The Enzymic Hydrolysis of Carrageenin[1]

W. Yaphe and Beverley Baxter

Maritime Regional Laboratory, National Research Council, Halifax, Nova Scotia, Canada

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Carrageenin is the water soluble polysaccharide extractable from the red algae *Chondrus crispus* and *Gigartina stellata*. The polysaccharide is essentially a polymer of alpha-D-galactopyranoside-4-sulphate. Smith *et al.* (1954) fractionated carrageenin with potassium chloride into two components and obtained a precipitable component which could form gels and a nongelling component which remained in solution. The former was designated kappa-carrageenin and the latter lambda-carrageenin. Smith, O'Neill, Perlin, and Cook (personal communication) found that these fractions differ in their content of D-anhydrogalactose.

Mori (1943*a*) demonstrated a hydrolase in marine molluscs which degraded the mucilage from *Chondrus ocellatus*. The bacterial decomposition of carrageenin has been commonly recognized but this has not been related to any particular organism or enzyme. The purpose of the work to be described was to attempt to isolate and characterize a bacterial carrageenase.

Materials and Methods

Cultures of bacteria were obtained from sea water and various marine algae collected in the neighborhood of Halifax, N. S. Pieces of algae or 10 ml of sea water were inoculated into 50 ml of synthetic sea water medium containing carrageenin, 0.5 per cent; NaCl, 3.0 per cent; $MgSO_4$, 0.05 per cent; K_2HPO_4, 0.1 per cent; $FeCl_3$, 0.002 per cent; $NaNO_3$, 0.05 per cent; dissolved in distilled water. Successive transfers were made in the same media and finally plated out on this medium containing 3.0 per cent carrageenin. The colonies of bacteria which hydrolyzed carrageenin were surrounded by an area of liquefaction. A pure culture of a strain which actively liquefied carrageenin was obtained by repeated subculture on the above medium containing 1.0 per cent agar and 0.5 per cent carrageenin.

The organism has the following characteristics: rods 1 to 2 μ by 0.5 μ, occurring singly and in pairs, motile, nonspore forming, nonencapsulated, gram negative. Agar colonies: punctiform, smooth, entire, raised, translucent, no depression in agar. Agar slant: growth moderate, dull. Broth: slight clouding, pellicle. Gelatin stab: stratiform liquefaction. Indole not formed. Nitrite produced from nitrate. Hydrogen sulfide produced. Starch hydrolyzed. Cellulose not hydrolyzed. Carrageenin hydrolyzed. Agar not hydrolyzed. Acid from arabinose, xylose, mannose, sucrose, maltose, cellobiose, raffinose, dextrin. Not utilized: lactose, trehalose, glycogen, glycerol, adonitol, mannitol, sorbitol, dulcitol, salicin, alpha methyl glucoside. Aerobic. Growth at 10 C, no growth at 37 C. Optimum growth 25 C. Isolated from red alga, Cow Bay, N. S. September 1953.

The enzyme was prepared by growing the organism in a culture medium containing carrageenin (Sea Kem No. 6), 0.5 per cent; NaCl, 3.0 per cent; $MgCl_2$, 0.05 per cent; NaH_2PO_4, 0.05 per cent; $NaNO_3$, 0.05 per cent; and $FeCl_3$, 0.002 per cent; dissolved in distilled water. Cultures were incubated in Erlenmeyer flasks, at 20 C to 25 C on a rotary shaker for 3 to 4 days. The material was then passed through a Sharples supercentrifuge, and the effluent was filtered through a Selas porcelain filter No. 03 to obtain a cell-free filtrate. This, and the Sharples effluent, were both tested for enzymic activity.

Specimens of *Chondrus crispus*, *Gigartina stellata*, *Ahnfeldtia plicata* were collected at Herring Cove, N. S. *Hypnea musciformis*, *Pterocladia* extract, *Furcellaria* extract, *Gracilaria confervoides*, *Gigartina acicularis*, *Iridophycus* sp., and *Gelidium cartilagineum* were obtained from commercial sources. Extracts were prepared by heating the dried ground algae for 1 to 2 hours in a boiling water bath. The extract was centrifuged, filtered, and precipitated by adding 4 volumes of 95 per cent ethanol. The precipitate was dehydrated consecutively in absolute ethanol, acetone and ether.

The kappa fraction of commercial carrageenin was precipitated from aqueous solution with KCl. After centrifugation the supernatant fluid was treated with 95 per cent ethanol to establish a concentration of 36 per cent by volume. This precipitated the lambda fraction.

The rate of hydrolysis of carrageenin was followed by measuring the increase in reducing power (Somogyi 1952) with galactose as standard. One volume of the enzyme solution was added to 2 volumes of 0.6 per cent carrageenin in M/20 sodium phosphate buffer. Aliquots of 5 ml were removed at intervals for analysis. Determinations were done in duplicate and corrected for the small blank present in the enzyme solution. Activity was expressed as the amount of galactose formed after hydrolysis of carrageenin for 2 min at 25 C and pH 7.5.

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Results

Conditions of activity and assay. The rate of hydrolysis of carrageenin by the enzyme preparation under the optimum conditions of pH 7.5 and 25 C is shown in figure 1. The initial rate of hydrolysis was rapid. At the end of the reaction approximately 30 mg of reducing sugar were obtained from 400 mg of carrageenin.

The relationship between pH and enzymic activity is shown in figure 2. The enzyme was active over a pH range of 5 to 9 with the optimum about 7.5.

Variation in the concentration of substrate established that activity of the enzyme preparation was independent of concentration above 0.3 per cent.

Relation of activity to temperature is shown in figure 3. There is a peak at 40 C and inhibition at 60 C. The amount of reducing sugar formed at 25 C was approximately 50 per cent of the total reducing sugar obtained

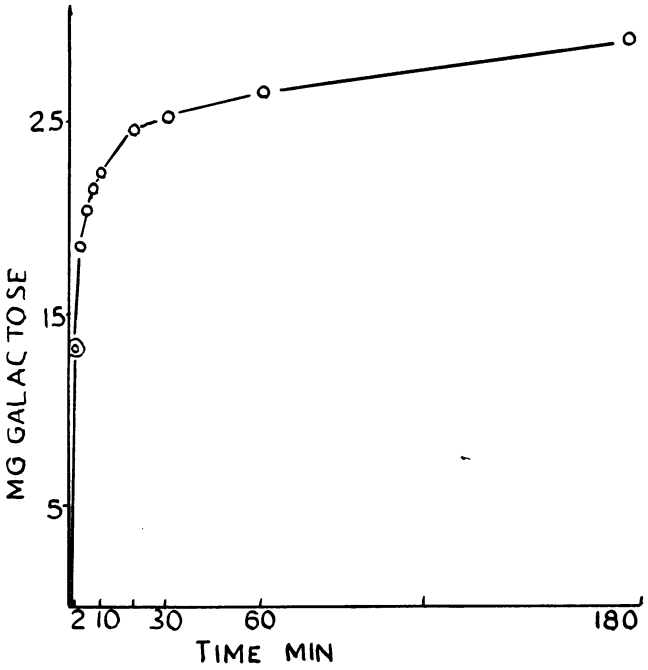

Fig. 1. Time activity curve. Rate of hydrolysis of 0.4 per cent carrageenin at pH 7.5 and 25 C.

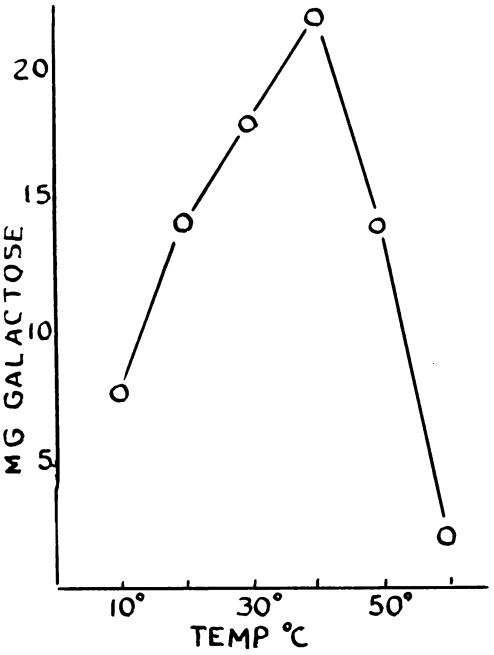

Fig. 3. Effect of temperature on enzyme activity. 0.4 per cent carrageenin, pH 7.5.

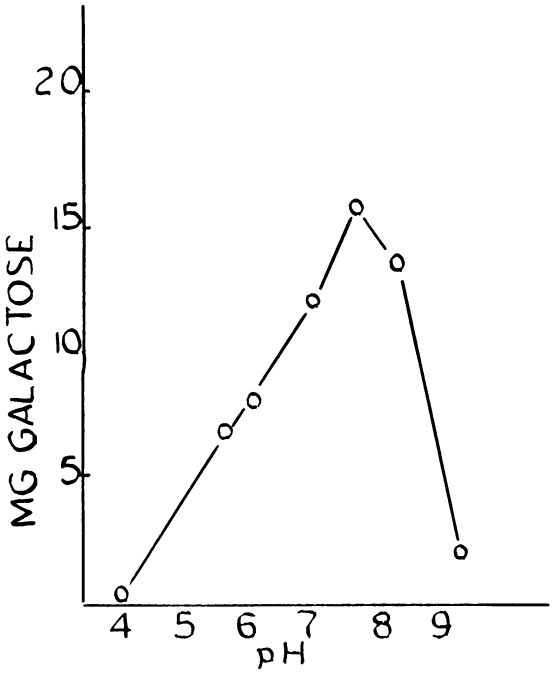

Fig. 2. Effect of pH on enzyme activity. Phosphate buffer 0.4 per cent carrageenin, 25 C.

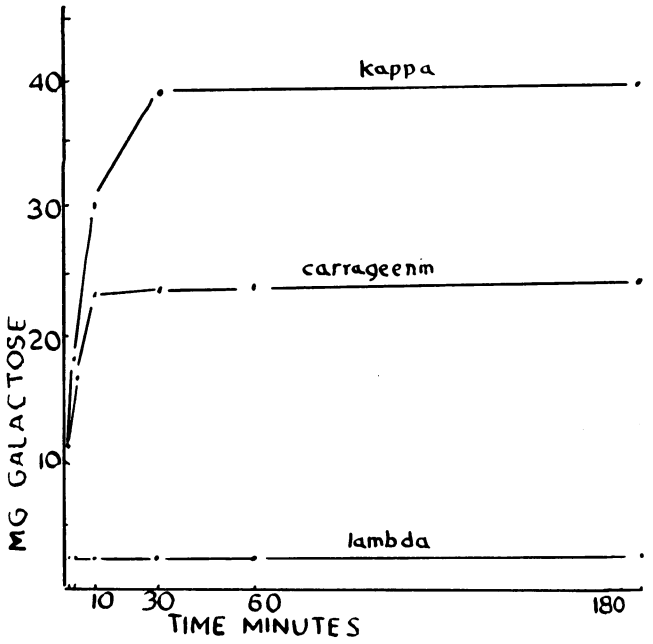

Fig. 4. Hydrolysis of kappa, lambda fractions and unfractionated carrageenin. Concentration 0.4 per cent, pH 7.0, 25 C.

on complete enzymic hydrolysis. The enzyme preparation was stable and no loss of activity occurred in solutions incubated for nine days at 10 C.

Specificity. The enzyme preparation did not degrade inulin, salicin, starch or dextrin. Galactoside linkages in raffinose and in the gums ghatti, benzoin, damar, and guaiac were not attacked.

The enzyme was essentially specific for the kappa fraction of carrageenin. Figure 4 shows that the kappa fraction was hydrolyzed to a greater extent than unfractionated carrageenin and that the lambda fraction was only slightly affected.

Rate of hydrolysis of the polysaccharides from various algae is shown in figure 5 and table 1. The carrageenase did not hydrolyze commercial agar or extracts from the known agarophytes, *Gelidium cartilagineum* (Difco agar), *Gracilaria confervoides* (Australian agar), *Pterocladia* (New Zealand agar), *Ahnfeldtia plicata* (Russian agar).

Extracts from *Chondrus crispus* and *Gigartina stellata* were hydrolyzed at the same rate as commercial preparations from these algae. An extract from *Furcellaria fastigiata* was hydrolyzed at the same rate as carrageenin. The rate of hydrolysis of an extract from *Hypnea musciformis* was similar to that of the kappa fraction. Extracts from *Gigartina acicularis* and *Iridophycus* sp. were hydrolyzed at a slower rate than carrageenin.

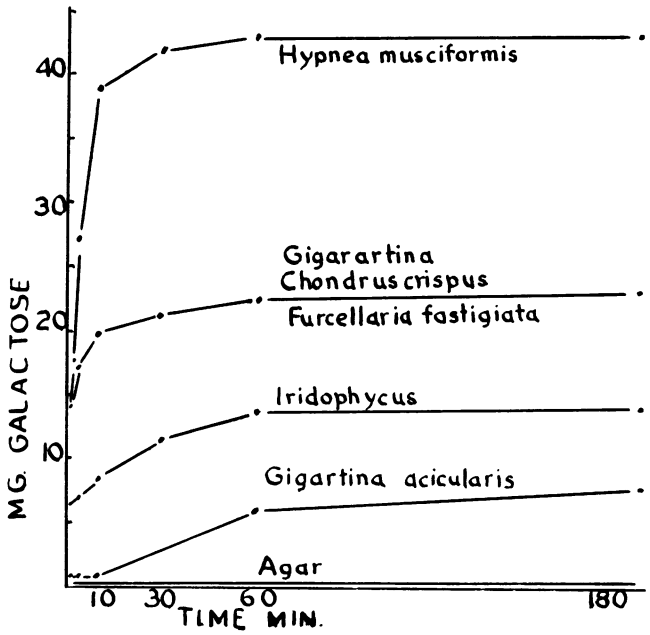

FIG. 5. Hydrolysis of extracts from marine algae. Concentration 0.4 per cent, pH 7.0, 25 C.

TABLE 1. *Enzymic hydrolysis of extracts from marine algae*

		Reducing sugar*
Agarophytes	*Gracilaria confervoides*†	0
	Pterocladia sp.	0
	Ahnfeldtia plicata	0
	Gelidium cartilagineum	0
	(Commercial Difco agar)	0
Carrageens	*Chondrus crispus*‡	22
	Gigartina stellata	21
	Gigartina acicularis	7
	Iridophycus sp.	15
	Hypnea musciformis	44
	Furcellaria fastigiata	23

* Reducing sugar mg galactose/100 ml from 400 mg extract in 180 minutes.

† pH 6.0 and pH 7.0 at 40 C.

‡ pH 7.0 25 C.

DISCUSSION

It may be claimed from the above results that the enzyme preparation which hydrolyzes carrageenin is specific for the kappa fraction. The recent finding of Smith, O'Neill, Perlin, and Cook (personal communication) of the occurrence of anhydrogalactose units in kappa-carrageenin provides a reason for this specificity. The sterioisomerism of the polysaccharides in *Chondrus* would thus be different. This key may thus be applied to extracts obtained from various algal species. The enzymic hydrolysis of such extracts suggests that the kappa or gel fraction of carrageenin may be present in other red algae. Smith and Cook (1953) have shown that extracts from *Gigartina* species contained less kappa carrageenin and those from *Hypnea musciformis* more kappa carrageenin than does *Chondrus crispus*, while *Gracilaria confervoides* contained no kappa-sensitive material. The rate of enzymic hydrolysis of the *Hypnea* extract in our results suggests that it contains a high concentration of kappa. The concentration of kappa in *Furcellaria fastigiata* is not known but potassium ions are usually added to increase the gel strength of the extract from this alga. Dillon (1952) reported that the polysaccharide of *Furcellaria fastigiata* appeared to resemble carrageenin fairly closely but contained less sulphate. Our results suggest that the extract from *Furcellaria* is similar to that of *Chondrus*. The extract from *Gigartina acicularis* forms a viscous nongelling solution. The low yield of reducing sugar formed on hydrolysis suggests that it contains only a small amount of kappa carrageenin. The polysaccharide of *Iridophycus* was found to be similar to carrageenin as previously claimed (Mori 1943*b*).

The enzyme preparation did not hydrolyze extracts from known agarophytes and this is consistent with the results of chemical studies differentiating agar and carrageenin.

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Summary

An extracellular enzyme which can hydrolyze carrageenin was obtained from a marine bacterium. Optimum conditions of activity were at 0.4 per cent carrageenin, 40 C and pH 7.5. Activity was measured by amount of reducing sugar formed after 2 minutes. The enzyme was specific for the kappa fraction of carrageenin. Extracts from *Furcellaria fastigiata*, *Hypnea musciformis*, *Gigartina acicularis* and *Iridophycus* sp. were hydrolyzed. The enzyme did not hydrolyze agar or extracts from several agarophytes.

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