhancement of their study.

KEYWORDS

α-Hemolysin, β-Hemolysin, HPLC, FT-IR.

INTRODUCTION

α-Hemolysin is the most characterized virulence factor of S. aureus. Upon binding to the cell surface, α-hemolysin monomers assemble into a homoheptamer, forming a prepore. The prepore subsequently transitions to a mature β-barrel transmembrane pore [1], thereby leading to the formation of a 14-Å diameter aqueous channel [2]. This pore allows the transport of molecules smaller than 2 kD [3], such as K+ and Ca2+ ions, leading to necrotic death of the target cell. β-hemolysin does not form pores in the plasma cell membrane but instead is a neutral sphingomyelinase hydrolysing sphingomyelin, which is a plasma membrane lipid. β-hemolysin's enzymatic activity is required for its hemolytic activity [4] [5]. β-hemolysin lysis of red blood cells is only observed after the cells are switched to low temperature, suggesting that the lytic activity of β-hemolysin is not as efficient as that of other hemolysins, at least toward erythrocytes. β-hemolysin digests sphingomyelin into ceramide and phosphorylcholine [6]. The mechanism leading to cytotoxicity is still poorly understood. Sphingomyelin is enriched in lipid-ordered membrane microdomains with high content in cholesterol. Sphingomyelinase treatment of synthetic lipid bilayers leads to aggregation of cholesterol-rich microdomains [5]. β-hemolysin lysis of red blood cells is only observed after the cells are switched to low temperature, suggesting that the lytic activity of β-hemolysin is not as efficient as that of other hemolysins, at least toward erythrocytes. β-hemolysin digests sphingomyelin into ceramide and phosphorylcholine [6].

High-performance liquid chromatography (HPLC) involves the separation of molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase, i.e., the sorbent. [7-8]. The most commonly employed experimental procedure for the RP-HPLC analysis of peptides and proteins generally involves the use of a C18-based sorbent and a mobile phase. The chromatographic packing materials that are generally used are based on microparticulate porous silica which allows the use of high linear flow velocities resulting in favorable mass transfer properties and rapid analysis times [9-10]. FTIR spectroscopy is recognized as a valuable tool for the examination of protein conformation in H2O-based solution, as well as in deuterated forms and dried states, resulting in a greatly expanded use in studies of protein secondary structure and protein dynamics in the past decade [4−17]. In the present study comparison of two different protein extracted from microorganisms α-Hemolysin and β-Hemolysin was done using HPLC and FT-IR analytical method.

MATERIALS AND METHODS

Cultivation of microorganisms on culture media

Microorganisms were cultivated on blood agar plates and were incubated at 37 until the zones of hemolysin were observed. Then isolated zone producing microorganisms were inoculated for cultivation into respective nutrient broth. The broth was kept on an orbital shaker and the temperature was maintained at 37 up to 5 days.

Extraction of α-Hemolysin and β-Hemolysin

Hemolysin toxins were extracted by salting out of proteins using ammonium sulfate method given by Duong-Ly KC & Gabelli SB [11].

Method for HPLC

The HPLC analysis was done in Shimadzu LC-20AD High Performance Liquid Chromatography instrument by using C18 column. Water : acetonitrile was used as solvent system in the ratio of 70:30. Run time for the sample was 20min and sample injection volume was 20µl. Column Temperature was 37°C, flow rate-0.5 ml/min with detector wavelength of 214nm.

Method for FT-IR

Purified Toxin samples were dissolved in 2ml TE buffer. FTIR measurements were performed in absorption mode with FTIR Spectro-microscopy coupled to the FTIR spectrometer Panorama software12. The spectra were obtained in the wave number range of 450–4000 cm-1. Baseline correction and normalization were performed for all the spectra by Panorama software. Baseline correction was done by line Algorithm. Data smoothing was done at 9 points smoothing window with polynorm of order 3 and the spectral resolution was at 4 cm-1. Peak Normalization was performed to bring the y value to zero. Derivative and peak finding were performed using OPUS 7.5 software.

RESULTS AND DISCUSSION

HPLC Analysis

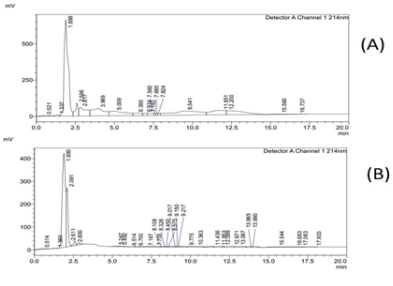

Fig.1 HPLC Chromatograms of both the sample α-Hemolysin and β-Hemolysin; (A) peak obtains from the analysis of α-Hemolysin which was around 1.888 min up to 670 mv, (B) Two main peaks obtain from the analysis of β-Hemolysin at 1.890 and 2.091 min up to 400 mV and 270 mV respectively.

Fig.1 (A) shows that the peak was observed at 1.888 min of α-Hemolysin at 720 mV, while fig.1 (B) shows two peaks which were observed at 1.890min at 410mV almost same as observed in α-Hemolysin chromatogram and 2.96 min of β-Hemolysin at 270 mV. From these observations, it can be concluded that both α-Hemolysin and β-Hemolysin may have same structure with slight changes in functional properties.

FT-IR

The most sensitive spectral region to the protein secondary structural components is the amide I band (1700-1600 cm-1), which is due almost entirely to the C=O stretch vibrations of the peptide linkages (approximately 80 %) (Jilie KONG et al. 2007). From the fig 2 (A) and (B), the bands observed at 1631.38 which confirms that both α-Hemolysin and β-Hemolysin have similar protein secondary structure.

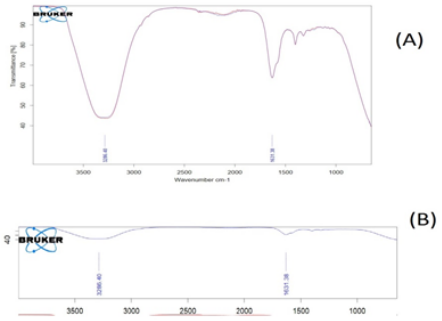

Fig.2 FT-IR spectra of (A) α-Hemolysin and (B) β-Hemolysin

CONCLUSION

Our approach was to distinguish the α-Hemolysin and β-Hemolysin using the HPLC and FT-IR analysis. Both α-Hemolysin and β-Hemolysin have different functional properties with almost same structural properties of protein which was observed from the chromatogram of HPLC and spectra of FT-IR. α-Hemolysin was distinguished from β-Hemolysin by HPLC analysis as they gives the peak at same time but at different absorbance 650 mV and 410 mV respectively. α-Hemolysin was distinguished from β-Hemolysin by FT-IR spectra as they gives the band at same wavenumber but at different transmittance % which was 65 % and 40 % respectively.

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Conflict of interests: The authors declare that they have no conflict of interests.

5. REFERENCES

1. Bhakdi, S., & Tranum-Jensen, J. (1991). Alpha-toxin of Staphylococcus aureus. Microbiological Reviews, 55(4), 733–751.
2. Song L., Hobaugh M. R., Shustak C., Cheley S., Bayley H., Gouaux J. E. (1996). Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science 274, 1859–186610.1126/science.274.5294.1859.
3. Menestrina G. (1986). Ionic channels formed by Staphylococcus aureus alpha-toxin: voltage-dependent inhibition by divalent and trivalent cations. J. Membr. Biol. 90, 177–19010.1007/BF01869935.
4. Huseby M., Shi K., Brown C. K., Digre J., Mengistu F., Seo K. S., Bohach G. A., Schlievert P. M., Ohlendorf D. H., Earhart C. A. (2007). Structure and biological activities of beta toxin from Staphylococcus aureus. J. Bacteriol. 189, 8719–872610.1128/JB.00741-07.
5. Ira J., Johnston L. J. (2008). Sphingomyelinase generation of ceramide promotes clustering of nanoscale domains in supported bilayer membranes. Biochim. Biophys. Acta 1778, 185–19710.1016/j.bbamem.2007.09.021.
6. Doery H. M., Magnusson B. J., Cheyne I. M., Sulasekharam J. (1963). A phospholipase in staphylococcal toxin which hydrolyses sphingomyelin. Nature 198, 1091–109210.1038/1981091a0.
7. Aguilar, M. I. and Hearn, M. T. W. (1996) High resolution reversed phase high performance liquid chromatography of peptides and proteins. Meth. Enzymol. 270, 3–26.
8. Mant, C. T. and Hodges, R. S. (1996) Analysis of peptides by high performance liquid chromatography. Meth. Enzymol. 271, 3–50.
9. Unger, K. K. (1990) Silica as a support, in HPLC of Biological Macromolecules: Methods and Applications (Gooding, K. M. and Regnier, F. E., eds.), Dekker, New York, pp. 3–24.
10. Henry, M. (1991) Design requirements of silica-based matrices for biopolymer chromatography. J. Chromatogr. 544, 413–443.
11. Duong-Ly KC & Gabelli SB 2014 Salting out of proteins using ammonium sulfate precipitation. Methods in Enzymology 541 85–94. (doi:10.1016/b978-0-12-420119-4.00007-0).
12. Jilie KONG and Shaoning YU (2007) Fourier Transform Infrared Spectroscopic Analysis of Protein Secondary Structures. Acta Biochimica et Biophysica Sinica. 39(8): 549–559.