

Purification and characterization of chondroitinase ABC from *Proteus vulgaris*, an Iraqi clinically isolate

Murtadha Nabeel Abdul-Gani^{1,*} and Bahaa Abdullah Laftaah²

¹Department of Biology, University of Madinat Al-Elem, Al-Kadhmiya, Baghdad, Iraq

²Department of Biology, College of Science, Baghdad University, Baghdad, Iraq

Forty-eight isolates were found belonging to *Proteus* sp. with isolation percentage of 38 out of 125 urine samples which were collected from urinary tract infected patients. *Proteus vulgaris* and *Proteus mirabilis* represent 18.7% (9 isolates), and 81.25% (39 isolates) respectively. The production of chondroitinase enzyme was studied in a medium which contained chondroitin sulphate as substrate. One hundred per cent of *P. vulgaris* showed the ability to produce chondroitinase. P.v 8 isolate produced the highest enzymatic activity which reached about 150 units/ml. Chondroitinase was extracted and purified by precipitation with 60% saturation of ammonium sulphate, dialysis, and followed by column chromatography on Sephadex 6B. The specific activity increased to 5000 (units/mg), with 35.2% yield and 17.4 fold purification. Optimum pH for activity and stability was 8.0; the optimal temperature for activity was 37°C, but the stability of chondroitinase was maintained 100% at 20–40°C for 30 min. Chondroitinase activity increased to 120% and 113% when the enzyme was incubated with Mg⁺² and Ca⁺² respectively, while NH₄Cl and KCl reduced the activity to 95% and 86% respectively. The human cartilage was degradable by the purified enzyme after incubation for 14 days at 37°C. Direct injection of chondroitinase at 0.1 mg/ml in knee cartilage of mice, showed changes in tissues. It may be concluded that chondroitinase enzyme may work as a virulence factor by catalysing the hydrolysis of chondroitin sulphate of cartilage and increasing the tissue permeability to invade the cartilage tissue by *P. vulgaris* which causes destruction of cartilage and inflammation in humans.

Keywords: Chondroitinase ABC, chondroitinase extraction, *Proteus vulgaris*, purification of chondroitinase.

PROTEUS VULGARIS is a well-known opportunistic pathogen that causes urinary tract infections and wound infections. It causes complicated UTIs with high frequency, when compared to other uro-pathogens. *Proteus* infections are accompanied by a formation of urinary stones containing struvite and carbonate apatite. The virulence of *Proteus* rods has been related to several factors including fim-

brae, flagella motility, outer membrane proteins, capsular antigens, enzymes (urease—hydrolysing urea to CO₂ and NH₃, proteases degrading antibodies, tissue matrix proteins, proteins of the complement system), iron acquisition systems and toxins like hemolysins. *Proteus* toxins such as agglutinin (Pta), endotoxin lipopolysaccharide (LPS) and *Proteus* rods could form biofilm, particularly on the surface of urinary catheters, which may lead to serious consequences for patients¹. Urease production, bacterial motility and fimbriae formation may favour the production of upper UTIs. The majority of *Proteus* bacteremia episodes originate from the urinary tract. Endovascular infection is rare. *Proteus* species are occasional agents of sepsis in neonates and of bacteremia in neutropenic patients². The development of wound infections caused by *P. vulgaris* depends on the interplay of many factors. The breaking of the host protective layer, the skin, thus disturbing the protective functions of the layer, will induce many cell types into the wound to initiate host response³. *Proteus* spp. are causative agents of a variety of opportunistic nosocomial infections including those of the respiratory tract, ear, nose, skin, burns and wounds. It may also cause gastroenteritis⁴ and is most frequently recovered from immune-compromised patients or those on long-term antibiotic regimen⁵. Chondroitinase is an enzyme degrading hyaluronic acid, chondroitin sulphate, dermatan sulphate or the like into a mixture of an unsaturated disaccharides and oligosaccharides. The enzyme is known to be produced by bacteria such as *P. vulgaris*. Chondroitinase of *P. vulgaris* was first purified and studied by Smith and Willettin⁶. The chondroitinase ABC was less toxic to noncartilaginous tissues than chymopapain in clinical applications of chemonucleolysis. Acute tissue reactions and compression, chondroitinase ABC and chymopapain on the epidural space, yellow ligament, sciatic nerve, knee joint, and Achilles tendon were examined. Chymopapain damaged nervous and ligamentous tissues as well as cartilaginous tissues. While chondroitinase ABC did not damage nervous and ligamentous tissues, it affected only cartilaginous tissues and its action was chiefly limited to digestion of the matrix. They concluded that the chondroitinase ABC has high enzymatic specificity for matrix *in vivo*⁷. To the best

*For correspondence. (e-mail: Mortadana88@gmail.com)

of our knowledge, there are no studies in Iraq about chondroitinase produced by local isolates of *P. vulgaris*. Therefore, the aim of this study is to address the problem in Iraq by isolation and characterization of chondroitinase from the clinical isolates of *P. vulgaris* and to study their role in pathogenicity of human cartilage.

Materials and methods

Bacterial isolation

Urine samples ($n = 125$) were collected from patients suffering from UTI, during the period between September 2013 and December 2013. The samples were obtained from Al Kadhimiya teaching Hospital, Baghdad and El Saheed Ghazi Al Hariri Hospital, Baghdad. The samples were cultured on blood agar, MacConkey agar and Urea agar. The plates were incubated aerobically for 18 h at 37°C. *P. vulgaris* were isolated and identified according to their morphological characteristics and also by biochemical tests⁸. API 20 E system was employed for confirmation of *P. vulgaris* isolates.

Production and extraction of chondroitinase

The production media containing chondroitin sulphate, 1 g; meat extract, 2.3 g; peptone, 15 g and NaCl, 1.5 g were dissolved in 1000 ml of distilled water and the pH was adjusted to 8.0 (ref. 9). It was autoclaved at 121°C for 15 min; 10 ml with 1×10^5 CFU/ml adjusted with turbidity standard of McFarland 0.5 of the bacterial inoculum of *P. vulgaris* was inoculated into the media and grown for 48 h at 37°C with shaking incubator at 150 revolutions/min (rpm)¹⁰. The cells were harvested in a cooling centrifuge at 4°C at 9000 rpm for 10 min and washed with cold normal saline two times. One volume of washed cells was taken and mixed with 3 volumes of 5 mM Tris HCl (pH 8.0) and disrupted by sonication at 19,600 Hz for eight cycles (30 s on and 90 s off) and centrifuged in a cooling centrifuge at 4°C, at 9000 rpm for 10 min⁹. The supernatant fluid (50 µl) was added into wells made on solid medium which was prepared by mixing chondroitin sulphate with 5% bovine serum albumin (both were filter sterilized). The mixture was added into the cooled medium of brain heart infusion (the final concentration of chondroitin sulphate and bovine serum albumin were 400 µg/ml and 1% respectively) with continuous stirring. The positive results for enzyme activity was determined from the clear zones around the wells⁶.

Assay of chondroitinase activity

Chondroitinase was assayed according to the method of Lohse and Linhardt¹¹ from the released $\Delta 4,5$ -unsaturated disaccharides from lysis of chondroitin sulphate and

measured at 232 nm. A 300 µl of reaction solution containing Tris-HCl 20 mM, pH 8.0 and 0.2% chondroitin sulphate (W/V)¹² was mixed well with 60 µl of crude enzyme and incubated for 20 min at 37°C in a water bath. The reaction was stopped by adding 2.64 ml of stop solution and stored at -4°C in a refrigerator before use. The enzyme activity was measured according to Yamagata *et al.*⁹.

Purification of chondroitinase

The crude enzyme in the supernatant fluid was precipitated with 30%, 60% and 90% saturation of $(\text{NH}_4)_2\text{SO}_4$ in cooling condition, and the highest amount of precipitated in suitable saturation was collected by centrifugation at 4°C, at 9000 rpm for 10 min. Dialysis was done against 0.05 M Tris HCl, pH 8.0 for 24 h at 4°C with stirring. Five millilitre of the dialysed enzyme was applied gently onto the chromatography column (80 × 2 cm) containing Sephadex 6B, prepared and equilibrated previously with 0.5 M of Tris HCl, pH 8.0, and eluted with 0.5 M of Tris HCl pH 8.0 at a flow rate of 6 ml/min. Fractions of 5 ml were collected¹⁰ and the protein was estimated following the procedure of Whitaker and Granum¹³ with some modifications. Briefly, protein was precipitated with 1.5 ml of 5% TCA for each 1 ml of crude enzyme and then centrifuged at 3000 rpm for 15 min. The precipitate was dissolved with 1.5 ml (0.05 M NaOH) and the optical density was measured at 235 and 280 nm. Protein concentration was calculated by the formula: (mg/ml) = OD at 235 nm – OD at 280 nm/2.51; while the specific activity was calculated as units/mg protein. The chondroitinase activity in all the fractions and the fractions with high chondroitinase activity was pooled and the volume, specific activity and protein concentration were determined. The active enzyme was stored at 4°C after adding 1 M EDTA and 10% glycerol¹⁰.

Characterization of enzyme

Molecular weight for the purified protein of chondroitinase was estimated by electrophoresis under denaturing conditions by SDS-PAGE with 10% polyacrylamide gels. The gel was incubated in staining solution (10% acetic acid, 0.06% Coomassie brilliant blue R-250 and 50% methanol) for 2 h with shaking followed by incubation in destaining solution (5% methanol and 7.5% acetic acid). The molecular mass of the protein was determined by comparison with the mobility of molecular weight markers¹⁴.

Optimum pH for the enzyme activity was measured at 37°C by preparing the reaction solution in different buffers (0.2 M of acetate buffer pH (4–5), 0.2 M of phosphate buffer pH (6–7), Tris-HCl buffer pH (8–9) with chondroitin sulphate C (0.2% (W/V) as the substrate. 60 µl of the purified chondroitinase was added and the mixture was

incubated for 20 min and the enzyme activity was determined. Plot of enzyme relative activity versus pH was constructed to determine the optimum pH for the reaction.

Chondroitinase (60 μ l) was incubated in buffer of different pH values from 4 to 9 for 30 min. Then 300 μ l of reaction solution at pH 8 was added to each tube and incubated at 37°C for 20 min. The enzyme activity was determined.

(iv) The optimal temperature on chondroitinase activity was measured by incubating the reaction solution at pH 8.0 at different temperatures 20°C, 30°C, 40°C and 50°C for 30 min with chondroitin sulphate C (0.2 W/V%) as the substrate. Sixty μ l of purified chondroitinase was then added and the mixture incubated for 20 min and chondroitinase activity measured under standard assay conditions.

Chondroitinase (60 μ l) was incubated at different temperatures (20°C, 30°C, 37°C, 40°C and 50°C) for 30 min and then cooled at room temperature. The reaction solution (at 300 μ l pH 8.0) was added to each tube and incubated at 37°C for 20 min. The enzyme activity was assayed.

The purified enzyme solution (60 μ l) was incubated with and without metal ion solution at a ratio of 1 : 1 at 37°C for 30 min. Then the reaction solution (300 μ l) was added containing chondroitin sulphate C (0.2 w/v%) as the substrate at pH 8.0; and assayed at 37°C for 20 min. Metal ions include KCl, CaCl₂, MgCl₂, NH₄Cl at the final concentrations of 1 and 5 mM.

Effect of purified chondroitinase on human cartilage

The normal knee cartilage of human, obtained from Al-Wasti Hospital, was transported to the laboratory in transporting fluid, Dulbecco's Modified Eagle Medium (DMEM). It was prepared according to instructions provided (by US Biological Company) by dissolving 13.55 g of media in 800 ml of distilled water; 3.7 g of sodium bicarbonate (NaHCO₃) was added and the pH was adjusted to pH 7.2–7.5. The volume was completed to 1000 ml by distilled water and sterilized by membrane filtration through 0.2 mm filter and distributed into small flasks. The cartilage was washed in Tris HCl pH 8.0 for 4–5 times to remove the transporting fluid and cut into small pieces around 1–2 mm in size and washed again; cartilage pieces were separated in two tubes each tube containing 6 small pieces of cartilage; First tube was filled with 3 ml Tris HCl, pH 8.0 and kept as control while the second tube was treated with 3 ml purified chondroitinase. Degradation of cartilage was observed and the reaction in triplicate was then measured for 14 days at 232 nm (ref. 10).

Histological changes after chondroitinase treatment in knee of mice

Female mice ($n = 8$) age 5–6 weeks, were obtained from the National Centre for Control and Pharmaceutical

Research in Baghdad. They were divided into two groups, each group containing four mice. The first group was injected with purified chondroitinase (100 μ l at 0.1 mg/ml) in knee and the second group was injected with 100 μ l normal saline and treated as control. All animals were injected two times (day 1 and day 7). They were kept in cages for two weeks after injection and were then dissected. The knee tissues were aseptically removed from animals and kept in 10% formalin solutions to study histological changes. The mice were anaesthetized with chloroform, and the skin around the knee joints was shaved. The histological sections were made according to Humason¹⁵. The knees joints were fixed in 10% formalin for 24 h and then washed in tap water for 3–4 times for 3 min. They were then passed through serial concentrations of alcohol (70%, 80%, 90% and 100%) for 2 h in each concentration, cleared in xylol for 2 h, saturated with melted paraffin at 60–65°C for 2 h and then embedded in pure paraffin until they became solid in templates. The blocks were then cut into sections of 5 μ m thickness by using a microtome. These sections were placed on glass slides using Myer's albumin and then left for drying at 37°C. The slides were left in xylene to dissolve the paraffin wax and then washed in different concentrations of alcohol (100%, 90% and 70%), for 3 min in each concentration. The slides were stained with haematoxylin stain for 5–10 min, washed in tap water, then with acidic alcohol and then finally washed in tap water again. Later, the slides were stained with eosin stain for 15–30 s and then rinsed in distilled water. The slides were incubated in increasing concentrations of alcohol (70%, 90% and 100%) for 2 min in each concentration and then cleared in xylol for 10 min. Canada balsam was added gently to the slides and cover slips were placed and then examined in light microscope.

Results and discussion

Bacterial isolates

Results showed that the overall incidence of *P. mirabilis* and *P. vulgaris* was 81.25% and 18.7% respectively. Out of 48 *Proteus* spp., 38% were obtained from 125 urine samples from patients with urinary tract abnormalities admitted in Baghdad hospitals. The incidence of *Proteus* spp. agreed with study done by Al-Bassam and Al-Kazaz¹⁶ who found that strains of *Proteus* isolated from urine samples were 40%, while Uwaezuoke and Ogbulie¹⁷ reported that the isolation percentage of *Proteus* strains from UTI was 4.8% and this result agreed with Malik¹⁸ who found that the isolation percentage of *P. mirabilis* was 94.28%, while *P. vulgaris* was 5.72% from UTI. However, Orret¹⁹ reported 74.3% and 12.2% of *P. mirabilis*, *P. vulgaris* respectively. The reason for such differences may correlate with differences in the season of collecting samples, use of

antibiotics and differences in the environment and living area.

Characterization of chondroitinase

The production and assay of chondroitinase in bacterial extracts before and after sonication were studied. Before sonication of cells, there were no clearing zones around the colonies because chondroitinase was classified an intracellular enzyme. But after sonication, the extract of isolate P8 gave the largest clearing zone with enzymatic activity, 165 units/ml, while the isolate P2 showed the lowest clearing zone with enzymatic activity 54 units/ml; isolate P7 did not show any clearing zone (Figure 1 and Table 1). The positive results suggest that the non-degraded substrate precipitates as a conjugate with the albumin, leaving a clear zone around colonies which may produce the enzyme that degrade the chondroitin sulphate. In negative results no substrate degradation was observed, because no enzyme was produced. These results are in close agreement with other studies^{9,20}.

Purification of chondroitinase

The optimal conditions include size of inoculum (1×10^5 CFU/ml), pH (8.0), temperature (37°C), incubation period (48 h) to obtain heavy growth of bacteria with maximum production of the enzyme¹⁰. The results showed that the activity of crude enzyme obtained from sonicated extracts P8 isolate of 50 ml volume was 165 units/ml with a specific activity 288 units/mg; 60% of ammonium sulphate precipitated enzyme activity and a specific activity of 300 units/ml, 428 units/mg respectively. The precipitated chondroitinase after dialysis show decrease in enzyme activity to 240 units/ml while the specific activity increased to 533 units/mg. The eluted fractions of gel filtration column showed five peaks and the specific activity of chondroitinase reached maximum in the fourth peak to 5000 units/mg (fractions 53–57) (data not shown) with 35.2% yield and 17.4 fold of purification (Table 2).

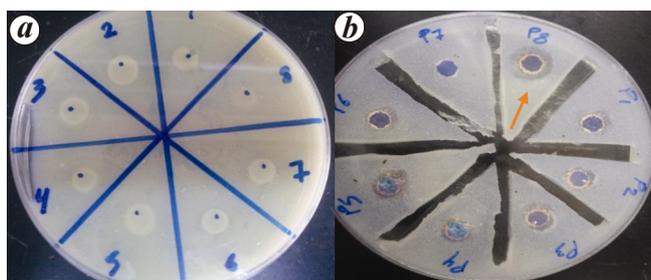


Figure 1. Screening of *P. vulgaris* isolates for chondroitinase on solid media. *a*, Before sonication; *b*, After sonication.

Characterization of purified enzyme

The molecular weight of *P. vulgaris* chondroitinase was determined by SDS-PAGE. The results showed only one band for the purified chondroitinase with a molecular mass of approximately 111.3 kDa (Figure 2). Previous studies have shown that the molecular mass for chondroitinase ABC was 150 kDa (ref. 9), while Hashimoto *et al.*²¹ found a single band in SDS-PAGE and a single peak in gel permeation chromatography (HPLC) with molecular mass of about 100 kDa in each technique. Hami reported²⁰ molecular mass of 100 and 105 kDa for chondroitinase ABC I and II extracted from *P. vulgaris*. Also, Prabhakar *et al.*²² reported a molecular mass of 110 kDa for chondroitinase I.

The highest enzyme activity was shown to be 500 units/ml at pH 8 which declined to 291 units/ml at pH 9 (Figure 3). Alkaline condition is better for activity of chondroitinase. Michelacci *et al.* found maximum activity for chondroitinase, produced by *Flavobacterium heparium* at pH 6.0–7.5 (ref. 23).

The stability of purified enzyme was found to be 100% at (pH 8.0) and decreased to 90%, 64%, 25%, 10% at pH values 7, 6, 5 and 4 respectively. At pH 9 the enzymatic activity was only 30% (Figure 4). Our result is in close agreement with Hashimoto *et al.*²¹ where they found that the highest activity was from pH 8–8.2 while the enzyme was incubated between 5 and 9 at 25°C for 24 h. The enzyme exhibited about 80% or more residual activity and lost most of its activity at pH 9 and 10. The reduction of enzyme stability at acid pHs is attributed to effect of ionization of the enzyme which led to irreversible denaturation of the enzyme²⁴.

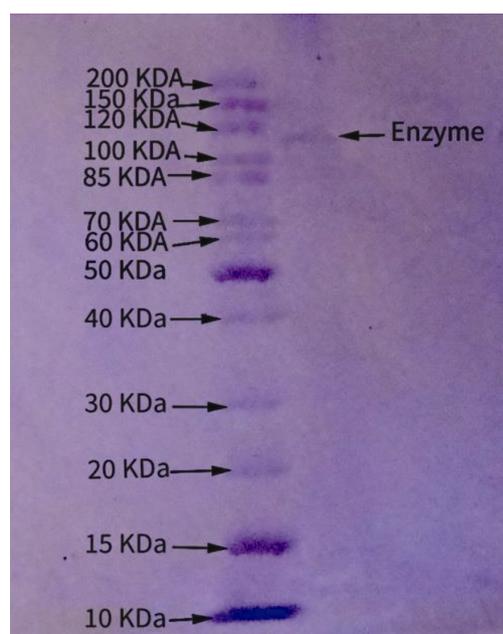


Figure 2. SDS-PAGE of purified chondroitinase from *Proteus vulgaris*.

Table 1. Enzyme activity of chondroitinase in each isolate

Isolate no.	P1	P2	P3	P4	P5	P6	P7	P8
Enzyme activity (units/ml)	67	54	90	73	125	60	00	165

Table 2. Summary of purification of chondroitinase from *P. vulgaris*

Purification step	Volume (ml)	Enzyme activity (units/ml)	Protein concentration (mg)	Specific activity (units/mg)	Total activity (units)	Fold purification	Recovery (%)
Crude enzyme	50	170	29.5	288	8500	1.00	100
Ammonium sulphate precipitations (60%)	4	300	2.8	428	1200	1.49	14
Dialysis	5.5	240	2.475	533	1320	1.85	15.5
Gel filtration on Sephadex 6B column	6	500	0.6	5000	3000	17.36	35.2

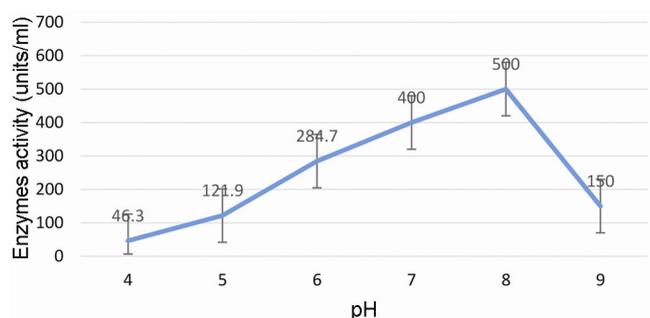


Figure 3. Effect of pH on the enzyme activity.

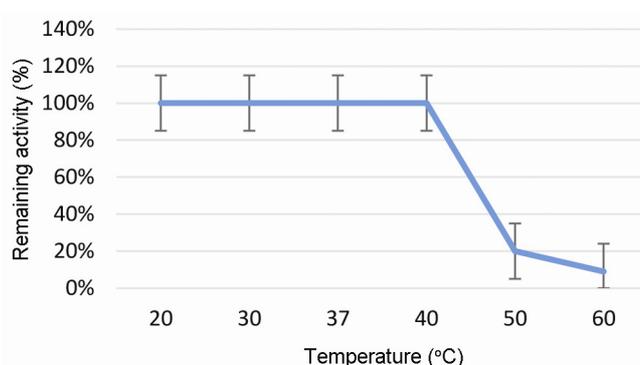


Figure 6. Effect of temperature on enzyme stability.

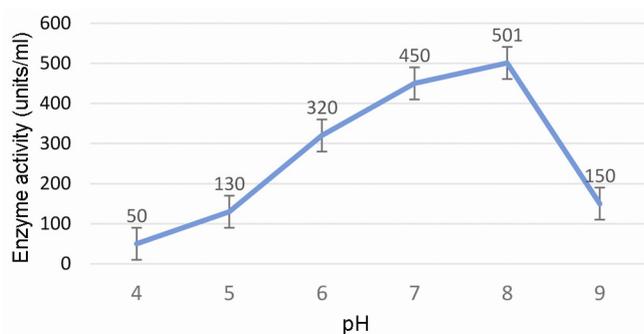


Figure 4. Effect of pH on enzyme stability.

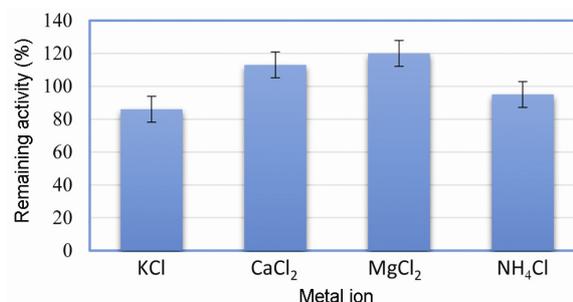


Figure 7. Effect of metal ions on chondroitinase activity.

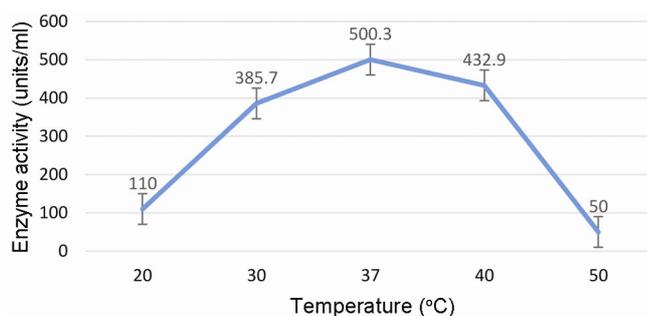


Figure 5. Effect of temperature on the enzyme activity.

The results showed that the enzyme activity of chondroitinase increased up to 500 units/ml with increase in temperature and the enzyme activity decreased drastically at 50°C (Figure 5). Prabhakar *et al.*²² found that chondroitinase ABC I from *P. vulgaris* had melting transitions at 45 ± 5°C.

The enzymatic activity maintained 100% between 20°C and 40°C for a 30 min incubation period, and decreased markedly with increase in temperature. At 60°C remaining activity was only 9% (Figure 6). Hashimoto *et al.*²¹ found that the enzyme was stable between 20°C and 30°C while it was inactivated at 50°C. Chondroitinase

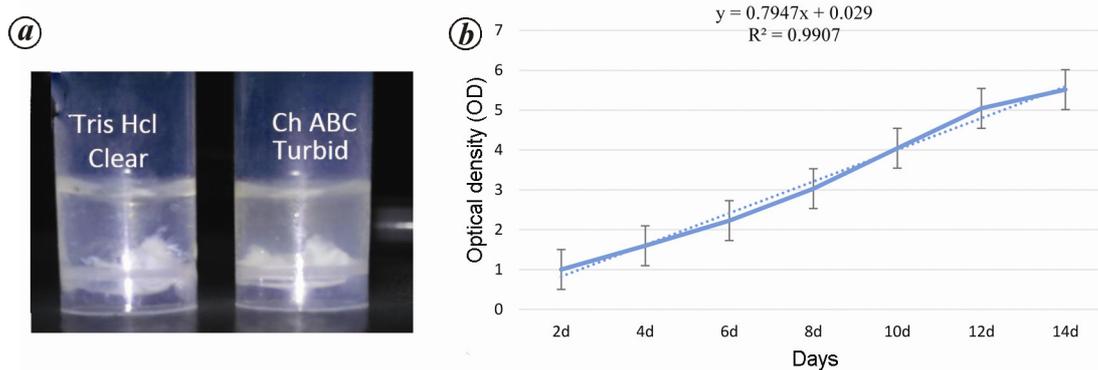


Figure 8. Degradation of human cartilage by purified chondroitinase. *a*, Effect A cartilage incubated with Tris HCl (control) and cartilage incubated with the purified enzyme. *b*, Relationship between OD and days of incubation, the suspension was measured at 232 nm.

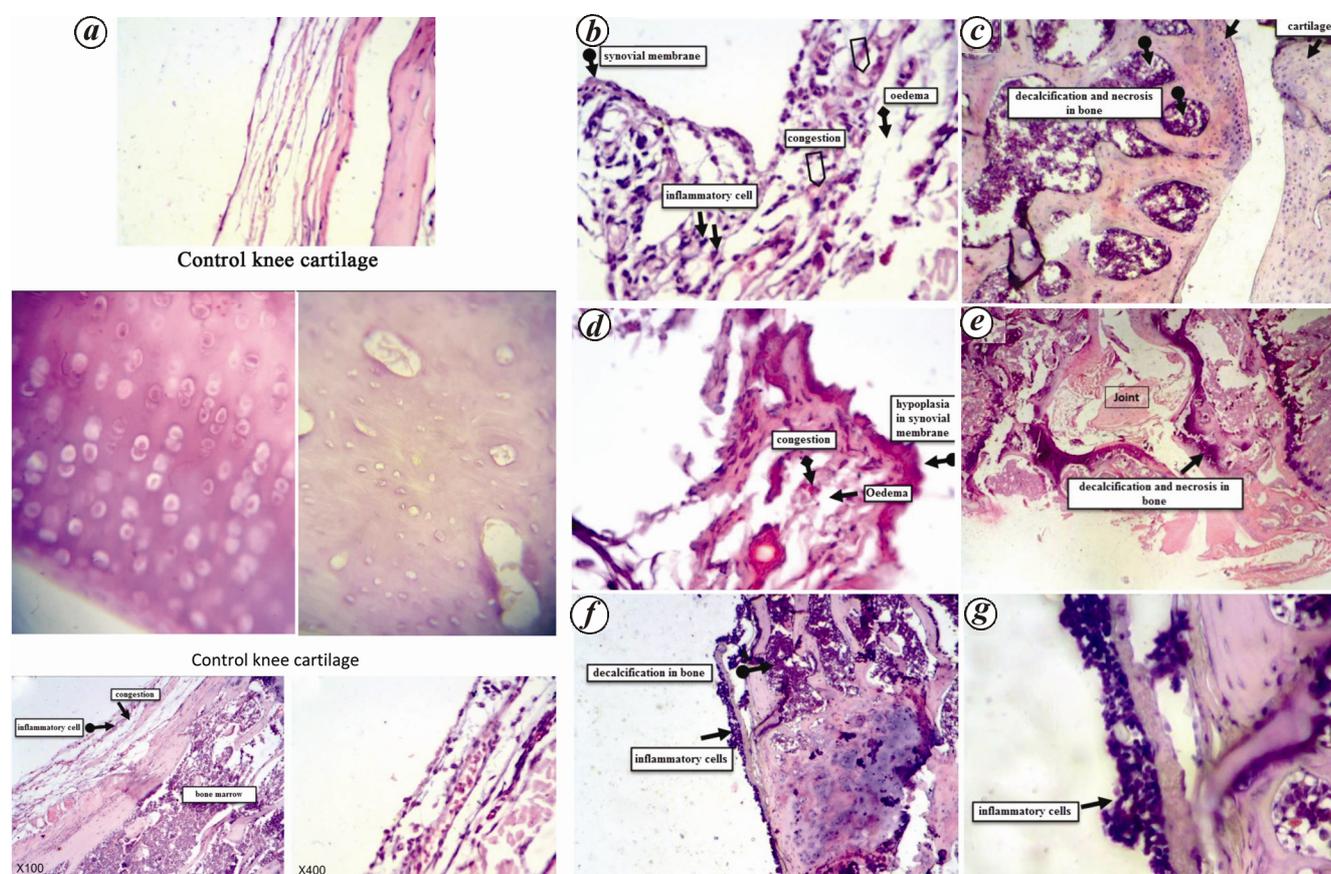


Figure 9. Effect of purified chondroitinase on knee cartilage. *a*, Section showing inflammatory cells in knee cartilage and congestion. *b*, Section in knee cartilage showing inflammatory cells with oedema and congestion ($\times 200$). *c*, Section in knee cartilage showing decalcification and necrosis in bone ($\times 200$). *d*, Section in knee cartilage showing hypoplasia in synovial membrane, congestion and oedema ($\times 400$). *e*, Section in knee cartilage showing decalcification and necrosis in bone ($\times 200$). *f*, *g*, Section in knee cartilage showing decalcification and necrosis in bone and inflammatory cells in synovial membrane (*f* ($\times 100$); *g* ($\times 400$)).

was found to be more stable when kept at lower temperatures (Figure 6).

Purified chondroitinase was treated with different metal ions and the activity was determined. Figure 7 shows that CaCl_2 and MgCl_2 were found to stimulate the enzyme activity. NH_4Cl and KCl reduced the enzyme activity to 95% and 86% respectively. Ionic interactions

between an enzyme-bound metal ion and a substrate can help orient the substrate for reaction or stabilize charged reaction transition states. Ca^{2+} and Mg^{2+} may be attached to amino acids that form the active site of chondroitinase ABC and increase enzyme reaction with substrate (chondroitin sulphate)²². Prabhakar *et al.*²² found that calcium, a divalent ion, preferentially increases the activity of

chondroitinase ABC I toward dermatan versus chondroitin substrates in a concentration-dependent manner. Calcium ions can bind to chondroitinase ABC I through specific amino acids that could potentially play a role in calcium coordination²³. However, Ca²⁺ and Mg²⁺ reduced the enzyme activity of chondroitinase AC and B extracted and purified from *F. heparinum* and K⁺, Ca²⁺ and Mg²⁺ increased chondroitinase B activity.

The human cartilage was found to be partially degraded after treatment with purified chondroitinase. The degradation effect of cartilage was found to increase gradually with incubation time for 14 days at 37°C (Figure 8).

The histological changes caused by injecting of purified chondroitinase in knee cartilage of mice were studied. The cross-sections showed the ability of chondroitinase to make changes in cartilage tissues and on bone formation which included appearance of inflammatory cells in knee cartilage, congestion, oedema, decalcification and necrosis, and also showed hypoplasia in synovial membrane (Figure 9). Thus, the chondroitinase works like other proteolytic enzymes that affect human cartilage and causes degradation similar to hyaluronidase enzymes produced by pathogenic bacteria such as *Clostridium perfringens*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* that cause serious diseases. Hyaluronidase works as a virulence factor to destroy the polysaccharide that holds animal cells together, making it easier for the pathogen to spread through the tissues of the host organism^{25,26}. Based on all the above data we conclude that the chondroitinase enzyme may work as a virulence factor by catalysing the hydrolysis of chondroitin sulphate, a constituent of the extracellular matrix of cartilage and increase tissue permeability to invade the cartilage tissue by *P. vulgaris*.

- Mobley, H. L. T. and Belas, R., Swarming and pathogenicity of *Proteus mirabilis* in the urinary tract. *Trends Microbiol.*, 1995, **3**, 280–284.
- Longo, D. L., Kasper, D. L., Jameson, J. L., Fauci, A. S., Hauser, S. L. and Loscalzo, J. (eds), *Harrison's Principles of Internal Medicine*, McGraw Hill, New York, 2012, 18th edn.
- Collier, M., Understanding wound inflammation. *Nurs. Times*, 2003, **99**(25), 63–64.
- Jacobsen, S. M., Stickler, D. J., Mobley, H. L. T. and Shirtliff, M. E., Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clin. Microbiol. Rev.*, 2008, **21**(1), 26–59.
- Mordi, R. M. and Momoh, M. I., Incidence of *Proteus* species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. *Afr. J. Biotechnol.*, 2009, **8**(5), 725–730.
- Smith, R. F. and Willett, N. P., Rapid plate method for screening hyaluronidase and chondroitin sulfatase-production microorganisms. *Appl. Microbiol.*, 1968, **16**(9), 1434–1436.
- Kato, F., Mimatsu, K., Iwata, H. and Miura, T., Comparison of tissue reaction with chondroitinase ABC and chymopapain in rabbits as the basis of clinical application in chemonucleolysis. *Clin. Orthop. Relat. Res.*, 1993, **288**, 294–302.
- Garrity, G. M., Brenner, D. J., Krieg, N. R. and Staley, J. T., *Bergey's Manual of Systematic Bacteriology*, Springer, 2005, pp. 745–751.
- Yamagata, T., Saito, H., Habuchi, O. and Suzuki, S., Purification and properties of bacterial chondroitinases and chondrosulfatases. *J. Biol. Chem.*, 1968, **243**, 1523–1535.
- Abdul-Gani, M. N., Investigation chondroitinase from *P. vulgaris* and studying its effect on animal tissue. M.Sc. thesis. Biology Department, Collage of Science, Baghdad University, Iraq, 2015.
- Lohse, D. L. and Linhardt, R. J., Purification and characterization of heparin lyases from *Flavobacterium heparinum*. *J. Biol. Chem.*, 1992, **267**, 24347–24355.
- Ke, T., Zhangfu, L., Qing, G., Yong, T., Hong, J., Hongyan, R., Kun, L. and Shigui, L., Isolation of *Serratia marcescens* as a chondroitinase-producing bacterium and purification of a novel chondroitinase AC. *Biotechnol. Lett.*, 2005, **7**, 489–493.
- Whitaker, J. R. and Granum, P. E., An absolute method for protein determination based on difference in absorbance at 235 and 280 nm. *Anal. Biochem.*, 1980, **109**, 156–159.
- Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 1970, **227**, 680–685.
- Humason, C. L., *Animal Tissue Techniques*, W.H. Freeman Company, 1972, 3rd edn, p. 641.
- Al-Bassam, W. W. and Al-Kazaz, A., The isolation and characterization of *Proteus mirabilis* from different clinical samples. *J. Biotech. Res. Center*, 2013, **7**, 24–30.
- Uwaezuoke, J. C. and Ogbulie, J. N., Antibiotic sensitivity pattern of urinary tract pathogens in Port-Harcourt, Nigeria. *J. Appl. Sci.*, 2006, **10**(3), 103–107.
- Malik, S. N. A., Extraction and purification of lipopolysaccharide of locally isolated *Proteus mirabilis* and study of its effect to prevent urinary tract infection in an animal model. M.Sc. Thesis. College of Science, Biology Department, Baghdad University, Iraq, 2006.
- Orret, F. A., Prevalence of *Proteus* species in urinary tract infections in a regional hospital in Trinidad. *Zhonghua Yi Xue Za Zhi (Tapei)*, 1999, **62**, 438–442.
- Hamai, A., Hashimoto, N., Mochizuki, H., Kato, F., Makiguchi, Y., Horie, K. and Suzuki, S., Two distinct chondroitin sulfate ABC lyases: an endoeliminase yielding tetrasaccharides and an exoeliminase acting on oligosaccharides. *J. Biol. Chem.*, 1997, **272**, 9123–9130.
- Hashimoto, N., Mochizuki, H. and Harnai, A., Producing pure chondroitinase ABC from, US Pat. 5,763,205, 9 June 1998.
- Prabhakar, V., Capila, I., Bosques, C. J., Pojasek, K. and Sasisekharan, R., Chondroitinase ABC I from *Proteus vulgaris*: cloning, recombinant expression and active site identification. *Biochem. J.*, 2005, **386**, 103–112.
- Michelacci, Y. M., Horton, D. S. and Población, C. A., Isolation and characterization of an induced chondroitinase ABC from *Flavobacterium heparium*. *Biochim. Biophys. Acta*, 1987, **923**, 291–301.
- Sawheney, S. K., *Introductory Practical Biochemistry*, Narosa Publishing House, Mumbai, 2008, p. 362.
- Berry, A. M., Lock, R. A., Thomas, S. M., Rajan, D. P., Hansman, D. and Paton, J. C., Cloning and nucleotide sequence of the *Streptococcus pneumoniae* hyaluronidase gene and purification of the enzyme from recombinant *Escherichia coli*. *Infect. Immun.*, 1994, **62**, 1101–1108.
- Howell, D. S., Sapolsky, A. I. and Pita, J. C., *The Pathogenesis of Degenerative Joint Disease*, UpJohn Company, Kalamazoo, MI, 1976, p. 28.

Received 4 September 2016; revised accepted 11 July 2017

doi: 10.18520/cs/v113/i11/2134-2140