Biodegradation of Cross-Linked Chitosan Gels by a Microorganism

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Introduction

Cross-linked biological polymers in aqueous systems have long been an important class of materials, and they are used in a diverse assortment of practical applications as hydrogels including food chemical purposes. Among biological polymers, chitosan [(1→4)-2-amino-2-deoxy-β-D-glucan] is found in some microorganisms and is one of the most abundant natural amino polysaccharides. Chitosan is a polymer of glucosamine residues and is readily prepared from crystalline chitin [(1→4)-2-acetamido-2-deoxy-β-D-glucan] via N-deacetylation with alkali. Chitin and chitosan chemistry has been widely investigated from basic scientific interests as well as from practical applications, such as biomedical and agricultural applications, by many research groups.[1−4] In addition, since chitosan is a nontoxic biodegradable polysaccharide, the possibility for its use as a material for drug delivery systems was reported.[5]

Chitosan gels have been investigated from the point of view of preparative procedures, mechanisms of gel formation, changes in specific rotation of polarized light, and thermally reversible gel characteristics.[6−8]

During investigations of biodegradation of cationic biohydrogels,[9,10] we reported the biodegradation characteristics of chemically cross-linked chitosan gels by microorganisms from soils.[11,12] The present note describes the quantitative analysis of the ultimate degradation of chitosan and chitosan−GA gels by a fungus, *Penicillium caseicolum*, from soils.

Experimental Section

Chitosan was prepared from chitin of crab shells (Alaska king crab) treated with 50% NaOH as reported in our earlier article.[13] After preparation of chitosan film, the degree of deacetylation was determined to be 74% by quantitative infrared analyses of the absorption bands at 1655 and 3450 cm^{-1}.[14] The molecular weight of chitosan used was estimated to be 940 000 from the viscosity measurement in 0.1 M acetic acid−0.2 M sodium chloride.[15]

Colloidal chitin and chitosan were prepared as described in earlier articles.[8,11,16] To a clear solution of chitosan in 10% acetic acid [20 mg (1.24×10^{-4} mol residue) in 1 mL] were added 2/5 (0.01 mL, 5×10^{-5} mol) and 4/5 (0.02 mL, 10^{-4} mol) equivalent amounts of 25% glutaraldehyde (GA) to chitosan residues with stirring for 5 min at room temperature. Stirring was then stopped. After 24 h, the prepared gels were washed thoroughly to pH 6 with sterilized water in order to remove acetic acid as described in our article.[12]

The microorganism used here was obtained in the following approach. At the beginning of the present study, soils at 13 different places were randomly collected within a 15 km area from this laboratory. A small part of the degraded liquid, in which microorganisms degraded the chitosan−GA gels, was cultured on potato agar containing 200 g of potato extract, 10 g of sucrose, and 20 g of agar per liter. After being reisolated from single spores, the fungus *P. caseicolum* within two species was obtained.[17]

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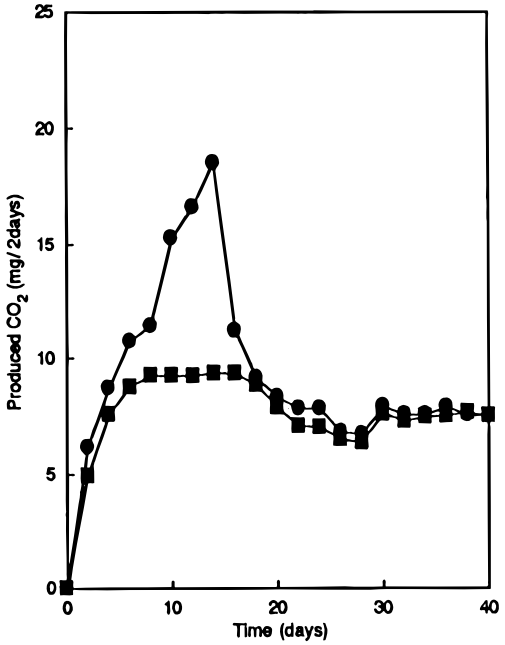

Figure 1. Time course of the biodegradation of the cross-linked chitosan (20 mg/mL)−2/5 GA gel (●) by *P. caseicolum* and control experiment with the fungus and without the cross-linked chitosan−GA gel (■). The biodegradability by the fungus was determined by measuring the amount of CO_2 trapped with and without the cross-linked chitosan−GA gel.

Quantitative determination of biodegradation (conversion to CO_2 and H_2O) was conducted using the CO_2 production test units described by Sturm and Larson.[18−20]

First, 3 L of sterilized water was added to a 4 L reaction bottle. Second, 20 mg/L of colloidal chitin or chitosan, or chitosan−GA gels containing 20 mg of chitosan, was added into the bottle. Third, the conidiospore of *P. caseicolum*, at 3300 colony forming units (CFU)/L, was added to the colloidal chitin and chitosan and to the chitosan−GA gel liquid media in the reaction vessels under aseptic conditions. The media contained only chitin, chitosan, or chitosan gels as nutrients in sterilized water, and CO_2-free air was bubbled through the test units (60−100 mL/min), which were statically maintained at 25 °C. The biodegradability of a given material was determined by measuring the amount of CO_2 trapped during a given time period (every 2 days) and relating the results to the calculated percent theoretical CO_2 production, on the basis of structure of the material under investigation.

Results and Discussion

The cross-linking of chitosan by GA has already been reported in our previous article.[12] For good chitosan gel formation, concentrations as high as 15 mg/mL chitosan and 1/9 molar equivalent of GA were necessary. Cross-linked chitosan (20 mg/mL)−2/5 and 4/5 GA gels were used for microorganism degradation experiments. Both chitosan−GA gels exhibited reversible expansion−contraction when immersed alternatively in water and in ethanol. The swelling degree in water was 2.0 in the case of chitosan−4/5 GA gels and 4.2 in the case of chitosan−2/5 GA gels. As in our previous paper, we have also reported here some data on degradation of amino polysaccharides, including cross-linked chitosan hydrogels, using microorganisms such as fungi and bacteria.[11] However, the question as to whether or not the chitosan and chitosan−GA gel degrading fungi degrade parent chitosan to CO_2 and H_2O remains unsolved.

As an example, Figure 1 shows the time course of the biodegradation of chitosan−2/5 GA gel by *P. caseicolum*,

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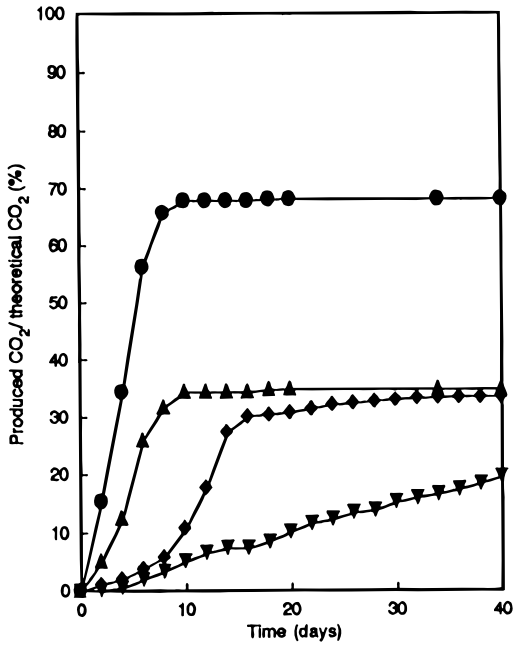

Figure 2. Percent theoretical CO_2 production of the biodegradation of colloidal chitin and chitosan, and the chitosan−GA gels with different cross-linking densities, by *P. caseicolum*: (●) chitin; (▲) chitosan; (♦) chitosan−2/5 GA gel; (▼) chitosan−4/5 GA gel.

together with a control experiment with the fungus and without chitosan−GA gel. CO_2 evolution due to ultimate degradation of the chitosan−GA gel started slowly after 2 days, accelerated after about 8 days, reached the highest after 12−14 days (17−19 mg/2 days), and slowed after 16 days. On the other hand, the control evolved an almost constant CO_2 amount (7−9 mg/2 days) after 6 days due to the respiration of the fungus. From these results, the percent theoretical CO_2 production was calculated. Figure 2 shows the percent theoretical CO_2 production of the biodegradation of chitosan−GA gels with different cross-linking densities by *P. caseicolum*. This also included the results of the biodegradation of chitin and chitosan. Original chitin produced the highest percent of theoretical CO_2 (∼70%) after 8 days. Chitosan with no cross-linking produced the second highest percent of theoretical CO_2 (∼35%) after 10 days. When chitosan was cross-linked, the biodegradation by *P. caseicolum* was slowed. The chitosan−2/5 GA gel produced CO_2 (28%) after 14 days and increased gradually to ∼33% after 40 days. The chitosan−4/5 GA gel with a higher cross-linking produced CO_2 gradually to ∼20% after 40 days. These experiments were repeated four times each in all cases. The experimental errors were within 30%. From these results it is clear that, when the chitosan gel has a higher cross-linking, the biodegradation is slower. Thus, it is possible to control the biodegradation of chitosan gel systems via cross-linking. In this connection, the biodegradation of cross-linked films of polycaprolactone has been studied using two different organisms, the yeast *cryptococcus* and the fungus *Fusarium* by P. Jarrett *et al.*[21,22] The presence of cross-links in polycaprolactone had a profound effect on biodegradability. Although the reason for this effect was not known, the cross-links themselves appeared to have a major effect on degradability. The present results of the biodegradation of cross-linked chitosan coincide with the earlier results of the biodegradation of cross-linked polycaprolactone and suggest the presence of both endo- and exoenzymes in organisms used.

We have reported here some data on biodegradation of amino polysaccharides, including cross-linked chitosan hydrogels, to CO_2 using the fungus *P. caseicolum*. The present biodegradation results by a soil microorganism might offer some clues to understanding degradation in biological cross-linked biohydrogels and also to future biotechnological uses of chitosan and chitosan hydrogels. These findings may also prove helpful in developing new biodegradable biohydrogel materials with very high water content. With regard to this, given the selective adsorption ability of anionic molecules such as benzoic acid and acidic amino acids in the cationic polyamino acid−GA gel matrices,[9,23] it may be possible to adsorb anionic medicines into cationic cross-linked biohydrogels. When the gels are biodegraded, the digested oligoglucosamine fragments are expected to exhibit anti-infection actions as reported earlier.[24] Thus, chitosan hydrogels appear very promising in their diverse agricultural and medical applications.

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