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Full Length Research Paper

Purification of kappa (k)-carrageenase from locally isolated *Cellulosimicrobium cellulans*

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Partial purification of the crude kappa (k)-carrageenase present in the culture filtrates of *Cellulosimicrobium cellulans* was carried out by fractional precipitation, using ammonium sulphate, acetone and ethanol individually. The highest recovered protein (37.08%) combined with enzyme activity was obtained with ammonium sulphate. The fraction precipitated by 90% ammonium sulphate was re-purified by anion exchange chromatography diethylaminoethyl (DEAE) cellulose, A-52 and 79 fractions were obtained. The loaded protein was separated into 4 peaks. The third protein peak was the major one which contained the most recovered enzyme activity (84.95%) from the eluted fractions. The collected fractions of this peak were subjected to further purification by re-chromatography on Sephadex G-100. The k-carrageenase activity was fractionated into 2 peaks. The first peak was the major one containing 95.622% of the total recovered activity. The pooled fractions of the major protein component showed a specific k-carrageenase activity of 46.22 U/mg protein, yielding about 4.6 fold purification of the crude enzyme preparation. Some properties of purified k-carrageenase obtained from *cellusimicrobium cellulans* cultures were studied. The optimum reaction temperature of the purified k-carrageenase was 30°C and the maximum activity occurred at a reaction pH of 6.

Key words: *Cellulosimicrobium cellulans*, k-carrageenase, purification, sephadex G-100, diethylaminoethyl (DEAE) sephadex A-52.

INTRODUCTION

Carrageenans are the main components of the cell wall of various marine red algae (Rhodophyceae) where they play a variety of structural (cell-cell cohesion and exchange boundary) and signaling (cell-cell recognition) roles (Kloareg and Quatrano, 1988; Potin et al., 1999). They exhibit unique rheological properties and are widely used as texturing agents in various industries (De Ruiter and Rudolph, 1997). K-Carrageenan consists of repeated units of the disaccharide 4-sulfate-O-1,3-β-D-galactopyranosyl-1,4-α-3,6-anhydro-D-galactose, also known as neocarrabiose sulfate. K-Carrageenase has been found in several marine bacteria (Bellion et al., 1982). Many bacterial strains were reported such as *Pseudomonas* (Weigl and Yaphe, 1966; McLean and Williamson, 1979, 1980; Greer and Yaphe, 1984), *Cytophaga* (Sarwar et al., 1983a, b; Sarwar et al., 1987; Potin et al., 1991), and some unidentified species (Greer, 1985). Enzymes, which degrade carrageenans, are called k-, ι-, and λ-carrageenases. They all are endohydrolases that cleave the internal β (1-4) linkages of carrageenans yielding products of the neocarrabiose series (Johnston and McCandless, 1973; Barbeyron et al., 1994, 1998, 2000). It was reported that k-carrageenase degrades k-carrageenan by hydrolyzing the β (1-4) linkages to a series of homologous, even-numbered oligosaccharides (Greer and Yaphe, 1984).

Carrageenan and its depolymerized forms as oligosaccharides, which are resulting from carrageenases activity, have been shown to have biological activities such as being an excipient or disintegrant, relieving cough, anti-viral, decreasing blood fat and cholesterin

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(Shi et al., 2000); there are also a few reports on their anti-tumor activities. Sulfated oligosaccharides from marine red algae can show potential anti-tumor activities on mammary adenocarcinoma, Meth-A tumor and Ehrlich ascites cells (Coombe et al., 1987; Noda et al., 1989). A much larger demand for enzymes such as carrageenases is developing rapidly within the new field of seaweed biotechnology, where cell wall-degrading enzymes are applied to obtain protoplasts. Also, breakdown products of molecular weight <40000, called poligeenans, have been implicated in gastrointestinal malignancy in animal models (Tache et al., 2000; IARC, 1983). Thus, additional biological evaluation of poligeenans is warranted (Tobachman, 2001; Gold et al., 1997; NRC, 1996). The aim of the present work was to purify the k-carrageenase enzyme from the culture filtrate of *Celluosimicrobium cellulans*. The determination of some characteristics of the purified enzyme was also fulfilled.

MATERIALS AND METHODS

Microorganism

Cellulosimicrobium cellulans was isolated from life specimens of the red algae *Gelidium sesquipedale*, *Alsidium corallium* and *Ceramium rubrum*. This strain was identified using 16s rDNA in the genetic laboratory in Norwegian University of Science and Technology (NTNU), Norway.

Culture medium

ZoBell medium was used for the maintenance of the bacterial strains throughout the work. This medium contained the following ingredients (g/l): filtered sea water 800 ml; distilled water 200 ml; yeast extract, 1; peptone, 5; kappa-carrageenan, 1.5; agar-agar, 15 and FeSO4.7H_2O traces, final pH was adjusted to 7.5. The inoculated slants were incubated for 3 days at 37°C then stored at 4°C until used. On the other hand, The mineral medium contained the following ingredients (g/l): Kappa-carrageenan, 15; $(NH4)_2SO_4$, 5; NH_4Cl, 0.7; NaCl, 20; $CaCl_2.2H_2O$, 0.2; K_2HPO_4, 3; $MgSO_4.7H_2O$, 0.5; KCl, 0.3; $FeSO_4.7H_2O$ traces and agar-agar, 15. The final pH was adjusted to 7.5 and was used to induce the production of k-carrageenase in specific.

Cultivation of bacteria for enzyme production

Fifty milliliter (50 ml) aliquots of the mineral medium were dispensed in 250 ml Erlenmeyer flasks. The medium was adjusted to pH 7.5 and then sterilized at 121°C for 15 min. After sterilization, each flask was inoculated with 1 ml of suspended bacterial cells obtained from over night culture slant and 10 ml culture volume; the flasks were then incubated in the shaker at 37°C and 250 rpm for 1 day. Afterwards, the content of each flask were taken for analysis. Each treatment was carried out in triplicates and the results obtained throughout the work were the arithmetic mean of at least 2 experiments.

Preparation of the crude enzyme

At the end of the incubation period, the bacterial cells were separated from the culture by centrifugation at 7000 rpm for 15 min in a cooling centrifuge (Chilspin, made in England) at 4°C. The clear supernatant was considered as the crude enzyme source.

Estimation of protein content

The protein content of the enzyme preparation was determined by the method of Lowry et al. (1951) and the developed color was measured at 750 nm (Shimadzu double beam spectrophotometer UV-150-02, Japan). The standard curve was previously constructed using crystalline bovine serum albumin (Sigma Chemical Company, St. Louis, U.S.A).

Assay for k-carrageenase activity

Assay was performed by incubating 100 µl of substrate suspension (0.1% kappa carrageenan in Tris-HCl buffer, pH 7.5) with 100 ul of kappa carrageenase for 10 min at 37°C. After incubation, 150 µl of reaction mix were removed and added to 150 µl of Kidby solution (1% Na_2CO_3 and 0.03% potassium hexacyanoferrate III). Color development was achieved by placing tubes in a water bath 100°C for 10 min. Finally, the spectral absorbance of samples at 420 nm was measured by (Kidby and Davidson, 1973). The amount of released sugar was estimated using a D-galactose calibration curve (0 to 400 µg/ml) and the activity unites (IU) were defined as the quantity of enzyme needed to release 1 µmol of equivalent galactose per minute under the assay conditions.

Partial purification of k-carrageenase enzyme produced by *Cellulosimicrobium cellulans*

Partial purification was achieved by salting-out with ammonium sulphate and by fractional precipitation with acetone and ethanol. The crude culture supernatant was obtained from 1 day old free cultures of *cellulosimicrobium cellulans* at log phase grown under optimal conditions (Beltagy, 2010). The supernatant was precipitated at different concentrations of each precipitant in a sequential manner.

Fractionation by salting-out with ammonium sulphate

A 250 ml volume of the crude culture filtrate was fast rotated in a cooling centrifuge 6000 rpm at 4°C to remove the residual waste and fungal cells. The protein content and the k-carrageenase activity of the enzyme solution were determined as described previously. The whole enzyme solution was kept in an ice bath. This was followed by adding ammonium sulphate very slowly while stirring to the ice cold enzyme solution until the desired saturation of ammonium sulphate was reached. The solution was left for 2 h and then centrifuged for 15 min at 7000 rpm in a cooling centrifuge. The precipitate (Fraction1) was removed and further ammonium sulphate was added to the supernatant fluid to obtain the next fraction. The process was repeated until 100% saturation was reached.

Each precipitated fraction was dissolved in about 10 ml distilled water and dialyzed against distilled water (or buffer) in a cellophane bag in a refrigerator until the water outside the bag gives no precipitate with 1% barium chloride solution, indicating that the enzyme solution inside the bag become free of sulphate. This was achieved by changing the water (or buffer) outside the bag several times. After complete dialysis, each enzyme solution was dried by evaporation under low temperature. The protein content and k-carrageenase activity of each solution was measured.

Fractional precipitation with acetone

Acetone (Analar Reagent) was cooled at 4°C one day before starting the precipitation. The culture supernatant sample was identical to that used for salting-out and was treated by the manner before precipitation with acetone. The whole enzyme solution was kept in ice bath; a certain volume of acetone was added slowly while stirring until the required concentration was reached. After removing the precipitated fraction by centrifugation at 5000 rpm at 4°C for 15 min, in cooling centrifuge, further acetone was added to the supernatant fluid and the process was repeated until the acetone reached a final concentration of 95%. Several enzyme fractions were thus obtained. Acetone fractions were dried over anhydrous calcium chloride under reduced pressure at room temperature and then dialyzed against distilled water.

Fractional precipitation with ethanol

Absolute ethanol (A.R.) was cooled at 4°C over night and precipitation with ethanol was performed using the same procedure as that of acetone precipitation.

Ion-exchange chromatography on DEAE cellulose A-52

DEAE-cellulose was used as an anion exchanger as recommended by Peterson and Sober (1962), it was equilibrated with 0.05 M Tris buffer (pH 7.5) to be used in chromatography. Preparing a suspension of the DEAE-cellulose A-52 ion exchanger in the starting buffer, the DEAE particles were allowed to settle, carried out equilibration and the excess buffer was decanted. This step was repeated several times until the pH of the DEAE particles was the same as that of the buffer. The cellulose buffer mixture was thoroughly dispersed with stirring. The mixture was placed in a cylinder and allowed to settle and the supernatant was then decanted to remove the fine particles.

The remaining uniform suspension was then poured into the column and the packing was processed as previously mentioned for gel filtration. The packed column was then equilibrated before use by passing several volumes of the starting buffer through the packed material of DEAE granules.

Gel filtration

Sephadex G-100 was used for gel filtration. The gel was soaked in Tris buffer (0.05 M, pH 7.5) and heated in a water bath at 90°C for 5 h, this step afforded swelling of the gel and degassing at the same time. The gel bead suspension was allowed to cool and settle down. Fine particles were removed by decantation. The gel suspension was adjusted to form thick slurry and gently mixed and poured down the column (28 × 1.8 cm). The column outlet was closed to allow the material to be packed. After packing the Sephadex G-100, the column was connected to a peristaltic pump and the flow adaptor was placed on the horizontal surface of the bed and connected to the eluent reservoir. To let the bed stabilize, 3 to 4 column volumes of the eluent were passed through the packing material in the column at a flow rate slightly higher than the one to be used for sample elution to avoid subsequent decrease in bed volume. The column was allowed to pack under these conditions until the bed height attained a stable value. Once the bed was packed, the pump was disconnected and flow from the bottom of the column was shut off. The top column unit was released and the enzyme sample (about 6 ml in 0.05 M Tris buffer, pH 7.5) was applied into the column. Acetate buffer was also used for elution of the enzyme protein at a flow rate 42 ml/h by using a peristaltic pump (Cole-Parmer, Multichannel cartridge pump, United States). The eluent was collected in 3.5 ml fraction manually. The protein content and k-carrageenase activity of each fraction was determined as described before.

Effect of temperature

To examine the effect of temperature of the reaction on the activity of the purified enzyme, the enzymatic reaction was carried out for 10 min at temperature 20, 30, 37, 40, 45, 50, 55 and 60°C using an enzyme protein and substrate concentration of 33 μg and 0.15 mg, respectively per 200 μl reaction mixture. In each case, a control was made using previously heated enzyme solution in the reaction.

Effect of pH of the reaction

The influence of the pH of the reaction on the activity of the pure kappa carrageenase was studied. The pH range of 4 to 8 was used (acetate buffer, 0.1 M; phosphate buffer, 0.1 M, Tris buffer, 0.05M). All the other assay conditions were at their optimum values as determined from the previous experiments.

RESULTS

Fractional precipitation

The results shown in Figure 1 which belong to *Cellulosimicrobium cellulans* indicate that fractional precipitation with ammonium sulfate brought about 37.08% of total protein. The recovered protein showed the highest peak value at the fraction precipitation with 90% ammonium sulfate saturation. This fraction represented about 52.12% of the total recovered protein. All other fractions showed a relatively lower protein recovery. The kappa carrageenase activity of the fraction precipitated with different ammonium sulfate saturations increased gradually up to the 75% fraction, where the maximum activity is obtained. The activity of this fraction was about 50.78% of the total recovered activity. Higher or lower ammonium sulfate saturations showed a lower kappa carrageenase activity. The total recovered activity obtained by ammonium sulfate fractions was 39.41% of the activity present in the crude enzyme solution. The results indicate that about 32.56% of the protein present in the crude enzyme solution was recovered with acetone. Although high yield of precipitation was obtained with first fraction, which was about 64.47% of total recovered protein and followed by sudden decrease in recovered protein, no enzyme activity was determined with any fraction, like acetone fractions. The same situation was observed with ethanol fractions, no activity was observed with all fractions although there was a recovered protein with first and second fractions. The rest of fractions gave undissolved precipitation (data not shown). At the end, ammonium sulfate was selected to be the precipitating agent that can be used to purify the enzyme protein from *Cellulosimicrobium cellulans*, 90% fraction of ammonium sulfate concentration was selected for further purification to cover almost all protein that

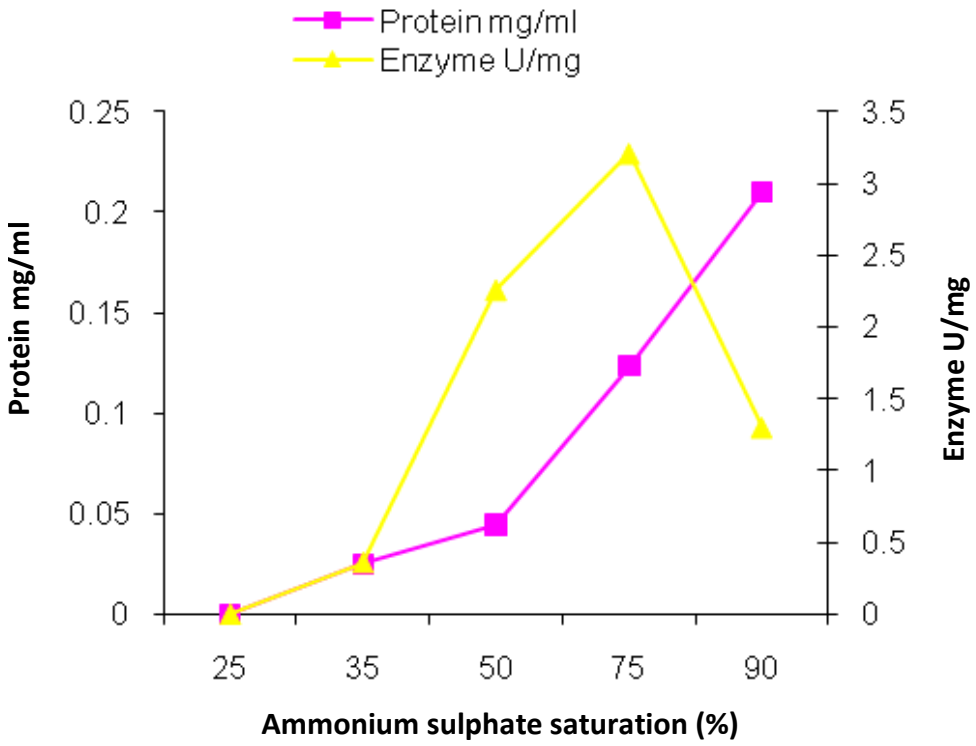

Figure 1. Fractional precipitation with ammonium sulfate of extracellular k-carrageenase from *Cellulosimicrobium cellulans.*

could produce k-carrageenase.

Ion exchange chromatography on Hi trap 5 ml QHP column

This experiment was conducted in NTNU's laboratories. 2000 ml culture volume of *Cellulosimicrobium cellulans* was implied for purification. The semi-purified sample volume from first step of purification, which was ammonium sulphate precipitation, was concentrated to 5 ml by ultra-filtration. This 5 ml of the concentrated solution was applied to anion exchange chromatography which was Hi Trap 5 ml QHP column connected to FPLC (data not shown). Fractionation with ammonium sulfate at 65% saturation removed the bulk of carbohydrates from the culture medium, without significant loss of carrageenase activity. After ultrafiltration, a significant loss of activity was observed. Running a 5 ml of ultrafiltrated sample on anion exchange column while using linear gradient of NaCl (0.1 to 1 M) gave protein peaks obtained at approximately 0.5 M NaCl, combined with kappa carrageenase activity (data not shown). Regarding so, it has been reported that it is the first case to get enzyme peak at 0.5 M NaCl. Due to insufficient amount of protein content and the dramatic lost of enzyme activity, step of purification using column chromatography was repeated to minimize the lost of protein and enzyme activity as possible, using 1300 ml culture volume of *Cellulosimicrobium cellulans*.

Ion exchange chromatography on DEAE cellulose A-52

Chromatography on DEAE cellulose A-52 column afforded 79 fractions as shown in Figure 2. A total of about 88.54% of the applied enzyme protein was recovered by the eluting solutions. The recovered protein by the first eluting solution (fraction no. 1 to 14) was 38.14%, while that obtained by the second eluting solution (fraction no. 15 to 36) was 38.11%. The third eluting solution (fraction no. 46 to 64) was 12.04% and finally the fourth eluting solution (fraction no. 70 to 75) was 0.29%. The obtained fractions were found to cover 4 protein components. The protein recovered in the first peak 43.07% of the total recovered protein from the column, while that of the 2nd, 3rd and 4th components in the other peaks represented 43.04, 13.62 and 0.003%, respectively. These results indicated that the first protein component and the second one were the majors while the other two components were minor ones. The total kappa carrageenase activity recovered from the anion exchange column reached about 56.12% of the activity of the sample applied to the column. Most of the recovered kappa carrageenase activity was present in the 3rd

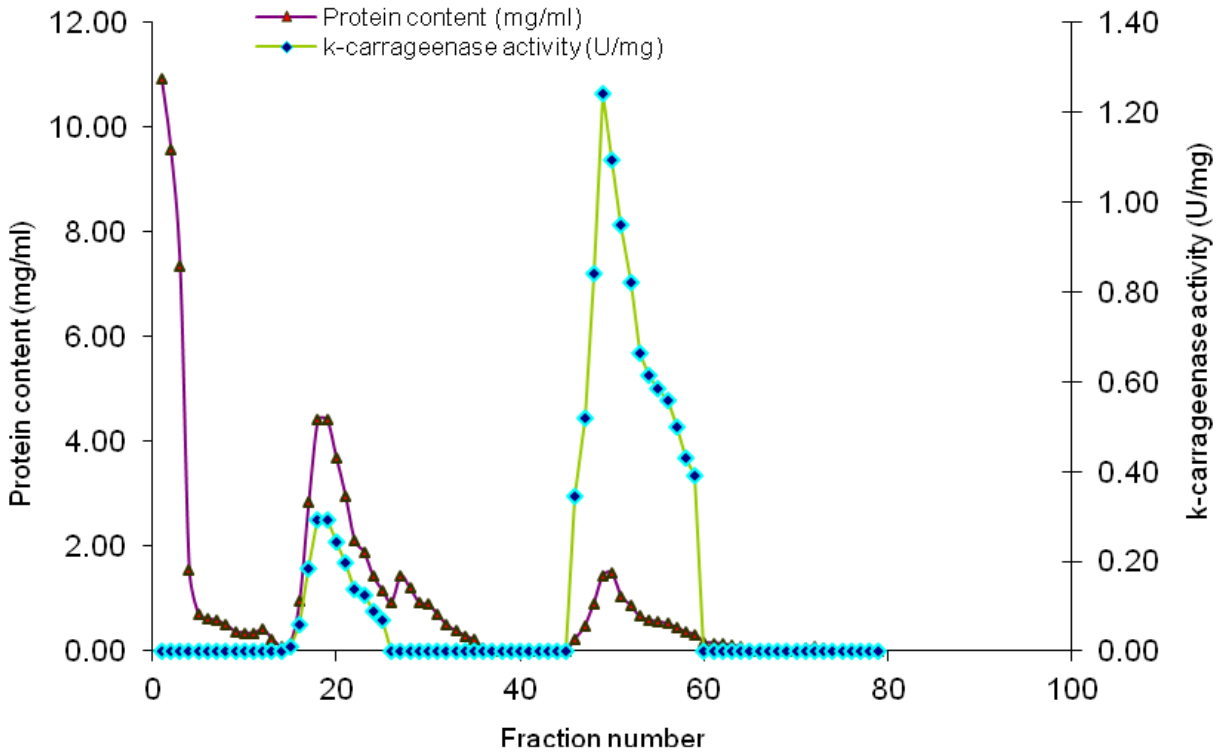

Figure 2. Ion exchange chromatography on DEAE Cellulose A-52 of a semipurified k-carrageenase obtained from *Cellulosimicrobium cellulans.*

protein peak as a major peak for enzyme activity while the rest was found to be in the 2nd protein peak as a minor activity peak. The major recovered kappa carrageenase peak reached 84.96% of the total recovered activity from the column. No activity peak was observed in the 1st and 4th protein peak. The specific activity of the collected pooled fractions from column regarding major peak of specific activity reached about 9.82 U/mg protein showing about 4.7-fold purification of the 90% ammonium sulfate fraction (semi-purified). Based on that, the recovered kappa carrageenase forming the major kappa carrageenase activity peak were combined and processed for further purification step which was Gel filtration on Sephadex G-100.

Gel filtration in sephadex-G100

The most active k-carrageenase components obtained from the anion exchange chromatography column was further purified by re-chromatography on Sephadex G-100. Re-chromatography of the enzyme sample (10.61 mg protein) afforded 33 fractions as shown in Figure 3. The protein recovered by the obtained fractions reached about 99.73% of the applied sample. The total kappa carrageenase activity recovered from the Sephadex G-100 column represented about 95.62% of the original activity. It was noticed that the protein was separated into 2 components in the column as one major and the other one was minor. The major protein peak was covered by fractions 4 to 12. It represented about 98.90% of the total recovered protein from the column. The second protein peak was a minor one and was covered by fractions 27 to 30. The protein recovered by this peak represented about 0.01% of the total protein recovered from the column. The kappa carrageenase activity was fractionated in the Sephadex column into one major peak. This peak was found to be compatible with the major protein peak. Fractions number 4 to 12 of this peak showed an enzyme activity approximately the same as that obtained from applied sample. The purification of kappa carrageenase in every step was quantified and summarized in the Table 1. The purity of the kappa carrageenase enzyme obtained from the Gel filtration chromatography was examined by disc gel electrophoresis. No bands were detected on the gel using Coomassie Brilliant Blue R-250 as stain for protein, even with highly concentrated sample (14 mg/500 ul) and silver stain also was applied for protein sample, but no band was detected as well.

Effect of temperature on the reaction

The data in Figure 4 showed that the kappa carrageenase

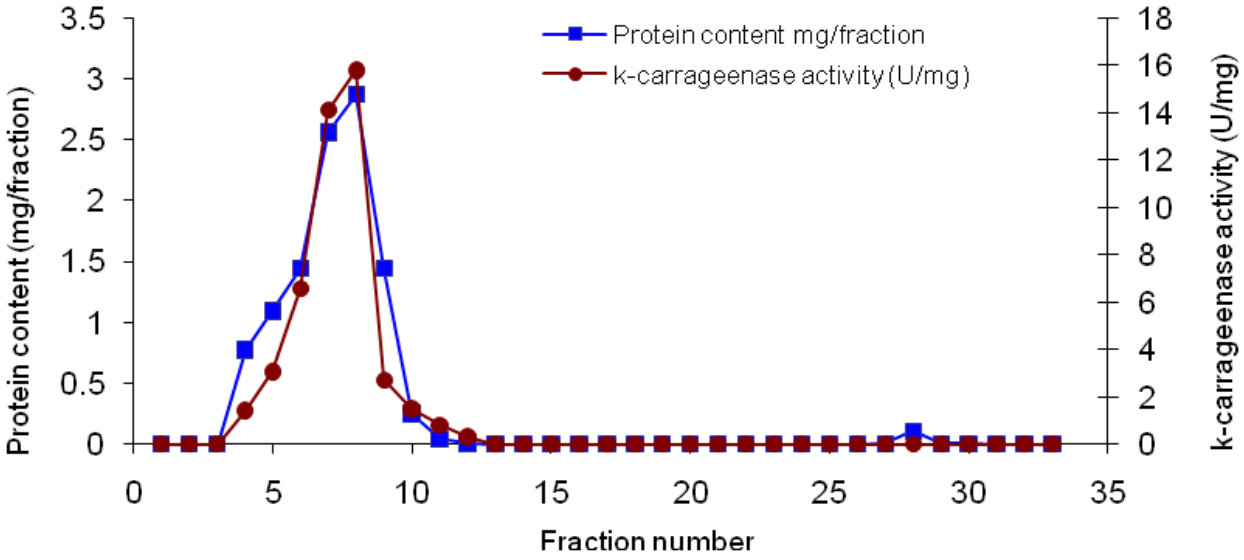

Figure 3. Gel filtration on Sephadex G-100 of the major active k-carrageenase obtained from anion exchange chromatography.

Table 1. Summarized table for all steps of k-carrageenase purification from *Cellulosimicrobium cellulans.*

Step	Volume (ml)	Protein content (mg)	k-carrageenase activity (U/ml)	Specific activity (U/mg)	Purification (fold)
Cell free medium	960	257	546.75	2.1	1
$(NH_4)_2SO_4$ precipitation	6	88	218.7	2.49	1.2
Ion exchange chromatography	6	10.61	104.16	9.82	4.7
Sephadex G-100	6	10.4	99.6	9.6	4.6

activity decreased gradually by increasing the reaction temperature starting from 30°C where the maximum kappa carrageenase activity was obtained 8.85 U/mg. At 37°C, the relative activity was 81.47% of that at 30°C. Therefore, in the next experiment, the temperature of the reaction was adjusted at 30°C.

Effect of pH on the reaction

The data in Figure 5 indicated that the optimum pH value for the pure enzyme was 6 where the highest specific activity was obtained (0.34 U/mg protein). Higher or lower pH values showed a minor effect on the activity and the lowest activity was obtained at pH 4 showing about 5.9% decrease of the value obtained at pH 6. However, the enzyme showed a relatively high activity in a pH range from 6 to 7.

DISCUSSION

Partial purification of the crude kappa carrageenase produced by *Cellulosimicrobium cellulans* was fulfilled by fractional purification with ammonium sulfate, acetone and ethyl alcohol. It was observed that no kappa carrageenase activity was achieved with acetone and ethyl alcohol fractions, although there were high precipitations with high protein recovery at first fractions. These results suggest that enzyme protein probably denatured during the precipitation. Partial purification with ammonium sulfate exhibited a good ability to partially purify the enzyme protein. A total of 15 fractions were obtained where the highest recovered protein was present in the fractions precipitated with ammonium sulfate (37.08%) followed by acetone (32.56%) and ethyl alcohol (24.56%). The only total recovered activity was obtained by ammonium sulfate (39.41%). Among all the obtained fractions, the 90% ammonium sulfate fraction showed the highest protein content. However, 75% ammonium sulfate fraction showed the highest kappa carrageenase activity as it gave about 1.7 fold specific activity higher than that obtained from the crude one. Therefore 90% ammonium sulfate was selected for subsequent purification to cover all possible protein of kappa carrageenase that might be in existence.

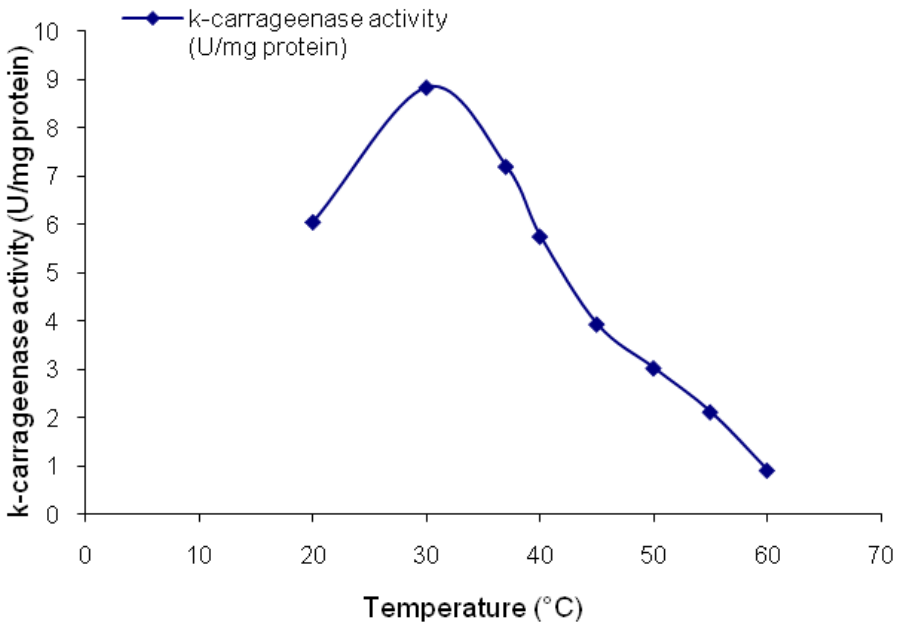

Figure 4. Effect of reaction temperature on the purified k-carrageenase activity obtained from *Cellulosimicrobium cellulans.*

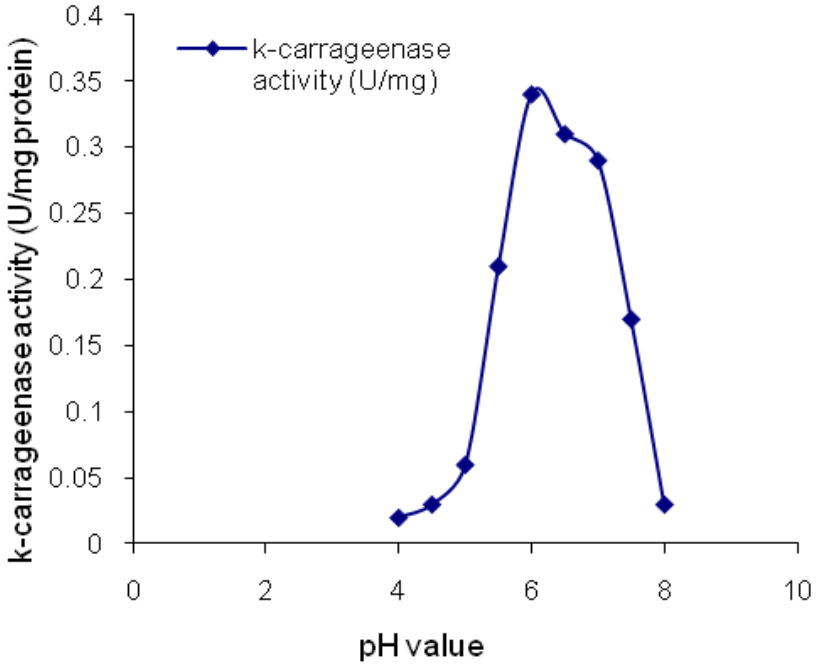

Figure 5. Effect of reaction pH on the activity of the purified k-carrageenase obtained from *Cellulosimicrobium cellulans.*

About 100% ammonium sulfate saturation fraction has been used to partially purify the kappa carrageenase from marine *Cytophaga*-like bacterium (Sarwar et al., 1987; Potin et al., 1991), and a 70% ammonium sulfate saturation fraction has been used to purify kappa carrageenase from *Pseudomonas carrageenovora* (Weigl and Yaphe, 1966; Mclean and Williamson, 1979). The specific activity obtained from supernatant of crude culture of *Cellulosimicrobium cellulans* was 1.82 U/mg. Weigl and Yaphe (1966) have reported nearly the same

specific activity which was 1.15 U/mg from the culture of *Pseudomonas carrageenovora*. It was noticed that with first trial to purify the enzyme protein using Hi Trap 5 ml QHP column (data not shown), the highest protein peak related to enzyme activity was determined at 0.5 M NaCl after using linear gradient from 0 to 1 M NaCl. This note was considered in second trial using stepwise gradient of NaCl. Subjecting the partially purified enzyme to anion exchange, chromatography of DEAE cellulose A-52 was carried out for further purification. The protein was separated into 4 peaks. The first peak was a major one but with no activity, while the second one showed a lowered activity comparing to the third protein peak which contained the most recovered enzyme activity form the column. These results indicate that more than one kappa carrageenase component are present in the partially purified sample. The major enzyme peak showed approximately an activity of 84.95% of the total recovered activity from the column.

The fractions of the major peak were further purified by rechromatography on Sephadex G-100. The protein was separated into 2 peaks. The first one was the major and contained 98.65 % from total recovered protein (99.73%) from the column, where the collected pooled fractions representing this peak showed a specific activity of 9.6 U/mg protein. It was noticed that most of the enzyme activity was recovered which was represented by 95.622 % of total activity. After trying with 3 bulk cultures (2500, 2000 and 1300 ml), and after using different techniques to purify this enzyme, starting from using Hi Trap column with FPLC for cultures 2500 and 2000 ml, and ending by using prestaltic pump with packed column of DEAE cellulose A-52 and Sephadex G-100 for culture 1300 ml, it was found the purified protein is very difficult to visualize on SDS-PAGE. No bands on SDS-PAGE were detected with Coomassie Brilliant blue R250 and even with Silver stain used normally to detect the proteins in nanograms. A likely explanation for this result emerged when we concentrated the samples. It has been noticed that with increasing concentration of the samples, the samples become more viscous. This viscosity referred to the degraded fragments of kappa carrageenan due to the activity of enzyme. By one way or another, these fragments have a role in not detecting any bands on the sodium dedecyl sulphate-polyacrylamide gel electrophresis (SDS-PAGE). In addition, the peptide chain of the protein may have glycosylation, phosphorylation or post transitional signal along the peptide chain which contributes in increasing the molecular weight and prevents the running of the samples on the gel. Actually, these results were found to be coincided with previous studies.

It has been reported by Sarwar et al. (1987) that purification of k-carrageenase presented a few problems. Mainly, the carrageenases were always found to be associated with the polysaccharide, bacterial cell wall materials, or the bacterial slime in the culture. The carrageenases were relatively unstable in all stages of purification which coincided with the previous reports (Yaphe and Boxter, 1955; Weigl and Yaphe, 1966; Johnston and McCankless, 1973). This instability was more pronounced after ion-exchange chromatography which removed the polysaccharide, possibly undigested kappa carrageenan, bacterial cell wall materials, or the bacterial slime. Stainer (1941) also faced the same problem with the enzyme that was produced by *Cytophaga sp*. It seems that some suitable colloid is necessary for complete purification of carrageenases. The study demonstrates that further study is required to find out a suitable colloid with which carrageenase will remain stable. After finding a suitable colloid, the peaks can be taken under the said protocol for purification using a large volume of cultures of *Cellulosimicrobium cellulans*. On the other hand, Sarwar et al. (1987) have succeeded in the purification of kappa carrageenase from *Cytophaga* species using ammonium sulfate for partial purification, while Sephadex G-200 and DEAE cellulose A52 for complete purification of the enzyme protein. Kappa carrageenase has been purified from *Cytophaga*-like bacterium using DEAE-Sepharose CL6B for ion exchange chromatography and Sephacryl S 200 HR for gel filtration (Potin et al., 1991). McLean and Williamson (1979) have purified the kappa carrageenase from *Pseudomonas carrageenovora* by CM-Sopharose CL-6B as ion exchange chromatography.

The optimum reaction temperature of the purified enzyme was 30°C, and the enzyme showed a relatively gradual decrease of activity with gradual increase in temperature starting from 30°C and ending by 60°C. These results are compared to other microbial kappa carrageenase. Sarwar et al. (1987) showed that the optimum temperature for the activity of kappa carrageenase produced by marine *Cytophaga* species was 25°C, while Mclean and Williamson (1979) proved that it was 40 to 45°C for kappa carrageenase produced by *Pseudomonas carrageenovora*. The optimum pH value for having a maximum kappa carrageenase activity was obtained at 6; besides, the enzyme showed a relatively high activity in a pH range from 6 to 7. These results are compatible to those of kappa carrageenase purified from other microorganisms. Potin et al. (1991) found that a kappa carrageenase from marine *Cytophaga*-like bacterium had a high activity at a pH 7.2. However, Sarwar et al. (1987) found that the optimum pH for having a high kappa carrageenase activity from marine *Cytophaga* species was a pH 7.6. Also, Weigl and Yaphe (1966) proved that the optimum pH for getting a high enzyme activity from *Pseudomonas carrageenovora* was a pH 7.5, but Mclean and Williamson (1979) reported that the optimum pH for having a high enzyme activity from *Pseudomonas carrageenovora* was pH 8.

It can be concluded that the purified k-carrageenase can be used in industrial applications in low pH medium at 30°C efficiently. Further investigations are required on

this enzyme produced from Gram positive strains.

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