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Desulfation of algal galactans

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Abstract

The desulfation of galactans was shown to be acid catalysed, and most likely to proceed through an intermediate complex in which SO_3 is transferred to an acceptor molecule. The reaction is inhibited by molecules such as methanol and water, frequently included as 'catalysts'. The reaction for ι-carrageenan appears to be stepwise, to be dependent on the nature of the acid and the sulfate acceptor, and is essentially independent of the initial counterion, unless these are the actual reagents. A useful preparative procedure is to dissolve the oven-dried sodium salt of the polysaccharide in anhydrous dimethyl sulfoxide and react it at about 120 °C for approximately three hours in the presence of a pyridinium salt of pyromellitic acid, excess pyridine, and an amphoteric nonhydrogen-bonding sulfate acceptor, of which arsenous oxide and antimony trioxide are preferable for removing all forms of sulfate ester in good yield. The procedure appears to work satisfactorily for each of the types of sulfate ester present in algal galactans, without significant degradation occurring of the underlying polysaccharide skeletons. © 1998 Elsevier Science Ltd. All rights reserved

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1. Introduction

A structural determination for a red algal galactan frequently requires the determination of the position of attachment of sulfate esters. This can be done by comparing a methylation analysis of the actual polysaccharide with a corresponding methylation analysis of the polysaccharide which has been desulfated, the difference in deduced linkage positions giving the positions of the sulfate esters. Accordingly, an accurate structural determination requires an efficient means of selectively removing the sulfate ester without causing the hydrolysis of polymer linkages.

Simple acid hydrolysis is not suitable, because the linkages from anhydrogalactosyl units are particularly susceptible to acid hydrolysis. Thus κ-carrageenan was converted in cold concentrated hydrochloric acid to the corresponding sulfated carrabiose in high yield [1].

At present, the most suitable technique for removing sulfate esters is what has been termed solvolytic desulfation. The technique usually involves heating the pyridinium salt of the sulfated polysaccharide in dimethyl sulfoxide and pyridine in the presence of methanol and/or water [2–6]. Other solvents have also been used to desulfate

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pyridinium salts of sulfated polysaccharides, and 1,4-dioxane was found to be a slightly better solvent than dimethyl sulfoxide for carrying out the desulfation, provided that the polymer dissolved in the dioxane [3]. However, the method was not ideal in that over 10% of the initial sulfate ester remained, and significant polymer depolymerisation occurred. It has been noted that triethylammonium salts of sulfated galactans do not desulfate well [4]. In addition to the general problem that preparation of a pyridinium salt is time consuming and inevitably results in loss of yield, in one typical linkage analysis almost 20% of the original sulfate ester remained [5], while in a recent structure determination [6] the evidence pointed towards 12% of 2,3-disulfation in the 4-linked residues, but the overall experimental error in the determination was ± 12%. In this structural determination, two sequential solvolytic desulfations had to be carried out, and while the recovered polymer had almost 90% of its sulfate esters removed, less than 10% of the initial weight was recovered. It has also been reported that when solvolytic desulfation was applied to *Iridaea undulosa*, only minor desulfation occurred [7].

Despite this reaction having been used many times as a critical element in a structural determination, the results obtained are far from optimal. Accordingly we have now examined some of the reaction variables with the objective of obtaining a better desulfation procedure.

2. Experimental

All ^{13}C NMR spectra were recorded for saturated solutions in Me_2SO-d_6 or D_2O at 80 °C on a Varian XL300 spectrometer using an acquisition time of 0.5 s and a flip angle of 64°. Chemical shift data are given in ppm relative to Me_2SO-d_6 at 39.6 ppm. Reagents and solvents were reagent grade materials used without further purification. ι-Carrageenan was a commercial product obtained from Shermberg Corporation, λ-carrageenan of fully characterised structure [8] was obtained by extraction from tetrasporophytic *Gigartina decipiens*, κ-carrageenan was obtained by extraction from gametophytic *Gigartina decipiens* [9] while the porphyran was obtained by extraction from *Porphyra columbina* [10]. The polysaccharides obtained by extraction were isolated by concentration followed by alcohol precipitation. No attempts were made to exchange the counterions obtained during the extraction.

In a typical reaction, Me_2SO (90 mL) was heated in an oil bath. Where drying was carried out, toluene (15 mL) was added and the solvent boiled under reflux under Dean Stark conditions, then the toluene was removed by distillation. The Me_2SO was then brought to the reaction temperature, as measured by the oil bath temperature, the oven-dried finely ground sulfated polysaccharide (2 g) was added and the mixture was stirred until dissolution took place. Pyridine (5 mL) was then added, followed by the other reagents, then additional pyridine (10 mL). Stirring at the reaction temperature was maintained for the given time, then the solution was cooled and poured into water (50 mL) containing $NaHCO_3$ (3 g). The solution was purified by dialysis, and the polymer recovered by evaporation.

3. Results and discussion

While obtaining a ^{13}C NMR spectrum of the triethylammonium salt of ι-carrageenan in dimethyl sulfoxide at 80 °C, the ι-carrageenan was converted to κ-carrageenan, the reaction having a half-life of less than 1 h. Addition of sufficient tetraethylammonium hydroxide to make the solution alkaline stopped the reaction. Forrester et al. [11] reported that during acid hydrolysis the sulfate ester adjacent to the anomeric carbon is more susceptible to hydrolysis. Reaction of the triethylammonium salt of ι-carrageenan in dimethyl sulfoxide in the presence of excess triethylammonium chloride at 120 °C led to excessive browning and polymer degradation, while the addition of triethylamine (2 mL) or methanol (2 mL) stopped the desulfation, and the ι-carrageenan was recovered unchanged. Methanol is often considered the 'reagent' in solvolytic desulfation, and in some examples may be present in amounts up to 10%. The inhibition of the reaction by the presence of approximately 2.5% methanol here explains why triethylammonium salts have been found to be unreactive under such conditions.

Accordingly we decided to examine the acid catalysed desulfation of some galactans, using the commercially available ι-carrageenan for examining general conditions, since it has two sulfate esters, and if desulfation occurs, the anhydrogalactosyl residue is particularly susceptible to hydrolysis. Four materials may occur in a reaction mixture: unreacted starting material, α-carrageenan (from removal of the 4-sulfate), κ-carrageenan (from removal of the 2-sulfate), and β-carrageenan (from removal of both sulfate esters). The ^{13}C NMR spectra of each of these species have been recorded [12,13]. We also examined the desulfation of λ-carrageenan, because it has sulfate esters in different positions, and porphyran, as it has the readily hydrolysable anhydrogalactosyl units remote from sulfate esters, and hence may offer a different susceptibility to polymer degradation. The ^{13}C NMR spectra of each of the species involved from these reactions have also been recorded [9,12,14], hence ^{13}C NMR spectroscopy was used as the method of analysis. The lower sensitivity of ^{13}C NMR spectroscopy means that small amounts of other materials ($<5\%$) cannot be excluded; however, the technique has the advantage of looking directly at the product and it does not require the forming and isolating of chemical derivatives with possible selective losses.

Acid catalysis can lead to desulfation, but the presence of acid can also lead to depolymerization, as well as other degradations. Pyridine was added to prevent this. Pyridine therefore controls the hydrogen ion concentration, and in principle other slightly less basic aromatic amines could have been used. This approach was not investigated further because pyridine was found to be quite suitable, and its ready availability makes it the amine of choice. With the levels of pyridine used, the only acid source would be the pyridinium ion, and accordingly the nature of the counterion should not have a significant effect.

However, when ι-carrageenan was used in the standard reaction together with 1,4-dioxane (5 mL) and pyromellitic acid (1.5 g), the completely desulfated product, β-carrageenan, represented 90% of the products; when trichloroacetic acid was used, β-carrageenan represented 20% of the products. Accordingly, a counterion effect was found, which may be because the pyridinium ion is freer with a large, diffuse counterion, or possibly the presence of nonionized carboxyl groups is also significant. Although the reason is unclear, the enhanced effect was not, hence pyromellitic acid was the acid of choice for the remaining experiments.

The effects of temperature, time and acid concentration are listed in Table 1. As expected, increasing the temperature leads to more efficient desulfation, but it also leads to polymer degradation, and the recovered yield was approximately 10% for reaction (7) (Table 1). There is a mild benefit in increasing the acid concentration, or of lengthening the time of reaction. In these reactions, only the β- or κ-carrageenan was found. Methanol or water inhibited the desulfation, as did the fluoride ion; a comparable experiment in which urea (3 g) was added and the reaction run for 3 h at 120 °C resulted only in κ-carrageenan and some unreacted starting material. Thus it appears that the reaction of ι-carrageenan proceeded essentially stepwise, in the sequence ι- → κ- → β-carrageenan. We found no reaction mixture with three carrageenans, nor could we find any conditions which produced α-carrageenan.

Table 1
Exploratory desulfation reactions[a] of ι-carrageenan

Reaction	PMA[b] (g)	Temperature (°C)	Time (h)	Reagents (g)	Products[c] (% β)
1	0.5	110	4	MeOH (5)	0
2	0.5	110	2	H_2O (4)	0
3	0.5	110	4	H_2O (4)	20
4	0.5	110	4	–	50
5	1.5	110	4	–	70
6	0.3	140	2	–	80
7	0.3	140	3	–	95
8	1.5	80	4	–	50
9	1.5	130	1.5	–	70
10	1.5	130	3	–	70

[a] For conditions see experimental section.
[b] Pyromellitic (benzene-1,2,4,5-tetracarboxylic) acid.
[c] Products consist of κ- or β-carrageenan only.

A further point of interest lies in comparing reactions (5), (9) and (10) in Table 1. It appears that the reaction reaches a certain point, then stops. Neither higher temperatures nor longer times make a detectable difference to the composition. It would appear that one of the products of the reaction, presumably SO_3, is inhibiting the reaction. If it is doing this by resulfating the polysaccharide, i.e. an equilibrium is being set up, all hydroxyl groups would be susceptible to such sulfation. While the observed faster desulfation of equatorial hydroxyl groups would ensure that the resultant equilibrium level of sulfate ester would be low, it should be kept in mind that very low levels of apparent sulfation in some analyses could be spurious.

The above observations suggest that for efficient desulfation a further reagent is needed that reacts irreversibly with SO_3. There are many such substances, although the reaction between SO_3 and a nitrogen, oxygen or fluorine atom appear most

Table 2
Recommended desulfation conditions[a] for carrageenans

Substrate[b]	Acceptor	Time (h)	Product[b]	Yield (g)	Yield[c] (%)
ι-Carrageenan	MeOH (5 mL)	1.25	κ-Carrageenan	1.0	63
ι-Carrageenan	MeOH (5 mL)	2.5	κ-Carrageenan	0.78	49
ι-Carrageenan	NaF (1 g)	3	κ-Carrageenan	1.31	82
ι-Carrageenan	As_2O_3 (2 g)	3	β-Carrageenan	0.80	67
ι-Carrageenan	Sb_2O_3 (2 g)	2–4[d]	β-Carrageenan	0.81	68
λ-Carrageenan	As_2O_3 (2 g)	3	D/S-λ[e]	0.73	71
λ-Carrageenan	NaF (1 g)	3	D/S-λ[f]	0.77	75
κ-Carrageenan	NaF (1 g)	3	See text	1.73	87
Porphyran	As_2O_3 (2 g)	3	D/S porphyran[g]	1.26	≈72[h]

[a] All reactions used 2 g of starting material, 90 mL of Me_2SO dried by Dean/Stark distillation, specified acceptor, 1.5 g pyromellitic acid, 15 mL pyridine, at 120 °C for the specified time.

[b] ι-Carrageenan contains anhydrogalactosyl-2-sulfate and galactosyl-4-sulfate, κ-carrageenan contains galactosyl-4-sulfate, λ-carrageenan contains 3-linked and 4-linked galactosyl-2-sulfate, and 4-linked galactosyl-6-sulfate (three sulfate ester groups per biose), β-carrageenan no sulfate ester, porphyran contains 3-linked galactosyl residues and an equal number of 4-linked residues, half of which contain galactosyl-6-sulfate and the others nonsulfated (and easily hydrolysable) 3,6-anhydrogalactosyl residues.

[c] Based on theoretical anhydrous polymers.

[d] The two times gave equal yields of identical material.

[e] All three sulfate esters completely removed.

[f] Both equatorial sulfate esters completely removed, approximately 85% of primary sulfate ester removed.

[g] All sulfate ester removed.

[h] Based on estimated value of sulfate ester in starting material.

likely to form compounds irreversibly [15]. Strongly basic materials can be excluded from consideration, as they would react with the added acid while others, such as phosphorus pentoxide and methanol, may assist other degradative reactions. From our results, it also appears that materials which form hydrogen bonds tend to inhibit the desulfation and so may not be desirable, except to carry out preferential desulfation. To minimize hydrolysis, the solvent should be dried, and for simple convenience we recommend the Dean Stark procedure. Accordingly, we carried out the desulfation reactions in anhydrous conditions with the SO_3 acceptors As_2O_3 or Sb_2O_3. As shown in Table 2, such conditions gave rise to high yields of the fully desulfated β-carrageenan.

Table 2 lists recommended reaction conditions for preparative desulfation, together with the corresponding yields based on air-equilibrated starting material. Each of these reaction products was essentially free of byproducts due to oxidation, and only one carrageenan was present in the isolated product. Both κ- and β-carrageenan could be prepared from ι-carrageenan. The percentage yield is based on the theoretical conversion of absolutely dry material and is almost certainly not achievable. While both starting materials and products will contain moisture, the sulfate salts would be expected to retain more water. The yields in Table 2 are those which are readily attainable from algal polysaccharides as obtained in the laboratory by usual techniques without requiring special prior purification techniques.

As can be seen, complete desulfation in reasonable yield is quite attainable. Effective desulfation procedures were also found for λ-carrageenan and for porphyran. The use of fluoride as a sulfate acceptor shows that the 2-sulfate in ι-carrageenan can be removed selectively. (The same result is also attainable with methanol or water, reactions 1–3, Table 1, but hydrolysis occurs with these. That less polymer degradation occurred with fluoride as the SO_3 acceptor was shown by the fact that only in this reaction was the resultant film nonbrittle and able to be readily folded.) Fluoride as the SO_3 acceptor removed both 2-sulfate esters in λ-carrageenan, but the 6-sulfate ester was also almost totally removed. Further, when κ-carrageenan was the substrate for the fluoride desulfation, approximately 30% of the sulfate ester was removed in 3 h. Thus while selectivity against the axial 4-sulfate ester is possible through the careful selection of conditions, the reaction cannot be used blindly as a 'test' for the 4-sulfate ester.

4. Conclusion

An improved procedure for desulfating sulfated polysaccharides has been found, in which the polysaccharide salt dissolved in dimethyl sulfoxide should be heated at about 120 °C for approximately 3 h in the presence of pyromellitic acid, pyridine, and an amphoteric nonhydrogen-bonding sulfate acceptor, of which arsenous oxide and antimony trioxide are preferable. The procedure appears to work satisfactorily for each of the types of sulfate ester present in algal galactans, without significant degradation of the underlying polysaccharide skeletons occurring. When sodium fluoride is the sulfate acceptor, selective desulfation of nonaxial sulfate esters is achievable.

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