Enzyme-Catalyzed Phase Transition of Alginate Gels and Gelatin−Alginate Interpenetrated Networks

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Received April 3, 2007

The enzyme-catalyzed gel−sol transition of calcium−alginate obtained by internal gelling strategy with the help of an entrapped alginate lyase is described. We show that alginate molecules and enzyme-produced oligoalginates shorten the gel time of physical gelatin gels (5% and 1.5%), probably due to local protein concentration increase. Interpenetrated networks composed of calcium−alginate and of gelatin were obtained only if elongation of gelatin helices inside a pre-existing calcium−alginate network could occur and only for low gelatin concentration (1.5%). The physical gelatin network is almost reversible inside the alginate one. Both networks can be obtained in the presence of alginate lyase, but gel−sol transition of calcium−alginate cannot be obtained in the presence of gelatin.

Introduction

Manufacturing of materials with new properties is a challenging purpose, and gels, defined as soft matter by De Gennes, are believed to be some of the most interesting materials of the 21st century.[1] A gel may be simply described as a continuous polymeric solid phase, assimilated to a single macromolecule, entrapping a continuous liquid phase unable to leak from the solid network.[2] Numerous applications of gels have been developed for years in cosmetics, pharmaceutics, or biotechnology for drug delivery or cell entrapment, and new ones are now emerging.[3]

Biological hydrogels offer the advantage of being biocompatible and biodegradable, a key point for medical purposes. Biological systems are able to organize their biomolecules into complex gels presenting unique physicochemical properties. Biological gels are mostly composed of proteins and/or polysaccharides, which form a gelling network through physical bonding (e.g., low energy) and/or through enzyme-mediated chemical bonding (e.g., high energy).[4] While chemical polymer composition and properties can be easily controlled, biological polymers are often heterogeneous with a chemical structure strongly depending on their biological origin and environmental parameters; accurate determination of the biopolymer structure is then of crucial importance.[5] Both physicochemical and biological (enzymatic) methods are suitable to elucidate the structure−function relationship between the polymer structure and its molecular association.[6]

The use of biopolymers at industrial scale is limited to the few ones available in large quantities, with low extraction costs. However, the association of different biopolymers ex vivo offers new opportunities to build new biomaterials. Genetically engineering the biological organisms on one side and tailoring the biopolymer by its specific enzymes on the other side enlarge the possible uses of biopolymers as described with alginates.[7]

Alginates are negatively charged polymers of mannuronic acid and guluronic acid (its C_5 epimer), produced by algae (mainly *Macrocystis pyrifera*) and some bacteria (*Azotobacter vinelandii* or *Pseudomonas sp.*). Alginate is biosynthesized as a polymannuronate polymer before action of specific epimerases, which introduce the guluronic residues. The various epimerase specificities lead to highly heterogeneous polymers containing polymannuronnic sequences (poly-M), polyguluronic sequences (poly-G), and alternating mannuronic−guluronic sequence (poly-MG).[7] Alginate molecules are able to assemble by the chelation of divalent ions, leading to an "egg-box" structure.[8] Starting from recombinant bacterial homogeneous polymannuronnate, it is possible to tailor alginate sequences as poly-G or poly-MG depending on the used epimerase. Alginate lyases, mainly isolated from sea-living organisms or from marine bacterial strains growing on alginate, can also be used to modify alginate molecules.[9] They act as β-eliminase leading to a α-unsaturated uronic acid at the non-reducing end of the alginate polymer[10] (Scheme 1).

Another well-known gel forming biopolymer is gelatin, a protein issued from the acidic or alkaline denaturation of collagen.[11] The physical network formation is due to the association of prolin-rich regions of the protein random chains into triple helices through hydrogen bonding. This network is thermally reversible.[12] Previous work in our laboratory and others has shown the possibility to obtain various gelatin networks with a microbial transglutaminase.[13] This enzyme catalyzes the formation of an isopeptidic bond (*N*-ϵ-(γ-glutamyl) lysine)[14] between two gelatin molecules, leading to various gels, depending on the relative amount of covalent to hydrogen bonds and on the history of the network formation.[13] Interestingly, the introduction of a protease directly in the gelling solution allows us to achieve a sol−gel transition followed by a gel−sol transition without external action.[15] One example of enzyme-catalyzed transient gel has been described when chitosan and gelatin are cross-linked by tyrosinase.[16] The authors proposed that the resulting material breaks due to physical interactions; we believe the low chemical stability of the covalent bond (imine) also contributes to the gel reversibility. In this example, no enzyme was used to catalyze the gel−sol transition.

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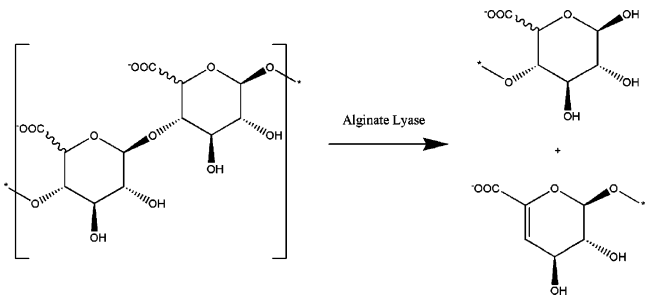
10.1021/bm700767u CCC: $37.00
Published on Web 10/19/2007

Scheme 1. Degradation of Alginate by Alginate Lyase



The introduction of a polysaccharidic fraction into these gelatin gels may allow the construction of new biomaterials, such as interpenetrated networks (IPN), if both biopolymers are able to gel independently. If only one of the biopolymers is subject to a sol−gel transition and the other one remains in the sol phase, we will define the resulting material as semi-IPN. For the IPN, after both networks have been generated, it should be possible to obtain the phase transition of one or both of the networks. Many examples of systems including both proteins and polysaccharides are described in the literature. Nevertheless, to our knowledge, in most of these systems, only one biopolymer forms a continuous phase while the other is entrapped, as an emulsion, within the first one.[17,18] Therefore, they are not true IPN. In the case where two gelling biopolymers form a bicontinuous phase,[19] the dynamics of the system was not related to enzyme reactions.

In this work, we describe the capacity of calcium−alginate gels to realize a sol−gel followed by an enzyme-catalyzed gel−sol transition as previously described for gelatin.[20] We further evaluate the influence of alginate on gelatin physical gels, and we study the dynamics of an artificial interpenetrated network formed by these two biopolymers of different chemical natures.

Experimental Section

Materials. Alginate lyase from *Flavobacterium sp.* (38730 U/g; lot #083K0695), alginate from *Macrocystis pyrifera* (A2158, low viscosity polymer [253 cps at 2% in water, 25 °C], lot #92H0255), and gelatin Type A extracted from pig skin (G2500, 300 Bloom, 50−100 kDa, pI 7.0−9.0, lot #102K0059) were obtained from Sigma-Aldrich (St-Quentin en Fallavier, France). All inorganic salts were from Research Organic (Cleveland, OH). Except the enzyme, which was purified, all materials were used as supplied by the manufacturer. All experiments were performed with the same lot number.

Optical Rotation. Optical rotation was measured on a Jasco P-1010 polarimeter. Temperature control was performed with a Julabo F25 bath. Cooling and heating ramps of 0.5 °C min^{-1} were applied.

The amount of helices was derived from the specific optical rotation.[21] The helix amount χ was derived from:

$$\chi = \frac{[\alpha]_\lambda^{\text{helix}} - [\alpha]_\lambda^{\text{coil}}}{[\alpha]_\lambda^{\text{collagen}} - [\alpha]_\lambda^{\text{coil}}}$$

where λ is the wavelength (λ = 435 nm), $[\alpha]_\lambda = \alpha/lc$ is the specific optical rotation of the protein in solution, c is the concentration (grams per cubic centimeter), l is the optical path (0.1 dm), α is the optical rotation angle (deg) measured experimentally, $[\alpha]_\lambda^{\text{collagen}}$ is the specific optical rotation of native soluble collagen ($\chi = 1$), which contains only triple helices, and $[\alpha]_\lambda^{\text{coil}}$ is the specific optical rotation of the coils ($\chi = 0$) ($[\alpha]_{435\text{nm}}^{\text{collagen}} = -800$ deg cm^3 g^{-1} dm^{-1} at 27 °C, $[\alpha]_{435\text{nm}}^{\text{coil}} = -274$ deg cm^3 g^{-1} dm^{-1} at pH = 7, 4 and 40 °C[21]).

Rheology. Rheology measurements were performed with a Rheostress 1500 from HAAKE operating in the oscillatory mode, with a strain of deformation of 5% and a frequency of 1 Hz. Deformation, storage modulus G', and loss modulus G'' were recorded as a function of time. Temperature was controlled via a Cryostat-F6 (HAAKE). A cone/plate geometry with a cone of 60 mm/2° was used, and water evaporation was prevented by the use of an adapted water trap. Temperature ramps of 0.5 °C min^{-1} were applied.

Alginate Lyase Purification. Commercial preparation of alginate lyase (10 mg/mL in 5 mM Tris-HCl pH 7.5) was purified on coupled DEAE- and CM-Sepharose (12 mL, Pharmacia, Uppsala, Sweden) after elution with NaCl gradient (0−500 mM). Typically, the main activity was recovered in 10−15 mM NaCl fraction (SI 1). The enzyme was further concentrated on a 10 kDa membrane (Amicon Ultra, Millipore Billerica, MA) before elution on a Gelatin-Sepharose 4B column (2.5 mL, Pharmacia, Uppsala, Sweden) and concentration over 10 kDa membranes. All purification steps were performed at 10 °C. Sodium azide (2%) was added at a final concentration of 0.02%, and the enzyme, stored at 4 °C, was used within a week. Enzymatic activity was assayed as described elsewhere.[22] The enzyme was purified 26 times with a yield of 85%.

Preparation of Polymer Solutions. Sodium alginate (0.1 g) was carefully dissolved in freshly prepared calcium−EDTA solution (282 mg of $CaCl_2 \cdot 2H_2O$ and 798 mg of $Na_4EDTA \cdot 2H_2O$ in 10 mL) without heating. Gelatin solution (up to 10% in water) was allowed to swell at 4 °C for at least 4 h before use. This solution was incubated at 40 °C for 30 min to allow the gelatin to melt prior to use. D-Glucono-δ-lactone (312 μL of a 3 M solution) was freshly prepared. Alginate lyase (100 μL) was added for specific experiments. Volumes were adjusted for a final volume of 5 mL. The solutions were mixed in this order: alginate, gelatin, lactone, and lyase. The same mixed solution was used for both polarimetric and rheological experiments.

Preparation of Oligoalginates. The appropriate amount of sodium alginate (usually 30 mg) was dissolved in Tris-HCl buffer (50 mM, pH 7.5, 985 μL) at room temperature without heating. Purified alginate lyase (5 U) and sodium azide (10 μL of a 2% solution) were added, and the solution was kept for 24 h at room temperature to ensure total lysis of alginate molecules (no increase of the UV-signal at 235 nm was observed after this time, even after further addition of lyase). Gelatin solution (2 mL) was added prior to polarimetric and rheological experiment.

Results and Discussion

Sol−Gel−Sol Transitions of Calcium−Alginate. The alginate sol−gel transition is induced by in situ liberation of calcium from a chelated form (e.g., Ca−EDTA, $CaCO_3$) through the hydrolysis of a lactone in a sodium alginate solution.[23] Briefly, the lactone is unstable in aqueous solution and spontaneously hydrolyzes, leading to proton release and pH drop. At acidic pH, calcium ions are released from their chelating agent (Ca−EDTA or $CaCO_3$). Here, Ca−EDTA is preferred to $CaCO_3$ because of its higher solubility, which avoids variations in rheological measurements. The gel formation of a 1% alginate solution is observed at 40 °C (SI 2), a temperature where a 5% gelatin solution does not gel. The gel time, here considered as the experimental time where $G' = G''$, is 37 min. After 600 min, the storage modulus (G') reaches 200 Pa and is then stable, indicating that no further physical bonds are formed in the network. During the same time, the loss modulus (G'') only reaches 6.9 Pa (7 Pa). These values are in good accordance with those found in the literature.[9]

The influence of a commercial alginate lyase on gel properties was measured. When the enzyme is added up to 0.19 U/mL to the alginate solution, a network is observed in which G' value is decreasing with increasing lyase concentrations (Figure 1). These enzyme concentrations are not high enough to prevent gel formation or to induce a further gel/sol transition. For higher

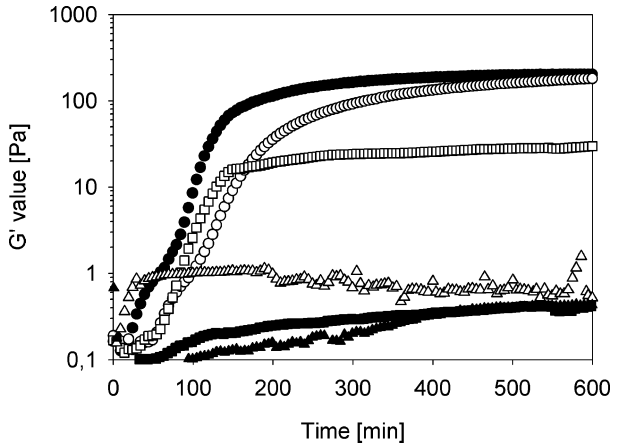


Figure 1. Rheological profiles of calcium−alginate gels in the presence of 0−0.76 U/mL commercial alginate lyase at 40 °C. 0 U/mL (●), 7.6×10^{-4} U/mL (○), 0.19 U/mL (□), 0.21 U/mL (■), 0.22 U/mL (△), 0.76 U/mL (▲).

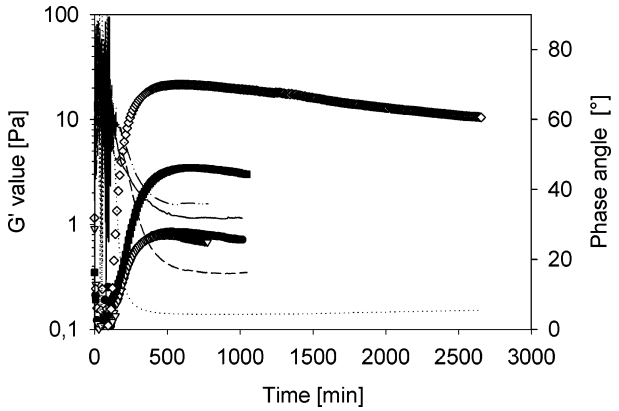


Figure 2. Rheological profiles of calcium−alginate gels in the presence of 0.19−0.21 U/mL commercial alginate lyase at 27 °C. *G*′ value for 0.19 U/mL (◇), phase angle for 0.19 U/mL (· · ·), *G*′ value for 0.198 U/mL (■), phase angle for 0.198 U/mL (− − −), *G*′ value for 0.205 U/mL (△), phase angle for 0.205 U/mL (− ·· − ·· −), *G*′ value for 0.210 U/mL (●), phase angle for 0.210 U/mL (−).

enzyme concentrations, no sol−gel transition occurs, the initial activity being sufficient to reduce alginate chain length to a size inadequate to realize an infinite network.

To prevent lyase thermal inactivation (half-life of 36 min at 40 °C), similar experiments were performed at 27 °C. When no lyase is added, at 27 °C, *G*′ value reaches 314 Pa in about 480 min (phase angle of 1.8°) and then does not decrease (SI 3). When a lyase concentration ranging from 0.19 to 0.21 U/mL is used, a different behavior is observed: sol−gel transition occurs and is followed by a decrease of the storage modulus (Figure 2). Macroscopic observations of the preparations reveal that clear solutions are obtained after a longer time reaction, indicating that a gel/sol transition has occurred. Similar results were observed for all tested lyase concentrations. The gel point corresponds here to $G' > G''$, as gels were observed with corresponding *G*′ values slightly below 1 Pa.

All of the obtained temporary gels present a weak elasticity modulus ($G' < 100$ Pa) and long gel time (between 140 and 280 min, compare to 37 min without the enzyme). The gels obtained with 0.205 and 0.210 U/mL enzyme are weak as shown by the phase angle, which never reaches less than 31°, while the gel prepared with 0.19 and 0.198 U/mL enzyme reaches 5° and 15°, respectively. The maximal *G*′ value and gelation velocity (dG'/dt) decrease with lyase concentration. *G*′ value at the gel time decreases with increasing lyase concentration. All of these data indicate a competition between the gel formation and its destruction. A sol/gel followed by a gel/sol transition has been described for gelatin upon the action of enzymes.[20] Here, we show for the first time a similar macroscopic behavior with calcium−alginate.

Next, the influence of alginate and enzyme-produced alginate fragments (oligoalginates) on the properties of a physical gel of gelatin was evaluated.

Semi-interpenetrated Networks of Gelatin and Alginate. Physical gels of gelatin are due to the association of random coil chains into triple helices reminiscent of the native collagen, which occurs below the coil−helix transition temperature. For various gelatins, a common curve usually expresses the relation between elasticity and helix content.

Gels were realized at 27 °C with 5% gelatin containing no, 1%, or 2% alginate without lactone and Ca−EDTA. A gelatin gel containing alginate molecules in solution is obtained, which may be considered as a semi-interpenetrated network (semi-IPN). Whatever the alginate concentration tested, the gel point occurs for the same helix content of 9%, indicating that alginate is not part of the network (SI 4). It must be pointed out that the gel formation is only due to the appearance of triple helices, that is, to gelatin. To better understand the effect of alginate and oligoalginate, various mixtures were compared through their gel time, *G*′, and *G*″ values after 4 h at 27 °C (Table 1). Alginate accelerates the sol/gel transition of gelatin, that is, its association into triple helices. This can be attributed to an excluded volume effect. This hypothesis is reinforced by the reduction of the water availability for gelatin due to the water shell necessary for alginate solvation. This is consistent with a phase separation phenomenon recently observed and currently under investigation. This would favor an increase of local gelatin concentration and consequently a higher rate of helix association.[21] The variations here observed cannot be attributed to a simple mass change. When a 6% gelatin gel is compared to a 5% gelatin gel containing 1% alginate in the liquid phase, gel time at 27 °C and *G*′ value at 240 min are similar (8.5 vs 11 min and 252 vs 256 Pa), but the *G*″ value increases from 17 to 44 Pa, indicating that the sol phases are different.

When 1% oligoalginates, previously obtained from alginate by lyase hydrolysis, are included into a 5% gelatin gel, gel time drops to 6.5 min (Table 1) and *G*′ value is the same as for a gel containing 1% alginate. As oligoalginates are shorter polysaccharidic chains, the viscosity of the solution estimated through *G*″ values is lower than in the presence of alginate. However, short polysaccharides are also able to favor the helix formation

Table 1. Properties of Gelatin Gels Formed in the Presence of Alginate or Oligoalginates

	gel time (min)	G' value$^{240\text{min}}$ (Pa)	G'' value$^{240\text{min}}$ (Pa)	helix content at gel point (χ, %)
gelatin 5%	22	102 ± 6	9 ± 0.6	9
gelatin 6%	8.5	252 ± 14	17 ± 1	
gelatin 5% + alginate 1%	11	256 ± 15	44 ± 3	9
gelatin 5% + oligoalginate 1%	6.5	264 ± 16	21 ± 2	

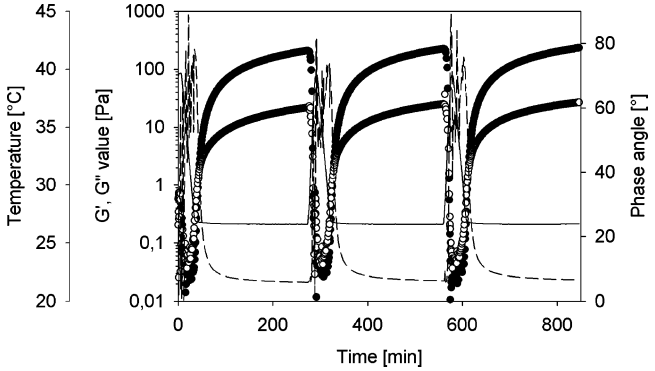


Figure 3. Rheological profiles of 5% gelatin gels supplemented by 1% alginate and 0.2 U/mL alginate lyase. G' value (●), G'' value (○), phase angle (− − −), temperature (−).

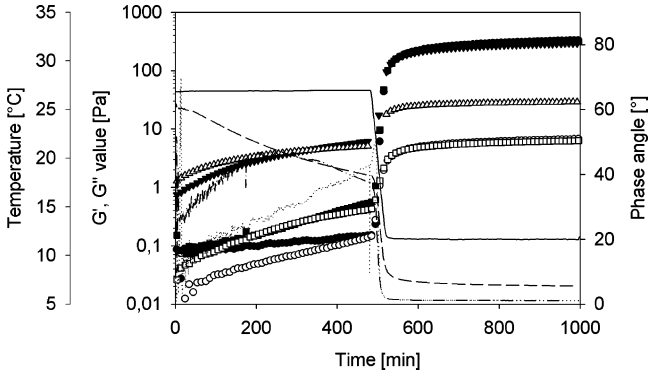


Figure 4. Rheological profiles of 1.5% gelatin gels supplemented by alginate or oligoalginates. G' value of gelatin 1.5% (●), G'' value of gelatin 1.5% (○), phase angle of gelatin 1.5% (· · ·), G' value of gelatin 1.5%/alginate 1% (▲), G'' value of gelatin 1.5%/alginate 1% (△), phase angle of gelatin 1.5%/alginate 1% (− − −), G' value of gelatin 1.5%/OA 1% (■), G'' value of gelatin 1.5%/OA 1% (□), phase angle of gelatin 1.5%/OA 1% (− ·· − ·· −), temperature (−).

as observed by shorter gel time. The oligoalginate charges are more dispersed than those of alginates, favoring helix formation. Moreover, the water shell needed to solvate all oligoalginates molecules is slightly higher, increasing the local gelatin concentrations.

Next, the alginate lysis inside a solution/gel of gelatin was studied. A 5% gelatin solution containing 1% alginate and 0.2 U/mL of alginate lyase was used. In these experiments, the thermal protocol is as follows: the temperature is maintained at 27 °C for 4 h to allow gelatin gel formation, then raised to 40 °C to melt the gel, and the whole process is repeated three times with the same sample. As shown in Figure 3, a new gel is formed each time the temperature is decreased to 27 °C; however, gel time is decreasing from 9 to 10, to 7 and then to 5.5 min. For the first gelation, the gel time is slightly lower than that for a gelatin gel containing 1% alginate, while for the third one, the gel time is slightly lower than that for a gel containing 1% of oligoalginates. As previously shown, reducing the alginate chain length reduces the gel time. These results confirm that alginate and oligoalginates favor the gelatin network formation. The viscoelastic properties of the gel are identical in the three successive runs.

The main question to answer at this stage is whether the lyase reacts inside the gel or just when gelatin is in a liquid state.

To better characterize the effect of alginate and oligoalginates, gelatin concentration was reduced. The effect of additives was investigated with different mixtures (Table 2) at 27 °C. A 2.5% gelatin gel is obtained in 193 min, while a 1.5% gelatin supplemented with 1% alginate needs 309 min to turn into a

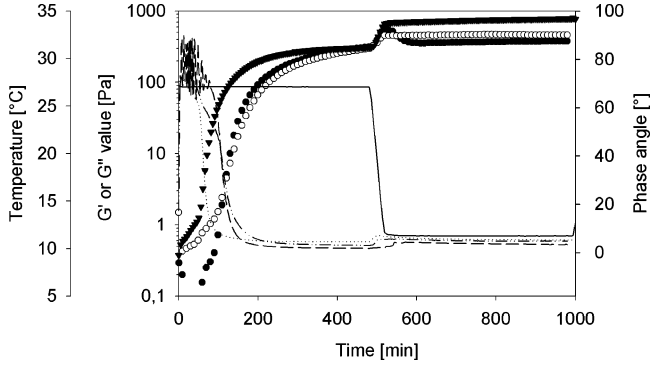


Figure 5. Calcium−alginate (1%) network formation in the presence of gelatin (0−1.5%) at 27 °C followed by the formation of the gelatin physical gel at 10 °C. G' value without gelatin (●), G' value with 1% gelatin (○), G' value with 1.5% gelatin (▲), phase angle without gelatin (− − −), phase angle with 1% gelatin (· · ·), phase angle with 1.5% gelatin (− ·· − ·· −), temperature (−).

gel. At low gelatin concentration (1.5%), alginate and oligoalginates favor and even allow the gelatin network formation as gelatin alone does not gel at 27 °C. Under such conditions, both additives lead to the same kinetic behavior, meaning the viscosity of the whole solution is probably not directly involved. Comparison of the rheological profiles (Figure 4) shows nearly superimposed profiles of G' values at 10 °C for gelatin alone or with alginate or oligoalginates. The effect of both additives on gelatin helix formation is also similar; in both cases, 14.7 ± 1.2% helices are measured at the gel time. G'' values obtained for gelatin gel and for gelatin containing 1% oligoalginates are also identical, while a higher value is measured with alginates as previously.

Interpenetrated Networks. The next step was to realize an interpenetrated network (IPN) where both gelatin and alginate were allowed to form their own independent network. Several combinations were assayed. Finally, the formation of a calcium−alginate network in which protein network is thus induced appears to be the easiest system to handle.

A mixture containing 1% alginate in the presence of the lactone, Ca−EDTA, and gelatin at concentrations ranging from 0 to 1.5% is maintained at 27 °C. A calcium−alginate gel is formed; thus after 8 h at 27 °C, temperature is decreased to 10 °C to induce the protein network formation (Figure 5). In such conditions, the calcium alginate network is formed in 104 min in the absence of gelatin. G' value is not significantly modified with temperature decrease (378 vs 314 Pa), while G'' value is doubled (22 vs 10 Pa). Addition of 1% gelatin in the solution has no influence on the gel time (103 min vs 104 min) nor on the G' value after 480 min at 27 °C (297 vs 314 Pa). G'' is slightly increased due to the increase in total polymer concentration. When the gel is cooled to 10 °C, gelatin network formation is induced as shown by the increase of the G' value (462 Pa). Observation of the kinetics with a linear scale reveals that G' value only increases during the temperature diminution and then is constant. This means that helices are formed during the temperature decrease but cannot continue their elongation as in a normal gelatin gel,[21] being constrained by the pre-existing alginate network.

This observation is amplified when gelatin concentration is increased to 1.5%. The gel time is greatly reduced (59 vs 103 min), but the G' value after 480 min at 27 °C is close to the one of the alginate gel alone (309 vs 314 Pa). In the previous experiment at the same concentrations, soluble alginate allowed slow gelatin network formation; here, this effect is not observed, as alginate rapidly turns to a gel and gelatin cannot jellify inside

Table 2. Viscoelastic Properties of 1.5% Gelatin Gels in the Presence of Alginate or Oligoalginates[a]

	gelatin 1.5%	gelatin 2.5%	gelatin 1.5% + alginate 1%	gelatin 1.5% + oligoalginate 1%
gel time (min)	no gel in 480 min at 27 °C	193 ± 11	309 ± 19	312 ± 19
G' value^{480min} at 10 °C (Pa)	334 ± 20	n.d.	293 ± 17	315 ± 19
G'' value^{480min} at 10 °C (Pa)	6.6 ± 0.4	n.d.	29 ± 1.5	6.2 ± 0.4

[a] n.d.: not determined.

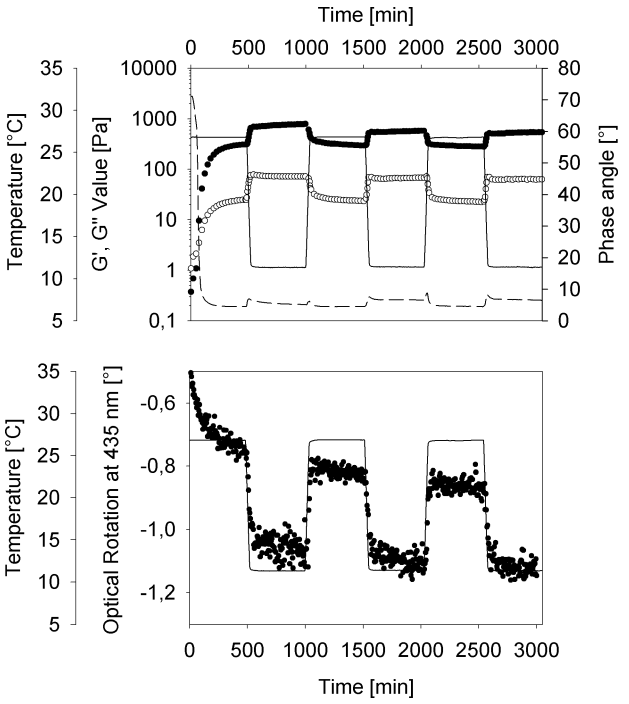


Figure 6. Reversible gelatin (1.5%) physical network formation inside a calcium−alginate gel (1%) followed by rheology (up: G' value (●), G'' value (○), phase angle (− − −), temperature (−)) and polarimetry at 435 nm (down: optical rotation (●), temperature (−)).

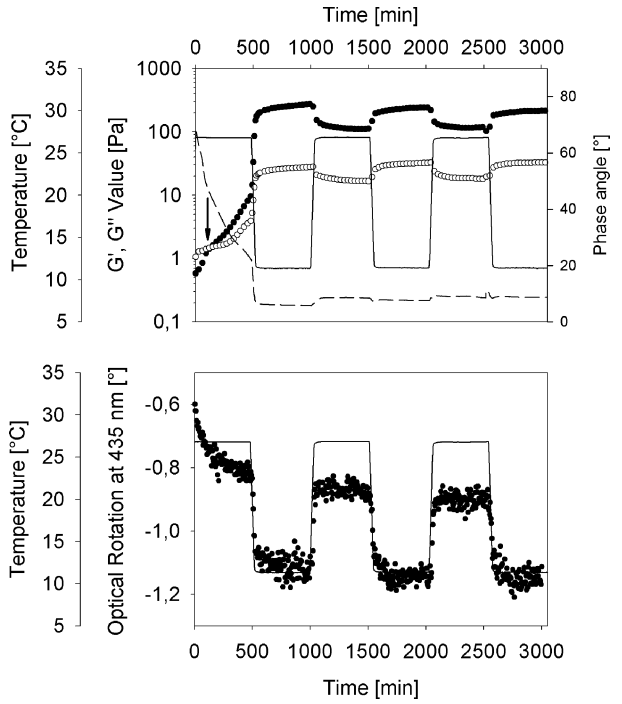


Figure 7. Influence of purified alginate lyase (0.19 U/mL) on an interpenetrated calcium−alginate (1%)/gelatin (1.5%) network followed by rheology (up) and polarimetry at 435 nm (down). Up: G' value (●), G'' value (○), temperature (−), phase angle (− − −), arrow indicated the gel point. Down: optical rotation (●), temperature (−).

this alginate gel at 27 °C. A temperature reduction to 10 °C induces helix formation, and a gelatin network is formed as shown by the large and rapid G' increase (from 309 to 787 Pa). More important, G' value increases continuously at 10 °C, which is characteristic of helix elongation: the gelatin network is growing inside the calcium−alginate gel.

Visual inspection of the material reveals translucent (but not transparent) material once the alginate gel is formed, while the initial solution is transparent. When the gelatin network is formed or melted, no significant differences of the macroscopic aspect of the gel are observed; in particular, no syneresis seems to occur.

Further temperature cycles from 27 to 10 °C induce successive melting and gelation of gelatin (Figure 6). Each time the gelatin is melted at 27 °C, the gel recovers the G' and G'' values of the calcium−alginate gel (∼300 and ∼24 Pa, respectively). Contrarily, the G' value after 480 min at 10 °C decreases (from 787 to 579 Pa after two runs) each time a new gelatin network is formed. In consequence, the gelatin physical network may be formed into alginate gel, but it is more difficult to form it again after melting.

If the same system is studied by polarimetry at 435 nm, a large optical rotation shift follows each of the three temperature decreases from 27 to 10 °C (Figure 6).[24] The corresponding values reach −0.32°, −0.29°, and −0.26°, respectively. Considering that the optical rotation variation is only due to helix formation, helix concentrations reach successively 40.5% (6 mg/mL), 37% (5.5 mg/mL), and 33% (4.9 mg/mL). Less and less helices are formed, but they are theoretically sufficient to generate an infinite network (>4.5 mg/mL).

A higher protein concentration generates a higher buffering effect and consequently prevents the liberation of calcium from Ca−EDTA, increasing the gel time of the calcium−alginate network. This phenomenon is currently under study.

The same experiment was performed in the presence of 0.19 U/mL of alginate lyase (Figure 7).

As expected from the previous results, a longer gel time than without lyase is observed (130 vs 59 min). The gel time is near the gel time of similar gels without gelatin (142 min). Also, the values for G' and G'' are drastically lowered (9.6 vs 309 Pa and 3.4 vs 25 Pa, respectively), which is due to the competition between network formation (the network is formed) and the lysis of the alginate by the enzyme (the rheological values are lowered). The alginate network is able to form in the conditions tested but is really weak. Each time the gel is cooled to 10 °C, both G' and G'' values increase concomitantly with the decrease of the polarimetric signal, indicating that gelatin molecules associate into helices and form a proteic network inside the alginate one. When gelatin helices are melted following a temperature increase, the resulting network is much stronger than the alginate network previously formed (G' value of 112 Pa), meaning the alginate network continues to form or is favored in the gelatin gel. Interestingly, in the present system, gelatin gel becomes nearly fully reversible as successive metling−gelling cycles give the same G' and G'' values at

27 °C. The slight decrease in G' at 10 °C is due to a slightly lower helix content as shown by polarimetry: the three successive cycles at 10 °C give 35% (5.25 mg/mL), 33% (4.95 mg/mL), and 31.6% (4.74 mg/mL) helix content, respectively. The total enzymatic dissolution of the alginate gel in the presence of gelatin was not yet achieved.

Conclusion

We have shown that calcium−alginate hydrogels can undergo sol−gel followed by enzyme-catalyzed gel−sol transition as it was recently demonstrated for protein gels.[20] Alginate lyases are appropriate enzymes for catalyzing such phase transitions. In the present study, we used a commercial lyase preparation; however, the selection of a lyase with a defined specificity, as well as alginates of homogeneous size and sequence such as poly-MG alginates, would allow a more accurate control of the system.

Alginate and its hydrolysis products, oligoalginates, are both able to diminish the gel time of gelatin gel without influencing strongly its elasticity. Alginate has only a kinetic effect on the gelatin gel formation, and the polysaccharide does not take part to the protein network as gelatin gels are obtained with the same helix content. This could be explained by the increase of the local gelatin concentration due to electrostatic repulsion or local water structuration. This is verified for the two gelatin concentrations (5% and 1.5%) assayed in this work.

Both networks can participate to an interpenetrated network if calcium−alginate gel is formed before the gelatin gel and if gelatin concentration is low enough to allow calcium liberation from Ca−EDTA. This work is the first achievement of an interpenetrated network entirely based on two biopolymers of different chemical nature, that is, the first totally biodegradable one.

Sol−gel and lyase-catalyzed gel−sol transition of the interpenetrated network was assayed. As long as the gelatin network was not induced, the calcium−alginate network behaves as in the absence of gelatin. The formation of gelatin helices leads to a stronger reversible network but inactivates alginate lyase by a still unknown way.

The gel−sol transition of the gelatin network inside the alginate one, as well as the whole sol−gel transition of both networks, is currently under study.

Acknowledgment. J. Bonnefoy (undergraduate student) is gratefully acknowledged for helping in the lyase purification.

Supporting Information Available. SI 1: Chromatographic profile of alginate lyase purification. SI 2: Rheological profile of 1% calcium−alginate at 40 °C. SI 3: Rheological profile of 1% calcium−alginate at 27 °C. SI 4: Relationship between helix content and elasticity and loss modulus of 5% gelatin gel including 0−2% sodium−alginate. This material is available free of charge via the Internet at http://pubs.acs.org.

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BM700767U