EXTRACTION AND CHARACTERIZATION OF TEICHOIC ACID FROM STAPHYLOCOCCUS AUREUS

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Abstract

Teichoic acid has been considered as surface reactive antigen (immunogens, biological response modifiers), as well as membrane components which mediate the attachment of *Staphylococcus aureus* to host cell tissues . The teichoic acid was extracted and purified in this study from an isolate of *Staphylococcus aureus* and the chemical analysis of teichoic acid showed that it was rich in carbohydrates (55 %) with small amounts of proteins (17.5 %).

Introduction

Staphylococcus aureus is a common cause of primary human skin infections including impetigo, pustules boils, carbuncles and cellulitis. It's also frequently implicated in postoperative sepsis, in wound infections, abscesses, and in colonizing intravascular prosthetic devices. Recently, many studies showed that teichoic acid can be mediate adherence of the bacterium to prosthetic devices, contributing to increasing number of hospital acquired infections [1] [2]. In addition, the antiteichoic acid antibodies can be used in diagnosis of deep seated *Staphylococcus aureus* infections such as endocarditis. Lung disease, meningitis especially in osteomyelitis cases where organisms cannot be isolated and therefore help in

Predicting the need for long term antimicrobial treatment [3]. Techoic acid was discovered in 1959 after discovering Nucleotide and was found to form important part of cell wall structure of Gve^{+} bacteria which linked with peptidoglycan or with lipid of membrane (membrane associated lipid) in this case techoic acid called lipotechoic acid (4, 5) teichoic acid formed 50 % of dry weight of cell wall and 10 % of total weight of cell.

Previously more than one method were used for extraction and purification of teichoic acid some of researcher used alkaline condition with NaOH at temperature 37 ^{o}C (6, 7). The purpose of the present study was to prepare a purified teichoic acid and characterize its chemical properties.

Material and method

Bacterial isolate : one clinical isolate of *Staphylococcus aureus* was isolated from patient with catheter related bacteraemia was used this strain was identified by biochemical tests including gram staining, catalase after growing on Blood Agar Medium, coagulase test and using Api staph. System (4), in order to confirm the diagnosis.

Preparation of crude techoic acid

Teichoic acid was extracted from *Staphylcoccus aureus* strain. Extraction of techoic acid was preformed by modified procedure which have been described previously [4], 5 ml of an 18 hr Trypticase soy broth culture of *Staphylococcus aureus* was inoculated in 1 L of Trypticase soy broth and incubated on a rotary shaker (200) rpm at 37 ^{o}C for 18 hr culture was centrifuged for 15 min at 6000 rpm at 4 C$^{\circ}$ and the precipitate of cells was washed with distilled water three times by resuspending and recentrifugation [8] Crude cell walls were obtained by vortexing the bacterial pellet with buffer (0.01M Phosphate buffer , pH 7.4), using glass beads (0.1–0.15 mm diameter, 2.4 g / 20 ml) for 15 min. Unbroken cells and glass beads were removed by filtration through asintered glass funnel, crude cell walls were then precipited by centrifugation at 2500 rpm for 20 min.

Preparation of purified Teichoic acid

The crude cell walls extraction were successively treated by suspension in 2% (w/v) sodium dodecyl sulphate with stirring for 16 hr, digestion with DNAase (1µg/ml), RNAase (3 µg/ml) for 16 hr stirring with phenol 40% for 20 min further centrifuging

step at 37 °C, pronase (1µg/ml) for 4 hr stirring with 40% phenol for 20 min, centrifuging for 30 min at 3200 rpm at 4 °C and extensive washing with distilled water. Techoic acid was removed from the purified cell walls by extraction with 10% (w/v) trichloroacetic acid TCA (20 ml) for 24 hr at 4 °C. After centrifugation, the supernatant solution was mixed with cold ethanol (100 ml) and kept at 2 °C. The wall residue was extracted with a further portion of TCA (20 ml) for 48 hr at 4 °C. The supernatant solutions were separately mixed with cold ethanol (100 ml). The precipitates were recovered by centrifugation, as above combined and dissolved in 10% TCA (5 ml). The small amount of insoluble material was removed by centrifugation and addition of 90% cold ethanol (25 ml). precipitately techoic acid as a fine white powder. And washed with ethanol and ether [1].

Aqueous solution of the extracted Techic acid (50 mg dry weight / ml) was chromatographed on Sephadex G-100 with diameter (40 × 2) cm and 45 fractions were obtained However, The volume of each fraction was 9 ml which eluted with phosphate buffer saline 500 ml, pH (7.2), and elution speed 60 ml in hour.

Chemical analysis

The collected fractions of 9 ml were assayed for protein by Lowry method (10) and using standard curve of protein Fig. (3). Nucleic acids content estimation was done at (260 nm) [9] and the total carbohydrate content was determined by the phenol–salphuric acid [11], using a standard curve of glucose Fig. (2).

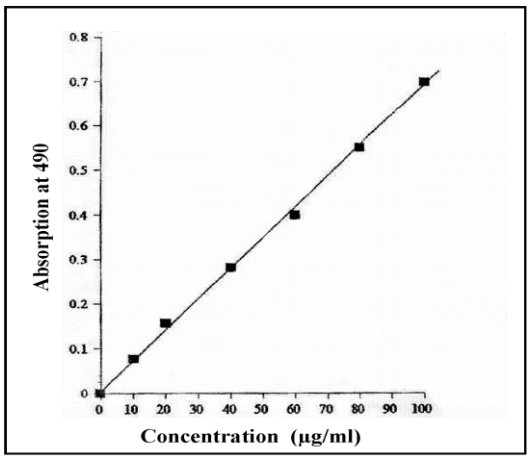

Fig. (2): Standard Curve of Carbohydrate.

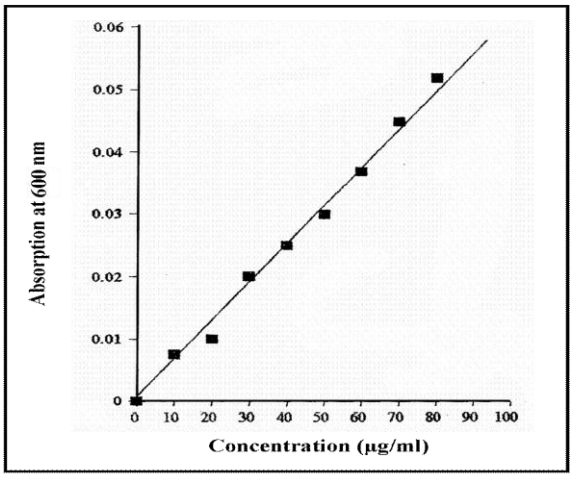

Fig. (3) : Standard Curve of Protein.

Results and Discussion

Techoic acid from cell wall of *S. aureus* was purified by treatment cell by TCA. This method is more efficient than other methods [1] because TCA as discarded other protein components of cell wall.

Through purification of techoic acid by using gel filtration two main peaks of carbohydrate appeared alongside with another four small peaks. and also Only one major peak of protein and five minor peaks have appeared, Fig.(1). The amounts of carbohydrate and protein were astimated according to the standard curves of carbohydrate and protein. It was found that the total amount of carbohydrate was 550 µg/ml (55%) and 175 µg / ml of protein (17.5 %) while nucleic acid were not obtained Table (1). This means the extracted teichoic acid was purified efficiently. In previous investigation which was studied the structure of teichoic acid of *S. aureus* found that Techoic acid was composed from number of oligosaccharide subunits [12] .

Structural studies of techoic acid showed that the major component of techoic acid was carbohydrate [13].

These results were astimated the amount of carbohydrate and protein in teichoic acid of genus *S. aureus* agreed with another previous studies which extracted teichoic acid by using the same method [14]. In previous studies which extracted teichoic acid by using another method and through purification was obtained only one main peak of carbohydrate [12].

Another studied prepared antiteichoic acid antibodies [6, 15].

From this result we conclude the using of phenol–TCA was more efficient method for extraction of teichoic acid with less contamination with protein.

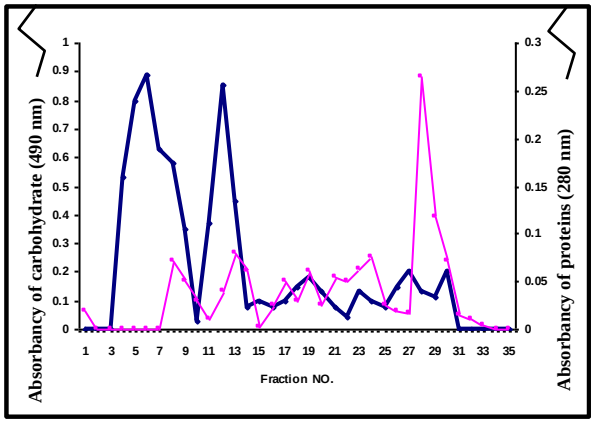

Fig.(1) : Purification of Techoic acid by chromatography gel filtration with ephadex G–100 diameter (40 × 2) cm .

Table (1)
Total amounts and percentage of carbohydrate, protein and nucleic acid in purified Techoic acid.

Carboh-ydrate µg/ml	% Carboh -ydrate	Protein µg/ml	% protein	RNA µg/ml	RNA µg/ml
550	55	175	17.5	zero	zero

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الخلاصة

يعد حامض التكويك من المستضدات السطحية (ممنعات ، محورات للاستجابة الحيوية). وهو من المكونات السطحية التي لها دور في التصاق بكتريا *Staphylococcus aureus* بانسجة المضيف استخلص في هذه الدراسة حامض التكويك ، وتم تنقيته من جدار عزلة البكتريا *Staphylococcus aureus* بواسطة (الفينول – TCA) والترشيح الهلامي وتم تحليله كيميائيا" . اظهر التحليل الكيميائي ان حامض التكويك غني بالكاربوهيدرات بنسبة 55% مع كمية قليله من البروتين وبنسبه 17.5 %.