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MOLECULAR-WEIGHT-DISTRIBUTION AND THE BEHAVIOUR OF KAPPA-CARRAGEENAN ON HYDROLYSIS*

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ABSTRACT

The molecular-weight-distributions for eight food-grade kappa-carrageenans have been determined by gel-permeation chromatography and found to be broad. On average, 25% of the total carrageenans in each batch had a mass of <100 kD. The rate of degradation of kappa-carrageenan in simulated gastric juice was considerable at pH 1.9.

INTRODUCTION

The molecular-weight-distribution for native kappa- and iota-carrageenan has been determined[1] by gel-permeation chromatography in combination with light-scattering measurements, and the hydrolysis behaviour under simulated physiological conditions has been studied. At pH 1.0, there was a considerable degradation of kappa-carrageenan, whereas iota-carrageenan was only slightly affected. It was found also that the native food-grade carrageenan investigated contained fractions of low molecular weight. From a toxicological point of view, carrageenans of low molecular weight are generally assumed to be more harmful when ingested than their counterparts of high molecular weight. Since kappa-carrageenan is the most commonly used carrageenan-type in the food industry and is easily degraded by acid, its study is important and its degradation in simulated gastric fluid is now reported.

EXPERIMENTAL

General. — Commercial chemicals of p.a. grade were used. Food-grade kappa-carrageenans were a gift from the Copenhagen Pectin Factory Ltd. (Denmark). The molecular-sieving equipment comprised a Miniplus II peristaltic pump (Gilson), a V7 seven-port injection valve (Pharmacia), K16 water-jacketed, borosilicate glass, chromatography columns (Pharmacia) containing 70 mL of Sepharose CL-4B (Pharmacia), a Multiref 902 refractometer equipped with cell

*Part II. For Part I, see ref. 1.

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911B/1 mm (Tecator), an AltroRac 7000 fraction-collector (LKB), a C3 bath circulator (Lauda), H and V bath circulators (Julabo Laboratory Equipment), four SRV-3 single-channel four-port valves (Pharmacia), and two SRV-4 double-channel four-port valves (Pharmacia).

Molecular-sieving chromatography. — All experiments were carried out with 0.2M LiCl as background electrolyte at 60°. The equipment was also used for determining molecular-weight-distributions of iota-carrageenans at temperatures which exceeded 57° in order to avoid helix formation. Viscosity measurements have showed that the kappa-carrageenan helices are uncoiled at temperatures above 25° with 0.2M LiCl as background electrolyte[1].

In developing the separation system, a commercial h.p.l.c. pump was used. The hot electrolyte (0.2M LiCl, 60°) caused marked corrosion of the stainless-steel parts in the pump and damaged both the pump and the refractive index (RI) detector. Attempts to use a piston pump (P-500, Pharmacia, Sweden), which has excellent resistance to chemicals, failed because fluctuations in the delivery pressure caused a non-acceptable level of background noise in the Multiref 902 RI-detector. A peristaltic pump was satisfactory, although not ideal because the delivered flow was not constant and required adjustment each day.

The 0.2M LiCl was filtered (0.45 μm) and degassed before being added to the reservoir which was kept at 65° in order to avoid degassing in the column. All transport tubes were made of polypropylene (Teflon is unsuitable at this temperature because of the high permeability of oxygen from the surrounding air). Problems with degassing in the column can arise even if the flow rate is low because of the pressure drop in the column. The increased exposure time results in a lowering of the temperature of the solution in the transport tubes which results in an increased solubility of gases.

The RI-detector cell was kept at 60 ±0.01° and the detector electronics at 35°. Since the cell unit contains materials which corrode easily when in contact with

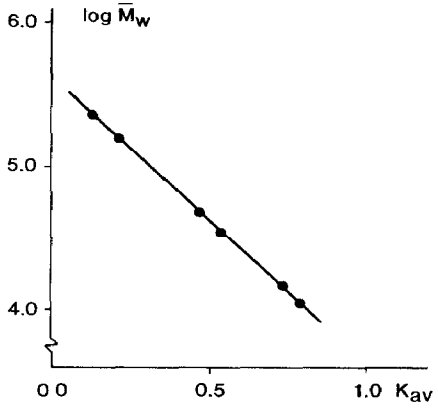

Fig. 1. Plot of log $\bar{M}_w$ against K_{av} for kappa-carrageenan.

the electrolyte, it is necessary to replace the latter with methanol when the equipment is not in use.

The electrolyte in the sample (1.2 mL) was adjusted to 0.2M with respect to chloride by addition of LiCl. The sample at 65° was filtered (1 μm) and degassed prior to use. The flow rate was 0.30 mL/min.

Column calibration. — The Sepharose CL-4B column was calibrated against fractionated carrageenans (Fig. 1) obtained from a semi-preparative column previously calibrated by light-scattering measurements and total concentration determinations of native and hydrolysed carrageenans[1]. The column covered the mass range 10–400 kD which corresponds to $0 < K_{av} < 0.8$. Impurities of low molecular weight gave a peak on the RI-detector in the vicinity of the total volume, V_T, making 0.8 the highest applicable K_{av} value.

Hydrolysis of kappa-carrageenan in simulated gastric fluid. — The simulated fluid was prepared in accordance with the recommendations in the United States Pharmacopoeia[2]. The pepsin was isolated from porcine gastric mucosa (lyphilisate). Equal volumes of solutions at 37° containing 0.4% (w/w) of kappa-carrageenan and 0.64% (w/v) of pepsin, 68mM NaCl, and 126mM HCl were mixed (final pH 1.2; in other experiments, the final pH was adjusted to 1.9). The mixture was kept at 37°, and aliquots taken during 0–6 h were each treated with solid LiOH to give a pH of 4.2, heated to 85° for 15 min, rapidly cooled, and centrifuged at 2000*g* for 5 min. The supernatant solution was decanted and neutralised with solid LiOH to pH 7.0 ±0.1. The Cl^- concentration was adjusted with solid LiCl to 0.2M.

RESULTS

Determination of distributions of molecular weight. — The elution profiles for 8 food-grade kappa-carrageenans on a Sepharose CL-4B column are shown in Fig.

TABLE I

MOLECULAR-WEIGHT-DISTRIBUTION FOR VARIOUS BATCHES OF FOOD-GRADE KAPPA-CARRAGEENAN

Batch	$\bar{M}_w \times 10^{-3}$	*Fractions (%)*	
		≤100 kD	*≤50 kD*
HF 19932-35	168	26	10
HF 10018-24	176	24	8
HF 19965-69	171	24	7
HF 19976-78	158	20	6
HF 19970-75	170	21	6
HF 19950-62	166	26	11
HF 19963-64	157	29	11
53253-54	171	28	11
Mean	167	25	9
± SD	7	3	2

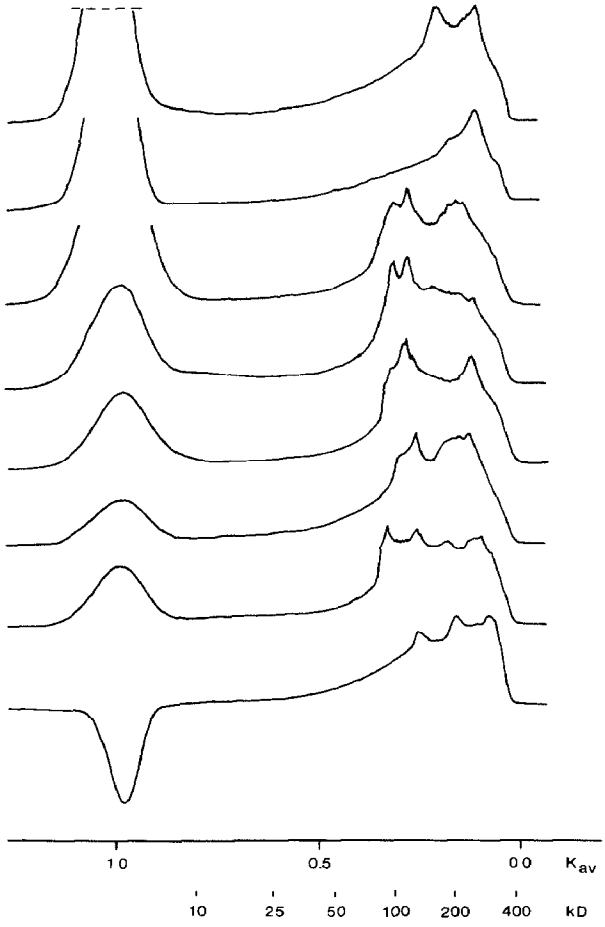

Fig. 2. Elution profiles of 8 samples of native food-grade kappa-carrageenans.

2. The mean molecular weights for these carrageenans were calculated by manual integration of the graphs, and the values are presented in Table I. The waste peak in the chromatograms (Fig. 2), which appeared at the elution volume V_T, was due partly to the counter-ions (mainly Na^+, K^+, NH_4^+, and Ca^{2+}) which have a refractive index different from that of Li^+. A further contribution to this peak could be due to traces of urea. Fractions from V_T containing the waste peak from all batches were concentrated to dryness prior to mass spectrometry which gave a spectrum characteristic[3] of urea (*m/z* 16, 17, 43, 44, and 60). It is conceivable that the urea is formed during the extraction of carrageenan from red seaweed in hot aqueous alkali since both carbon dioxide and ammonia are present.

Hydrolysis behaviour. — The distributions of molecular weight of kappa-carrageenan at different times of hydrolysis (0–6 h) in simulated gastric juice at pH 1.2 and 1.9 are presented in Fig. 3, from which the percentages with masses less than

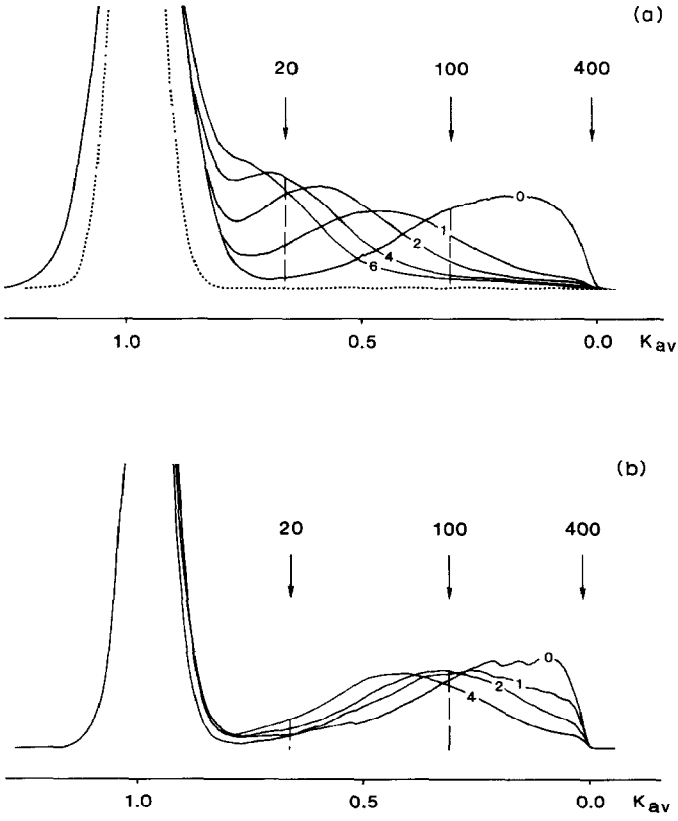

Fig. 3. Elution profiles for kappa-carrageenan after hydrolysis in simulated gastric juice at (a) pH 1.2 and (b) pH 1.9. The figures on the curves denote the time of hydrolysis (h) and the arrows indicate the positions of elution corresponding to 20, 100, and 400 kD. The dotted line in (a) indicates the elution profile for simulated gastric juice, with the pepsin precipitated prior to analysis.

100, 50, and 20 kD can be calculated. Fig. 4 shows the increase of these degraded carrageenans as a function of time of hydrolysis at pH 1.2 and 1.9. At zero time, some degradation of the carrageenan had taken place because of the lapse of time during the neutralisation to pH 4.25, the highest pH at which the denaturation of pepsin at 85° occurred; incubation at 85° for 15 min at pH 4.25 causes some degradation of carrageenan.

Since the RI-detector is non-selective, it is necessary to check that pepsin (mass 34.5 kD) is absent in the sample. A reference elution profile of simulated gastric juice not containing carrageenan, and from which the pepsin had been precipitated, was neutralised as described above and is shown in Fig. 3a. The co-precipitation of kappa-carrageenan with pepsin was 6–7%.

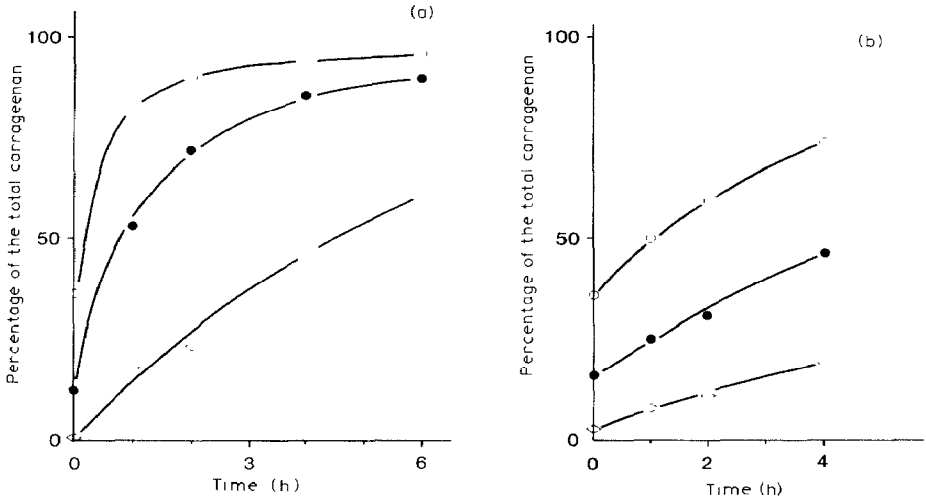

Fig. 4. Increase (%) of low-molecular-weight fractions of kappa-carrageenans after hydrolysis in simulated gastric juice at (a) pH 1.2 and (b) pH 1.9; ≤100 kD (—○—), ≤50 kD (—●—), and ≤20 kD (—◇—).

DISCUSSION

The weight-average molecular weight and the shape of the distribution curve of the molecular weight was similar for the various batches of kappa-carrageenan. The weight-average molecular weight was $167 \pm 7 \times 10^3$. An average of 25 ±3% of the carrageenan in the batches had a mass <100 kD and 9 ±2% <50 kD. These broad molecular-weight-distributions indicate that the parameters commonly used for classification of carrageenans, namely, viscosity and molecular weight, are much less reliable indicators of composition compared to that based on molecular-weight-distribution. After 2 h in simulated gastric juice at pH 1.2, almost 90% of the carrageenan had a mass of <100 kD and 25% had a mass of <20 kD. At pH 1.9, the rate of degradation was much lower; after 2 h, 65% of the carrageenan had a mass of <100 kD and 10% had <20 kD. The presence of pepsin did not affect the rate of the degradation although carrageenan has a strong protein-binding tendency. Thus, the acidity and the rate of passage through the stomach will determine the degree of degradation of carrageenan. The possible toxicological implications of the low-molecular-weight fragments present either in the food-grade carrageenans or formed by the digestion should be taken into account when evaluating the health-risks of these food additives.

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