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Thiolated polymers—thiomers: synthesis and in vitro evaluation of chitosan–2-iminothiolane conjugates

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Abstract

The aim of this study was to improve the properties of chitosan as excipient in drug delivery systems by the covalent attachment of thiol moieties. This was achieved by the modification of chitosan with 2-iminothiolane. The resulting chitosan–4-thio-butyl-amidine conjugates (chitosan–TBA conjugates) displayed up to 408.9 ± 49.8 μmol thiol groups per gram polymer. Because of the formation of disulfide bonds based on an oxidation process of the immobilized thiol groups under physiological conditions, chitosan–TBA conjugates exhibit in situ gelling properties. After less than 2 h, 1.5% (m/v) chitosan–TBA conjugate solutions of pH 5.5 formed covalently cross-linked gels. The viscosity increased in positive correlation with the amount of thiol groups immobilized on chitosan. In addition, also the mucoadhesive properties were strongly improved by the covalent attachment of thiol groups on chitosan. The adhesion time of tablets based on the unmodified polymer on freshly excised porcine intestinal mucosa spanned on a rotating cylinder in an artificial intestinal fluid was extended more than 140-fold by using the thiolated version. Drug release studies out of tablets comprising the chitosan–TBA conjugate demonstrated that an almost zero-order release kinetic was achieved for the model drug clotrimazole within the first 6 h. The modification of chitosan with 2-iminothiolane leads, therefore to thiolated polymers, which represent a promising tool for the development of in situ gelling and/or mucoadhesive drug delivery systems.
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Keywords: Chitosan; Thiomers; Mucoadhesion; Traut's reagent; In situ gelling

1. Introduction

Within recent years, the deacetylated form of chitin, which can be isolated from insects, crustacea such as crab and shrimp as well as from fungi (Felt et al., 1998) has received considerable attention as novel excipient in drug delivery systems (Illum, 1998). Apart from the deacetylation of chitin leading to so-called chitosans, also further chemical modifications have been performed in order to improve the properties of the polymer. These intentions led to the development of trimethylated chitosans (Thanou et al., 2000), mono-*N*-carboxymethyl chitosans (Thanou et al., 2001), *N*-sulfo-chitosan (Baumann and Faust, 2001) or chitosan–EDTA conjugates (Bernkop-Schnürch et al., 1998; Bernkop-Schnürch and Scerbe-Saiko, 1998). A further promising modification is based on the immobilization of thiol groups on the primary amino groups of chitosan. Recently, it was shown that

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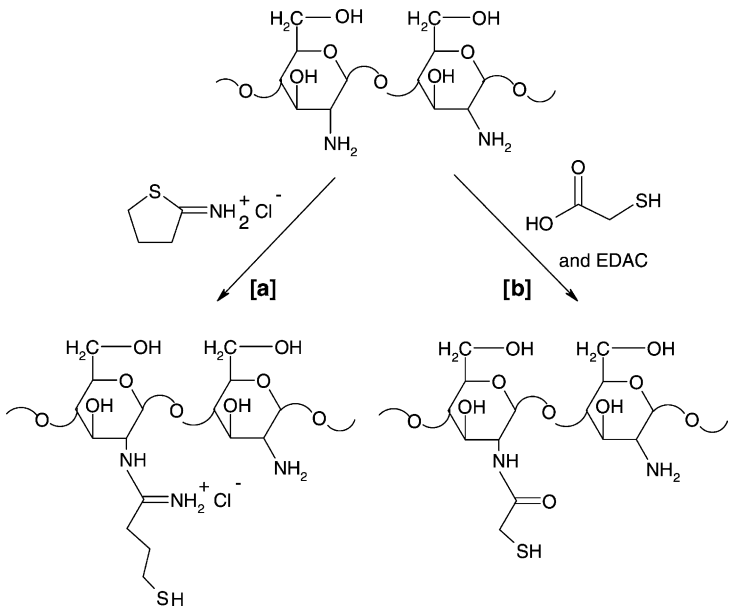

Fig. 1. Synthetic pathway for the modification of chitosan with 2-iminothiolane (a) and thioglycolic acid mediated by a carbodiimide (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EDAC) (b).

the mucoadhesive and permeation enhancing properties of the polymer could be strongly improved by the covalent attachment of L-cysteine or thioglycolic acid (Fig. 1b) on the polymer (Bernkop-Schnürch et al., 1999; Bernkop-Schnürch and Hopf, 2001; Kast and Bernkop-Schnürch, 2001). Based on thiol/disulfide exchange reactions between the thiolated polymer (=thiomer) and disulfide bonds within the mucus gel layer new disulfide bonds between the thiolated chitosan and the mucus are formed leading to even four-fold improved adhesive properties (Kast and Bernkop-Schnürch, 2001). The cationic character of chitosan is in addition responsible for ionic interactions with anionic substructures such as sialic acid and sulfonic acid of the mucus layer providing its mucoadhesiveness (Lehr et al., 1992) and for ionic interactions with the cell membrane resulting in a structural reorganization of tight junction-associated proteins leading to an enhanced paracellular uptake of hydrophilic drugs (Schipper et al., 1997). In contrast to the modification of chitosan with thioglycolic acid or L-cysteine, both leading to an uncharged amide bond linkage, the modification with 2-iminothiolane provides the introduction of a cationic amidine substructure. So by modifying chitosan with 2-iminothiolane as illustrated in Fig. 1a on the one hand, the cationic character of the polymer can be improved and on the other hand, thiol groups are immobilized on it. The combination of a raised cationic character with covalently attached thiol groups on chitosan should consequently lead to a second, further improved generation of thiolated chitosans, which can be produced by an even more simple coupling reaction. In order to verify this working hypothesis, it was the aim of this study to optimize the synthesis of such polymer conjugates (I), to evaluate the viscoelastic (II) as well as mucoadhesive properties (III) and to prove whether a controlled release can be provided out of such polymer conjugates used as drug carrier matrix (IV).

2. Material and methods

2.1. Modification of chitosan with 2-iminothiolane

One gram of chitosan (molecular mass: ~150 kDa; degree of deacetylation: 83–85%; Fluka Chemie, Buchs, Switzerland) was dissolved in 700 ml of 1% acetic acid for 5 h. Different amounts of 2-iminothiolane HCl (Traut's reagent; Pierce, Oud

Table 1
Comparison of different reaction conditions in order to evaluate the influence of the weight-ratio chitosan to 2-iminothiolane (I), the influence of the pH-value (II) and the influence of 2-mercaptoethanol (III) on the amount of immobilized thiol groups on the polymer. The resulting chitosan–4-thio-butyl-amidine (TBA) conjugates were called chitosan–TBA conjugates

Reaction mixture	Polymer	Chitosan dissolved in 700 ml 1% acetic acid (g)	2-Iminothiolane (g)	pH	Mercapto-ethanol (%)	Immobilized thiol groups (μmol/g)	S.D.
I		1.0	0.1	5	–	39.1	±12.5
II	Chitosan–TBA conjugate 60	1.0	0.2	5	–	59.8	±3.1
III	Chitosan–TBA conjugate 220	1.0	0.4	5	–	216.8	±10.1
IV		1.0	0.2	5	3	63.4	±3.5
V	Chitosan–TBA conjugate 100	1.0	0.1	7	–	95.1	±9.0
VI		1.0	0.2	7	–	225.7	±14.1
VII		1.0	0.4	7	–	408.9	±49.8
VIII		1.0	0.2	7	3	152.5	±15.9
IX		1.0	0.2	10	3	177.2	±21.9

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Beijerland, NL) were added and the pH adjusted with 5 M NaOH as listed in Table 1. In order to avoid an oxidation process during the coupling reaction optionally 2-mercaptoethanol (Sigma–Aldrich, Steinheim, Germany) was added in a final concentration of 3% (v/v). After an incubation period of 24 h at room temperature under continuous stirring, the resulting polymer conjugates were dialyzed against 5 mM HCl, two times against 5 mM HCl containing 1% NaCl, against 5 mM HCl and finally against 1 mM HCl.

Thereafter samples and controls were lyophilized by drying frozen aqueous polymer solutions at −30 °C and 0.01 mbar (Christ Beta 1-8K; Germany) and stored at 4 °C until further use.

2.2. Determination of the thiol group content

The degree of modification was determined by quantifying the amount of thiol groups on the modified chitosan with Ellman's reagent. First, 5 mg of each conjugate was dissolved in 2.5 ml of demineralized water. To aliquots (250 μl) of the conjugate solutions, 250 μl of 0.5 M phosphate buffer pH 8.0 and 500 μl of Ellman's reagent (3 mg of 5,5′-dithiobis(2-nitrobenzoic acid) (Sigma, St. Louis, MO) dissolved in 10 ml of 0.5 M phosphate buffer pH 8.0) were added. The reaction was allowed to proceed for 2 h at room temperature. Afterwards, the precipitated polymer was removed by centrifugation (24,000 × *g*; 5 min) and 300 μl of the supernatant fluid was transferred to a microtitration plate. The absorbance was immediately measured at a wavelength of 450 nm with a microtitration plate reader (Anthos reader 2001; Salzburg, Austria). The amount of thiol moieties was calculated from an according standard curve obtained by chitosan solutions with increasing amounts of L-cysteine HCl (Sigma, St. Louis, MO).

2.3. Decrease in the thiol group content within the polymer conjugate

Chitosan–TBA conjugate 100 was dissolved in demineralized water in a final concentration of 1.5% (m/v) and the pH-values were stabilized with a 100 mM acetate buffer at pH 4.0, 5.0 and 6.0, respectively. The samples and controls were incubated at 37 °C under permanent shaking. At predetermined time points, aliquots of 500 μl were withdrawn and 50 μl of 1 M HCl was added in order to quench any further reaction. The amount of remaining thiol groups was determined with Ellman's reagent as described above.

2.4. Rheological measurements

Oscillatory shear experiments were performed on a Haake rheometer Rotovisco RT20 (Haake, Karlsruhe, Germany, thermo controller Haake F6/8) with a thermostatically controlled cone/plate system (35 mm in diameter and 2° angle). After preliminary identification of the linear viscoelastic region, frequency sweeps were performed with the samples. Thiolated chitosan and unmodified chitosan serving as control were dissolved in 100 mM acetate buffer pH 5.5 in a final

concentration of 1.5% (m/v). In order to determine the increase in viscosity by the formation of disulfide bonds within the thiolated polymers as a function of time, all samples were incubated at 37 °C. At predetermined time points, aliquots (1.5 ml) were transferred on the plate of the rheometer and the samples were investigated over a 0.1–10 Hz frequency range at a constant temperature of 37 ± 1.0 °C. The parameters obtained are the complex modulus G^* and the phase angle δ. The elastic modulus G', the viscous modulus G'', and the dynamic viscosity η' are calculated by:

$$G' = G^* \cos(\delta)$$

$$G'' = G^* \sin(\delta)$$

$$\eta' = \frac{G''}{\omega}$$

where ω is the angular frequency, which is related to the oscillatory frequency ν by the relationship $\omega = 2\pi\nu$. Loss tangent ($\tan\delta$), a parameter that represents the ratio between the viscous and elastic properties of the polymer, was also calculated ($\tan\delta = G''/G'$).

2.5. In vitro mucoadhesion studies

In order to evaluate the time period of adhesion of thiolated chitosan to the mucosa, a slightly modified method as described previously has been used (Bernkop-Schnürch and Steininger, 2000). Thirty milligrams each of the lyophilized modified chitosans and control were compressed (Hanseaten Type EI, Hamburg, Germany) into 5.0-mm diameter flat-faced tablets. The compaction pressure was kept constant for all test tablets. These tablets were attached to freshly excised intestinal porcine mucosa, which had been fixed on a stainless steel cylinder (diameter: 4.4 cm; height: 5.1 cm; apparatus 4-cylinder, USP XXII). The cylinder was placed in the dissolution apparatus according to the USP containing 100 mM phosphate buffer pH 6.0 at 37 ± 0.5 °C. The fully immersed cylinder was agitated with 125 rpm. The detachment of the test tablets was determined during an observation period of one week.

2.6. Release studies

Release studies were performed with the model drug clotrimazole. Two hundred and fifty mg of chitosan–TBA conjugate 60 was dissolved in 20 ml of demineralized water and homogenized with 50 mg of clotrimazole dissolved in 1 ml of dioxane. The mixture was frozen at −80 °C and lyophilized at −30 °C and 0.01 mbar (Christ Beta 1-8K; Germany). Tablets were compressed out of the lyophilized polymer/drug mixture as described above. The in vitro release rate from this drug delivery system was then analyzed. The dosage form was placed in a beaker (Schott Duran 25 ml, Germany) containing 6 ml of release medium (100 mM acetate buffer pH 6.0/dioxane; 7 + 3). The vessels were closed, placed on an oscillating water bath (GFL 1092; 100 rev/min) and incubated at 37 ± 0.5 °C. Sink conditions were maintained during the study. Aliquots of 400 μl were withdrawn every 30 min for 6 h. The medium was replaced with an equal volume of release medium equilibrated to temperature. Released clotrimazole was assayed by measuring the absorbance photometrically (Lambda 16; Perkin-Elmer) at 255.5 nm. Concentrations were calculated by interpolation from a standard curve. The linearity interval established in the release medium was 35–1200 μg/ml (r^2: 0.9992).

2.7. Statistical data analyses

Statistical data analyses were performed using the *t*-test with $P < 0.05$ as the minimal level of significance.

3. Results and discussion

3.1. Modification of chitosan

2-Iminothiolane is well known as reagent for the immobilization of thiol groups to primary amino groups of proteins (Schramm and Duelffer, 1977). Within this study it was used for the modification of chitosan bearing comparatively much more primary amino groups. As listed in Table 1 in dependence on the weight-ratio chitosan to 2-iminothiolane and on the pH during the coupling reaction, different amounts of thiol groups were covalently attached to the polymer. Although chitosan remained insoluble at pH 10, a derivatization of the polymer was nevertheless to a certain extent possible. On the other hand, coupling reactions carried out at pH 5, at which pH chitosan is completely dissolved, led to an even lower

amount of immobilized thiol groups. A reason for these results seems to be the limited access of primary amino groups when chitosan remains solid at pH 10 and the low reactivity of the reagent at pH 5. Consequently, coupling reactions, which were performed at pH 7, led to the highest yield in polymer immobilized thiol groups. At this pH, chitosan is almost completely dissolved in an acetate buffer providing a good access of its amino groups and on the other hand, the coupling reagent exhibits already a high reactivity.

At pH 5 and 7, coupling reactions were also carried out at different ratios of chitosan to 2-iminothiolane. Results of this study demonstrated that the more reagent is added, the higher is the amount of immobilized thiol groups. At pH 7, even linearity was given. Accordingly, the addition of more 2-iminothiolane should lead to even higher coupling rates. In order to optimize also the reaction time, the degree of modification as a function of time was analyzed. Results of this study are shown in Fig. 2, demonstrating that already after 4 h the end point of the reaction was reached. This observation is in contrast to other studies. For instance, Weber et al. (2000) showed that the reaction of proteins with 2-iminothiolane was even after 24 h not finished, although carried out at pH 8.5, where the coupling reagent is more reactive.

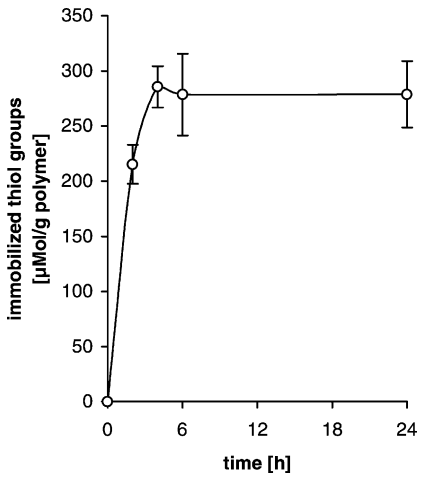

Fig. 2. Kinetic of the derivatization reaction. Synthesis was performed at a weight-ratio of chitosan to 2-iminothiolane 4 + 1 at pH 7.0 without 2-mercaptoethanol. Indicated values are means (±S.D.) of at least three experiments.

The lyophilized thiolated chitosans or so-called chitosan–TBA (=4-thio-butyl-amidine) conjugates appeared as white, odorless powder of fibrous structure. They were easily soluble in aqueous solutions at a pH below 6.0 and formed transparent gels of high viscosity. The lyophilized chitosan–TBA conjugates were stable towards air oxidation when stored at 4 °C.

3.2. Decrease in the thiol group content within the thiolated chitosans

Dependent on the pH of the thiomer solution, the thiol groups of chitosan were oxidized, thereby forming inter- as well as intramolecular disulfide bonds. These results are illustrated in Fig. 3. They demonstrate that under physiological pH-conditions of pH 4–6, which are prevalent on the surface of mucosal membranes such as the oral cavity, the gastrointestinal tract and the vagina, even after 6 h there are still at least 80% of the thiol groups available in their reactive form. The higher the pH of the solution, the more rapidly the thiol groups of the polymer were oxidized. This observation can be explained by the decreasing H^+-concentration at raising pH-values which leads in turn to a higher amount of negative thiolate anions, S^-, representing the active form for oxidation. The estimated apparent pK_a-value of the covalently attached 2-iminothiolane was calculated by the software Chem Sketch (Advanced Chemistry Development) to be 9.94 ± 0.25.

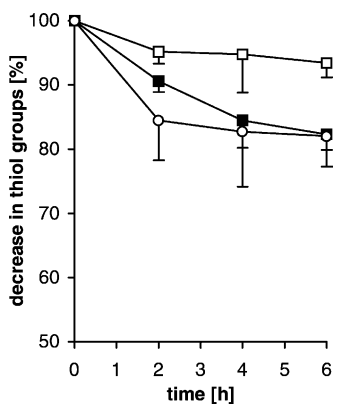

Fig. 3. Decrease in thiol groups within aqueous 1.5% chitosan–TBA conjugate 100 solutions buffered at pH 4 (□), pH 5 (■) and pH 6 (○) with 100 mM acetate buffer at 37 °C. Indicated values are means of three experiments.

3.3. Rheological studies

The oxidation of thiolated chitosans leads to the formation of inter- and intrachain disulfide bonds at physiological pH values. Because of this in situ crosslinking process, the viscoelastic properties of the thiomer will change as a function of time. The significant increase in the elastic properties of chitosan–TBA gels is evidenced by a decrease in $\tan\delta$, which describes the ratio between the elastic and the viscous modulus of the gel (Table 2). Before the formation of disulfide bonds could take place, G' shows a decline at low frequencies (Fig. 4a). This is typical for a system that is only physically entangled (Mortazavi et al., 1993). After 1 h, a significant increase in G' was already observed and after a gelation time of 2 h, G' and G'' were not influenced by the frequency of oscillation (Fig. 4b). Also $\tan\delta$ was smaller than 1 and these observations indicate the formation of a covalently cross-linked gel (Ross-Murphy, 1995).

The viscosity η' of unmodified chitosan remained constant over the whole observation period, whereas it increased 30-fold in case of the chitosan–TBA 100 and 90-fold in case of chitosan–TBA 220. The increase of G' was 1400-fold for chitosan–TBA 100 and 3200-fold for chitosan–TBA 220. It was also shown for chitosan–thioglycolic acid conjugates, with various degrees of modification (119.65 ± 3.59 μM, 208.56 ± 8.33 μM and 438.98 ± 13.22 μM thiol groups per g polymer), that the greater the amount of thiol groups immobilized on the polymer, the higher

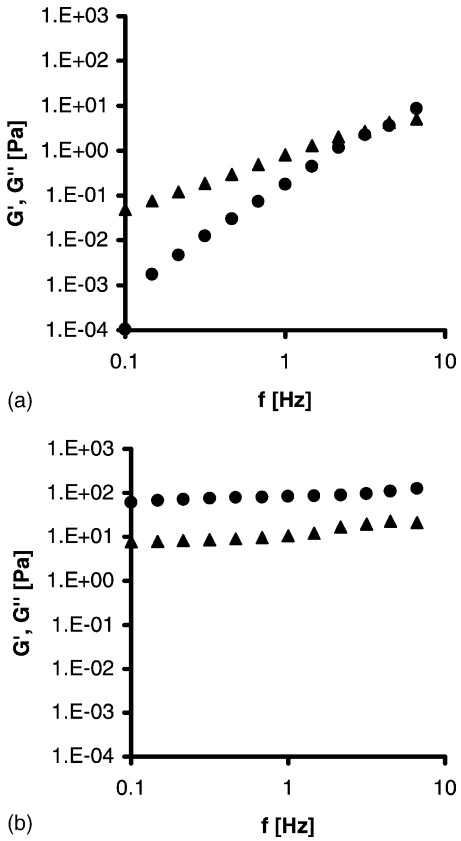

Fig. 4. Effect of the oscillatory frequency on the storage modulus G' (●) and the loss modulus G'' (▲) of a 1.5% (m/v) chitosan–TBA conjugate 100 solution at the beginning of the observation period (a) and after 2 h at pH 5.5 and 37 °C (b).

Table 2
Loss tangent ($\tan\delta$) and dynamic viscosity η' (mPas) measured at a frequency of 1 Hz of 1.5% (w/v) solutions of chitosan–TBA conjugate 100, chitosan–TBA conjugate 220 and unmodified chitosan

Polymer	Time (h)	$\tan\delta$	η' (mPas)
Chitosan–TBA conjugate 100	0	3.42 ± 0.83	91 ± 27
	1	4.70 ± 0.07	235 ± 15
	2	0.17 ± 0.04	1322 ± 320
	4	0.08 ± 0.00	1368 ± 59
	6	0.06 ± 0.03	2821 ± 242
Chitosan–TBA conjugate 220	0	8.34 ± 1.40	243 ± 20
	6	0.22 ± 0.02	21884 ± 2363
Chitosan	0	37.22 ± 9.64	145 ± 45
	6	34.80 ± 7.98	132 ± 10

Indicated values are means (±S.D.) of at least three measurements.

was the increase in viscosity (Hornof et al., 2002). According to this, chitosan–TBA conjugates of a comparatively higher coupling rate should lead to an even more pronounced increase in viscosity.

In case of vaginal delivery systems, mucoadhesive polymers used as carrier matrix offer the advantage to prolong the residence time of various drugs such as antimycotic, anti-inflammatory and disinfectant agents on the target tissue. Based on thiolated polycarbophil, a mucoadhesive vaginal delivery system for progesterone was developed by Valenta et al. (2001). In contrast to thiolated polycarbophil, where the crosslinking was investigated only at pH 7, chitosan–TBA has the advantage of forming highly elastic gels already at the physiological pH value of 5.5. As chitosan–TBA conjugates exhibit strongly

improved mucoadhesive properties as described below and the polymeric delivery system is stabilized in situ by this crosslinking process, they might be a promising new development for such applications.

Since the formation of disulfide bonds is also feasible at a pH of 6.8, chitosan–TBA conjugates might be of interest for in situ gelling nasal (Illum et al., 1994) or ocular drug delivery systems (Paulsson et al., 1999) as well.

3.4. Mucoadhesion studies

Results obtained by mucoadhesion studies demonstrated a significant improvement in the mucoadhesive properties of chitosan–TBA conjugates in comparison to unmodified chitosan and chitosan–thioglycolic acid conjugates as shown in Table 3. In comparison to chitosan–thioglycolic acid conjugates, which were tested under the same conditions, the mucoadhesive properties of chitosan modified via 2-iminothiolane were markedly improved. Tablets comprising chitosan–TBA conjugates remained on the agitated mucosa even for several days, whereas the best tablets comprising the chitosan–thioglycolic acid conjugate detached within 4 h (Kast and Bernkop-Schnürch, 2001). In comparison to anionogenic mucoadhesive polymers such as thiolated sodium carboxymethylcellulose (CMC), thiolated chitosan described here exhibits also stronger adhesive properties. Under the same test conditions, tablets consisting of thiolated CMC detached within 3 h (Bernkop-Schnürch and Steininger, 2000). The improvement in the mucoadhesive properties of chitosan by the immobilization of 4-thio-butyl-amidine ligands was determined to be at least 140-fold (see Table 3) and represents therefore the greatest so far made progress. An explanation for these strongly improved mucoadhesive properties of the chitosan–TBA conjugate can be given by the immobilization of thiol groups and by the improvement in its cationic character due to the introduction of an amidine substructure as well.

Snyder et al. (1981, 1983) could demonstrate that thiol groups with cationic neighbor groups react more rapidly with disulfide bonds having anionic substructures as neighbors. According to this observation thiol/disulfide exchange reactions between a thiomer and disulfide bonds within the mucus glycoproteins, often having neighboring asparaginic and glutamic acid substructures, will take place to a greater extent in the case of chitosan–TBA conjugate than in case of chitosan–thioglycolic acid conjugates. Moreover, according to the theory, that the mucoadhesive properties of chitosan are mainly based on ionic interactions with anionic substructures of the mucus layer such as sialic acid and sulfonic acid moieties (Hassan and Gallo, 1990), the introduction of more cationic groups to chitosan should also contribute to an improvement in its mucoadhesive properties.

Table 3
Comparison of the mucoadhesive properties of chitosan–TBA conjugates, chitosan–thioglycolic acid conjugates (chitosan–TGA) and unmodified chitosan

Polymer	Time (h)	Improvement ratio
Unmodified chitosan	1.2 ± 0.8	1
Chitosan–TGA conjugate 10[a]	1.05 ± 0.05	0.88
Chitosan–TGA conjugate 30[a]	4.0 ± 0.1	5
Chitosan–TBA conjugate 60	148 ± 25	123
Chitosan–TBA conjugate 100	>168	>140

Test discs of each polymer were attached to excised porcine mucosa, which has been spanned on a cylinder and agitated with 125 rpm in a 100 mM phosphate buffer pH 6.0 at 37 ± 0.5 °C. The indicated time of adhesion represents the mean (±S.D.) of at least three experiments. The improvement ratio is calculated by adhesion time of conjugates versus adhesion time of control.

[a] According to Kast and Bernkop-Schnürch (2001); chitosan–TGA 10 and 30 display 9.9 and 27.4 μM thiol groups per g polymer, respectively.

3.5. Drug release studies

Because of its high mucoadhesive properties and the strong in situ gelling properties, thiolated chitosan might be a promising carrier matrix for vaginal drug delivery systems. As model drug clotrimazole was therefore chosen. It is well established as antimycotic drug in treatment of vaginal infections. In order to improve its therapeutic efficacy sustained release of the drug over a period of several days might be highly beneficial. Hence, within this study, a proof of principle concerning a sustained release of the drug out of chitosan–TBA conjugates should be provided. As shown in Fig. 5, a controlled release of the drug is guaranteed. In this early phase of development, however, an optimization of the drug release does not seem to be necessary.

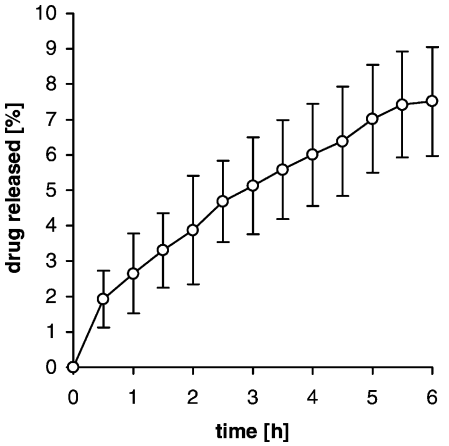

Fig. 5. Release profile of clotrimazole from tablets comprising chitosan–TBA conjugate 60. Dissolution studies were performed in 100 mM acetate buffer pH 6.0/dioxane (7 + 3) at 37 °C. Indicated values are means (±S.D.) of at least three experiments.

3.6. Presumptive additional features of thiolated chitosan

Apart from the in situ gelling and mucoadhesive properties, chitosan–TBA conjugates might exhibit also further features, which might be highly advantageous in drug delivery. Because of the capability of thiomers to bind divalent metal ions such as zinc ions, these polymers are capable of inhibiting zinc-dependent proteases such as carboxypeptidases A and B as well as most membrane-bound peptidases (Bernkop-Schnürch and Thaler, 2000; Bernkop-Schnürch et al., 2001). Accordingly, also chitosan–TBA conjugates should display such features. They would render the novel polymer useful in peptide drug delivery (Bernkop-Schnürch, 2000).

Moreover, thiolated polymers were shown to display a strong permeation enhancing effect for the paracellular up-take of drugs (e.g. Bernkop-Schnürch et al., 1999; Clausen and Bernkop-Schnürch, 2000; Clausen et al., 2002). Chitosan exhibits per se a permeation enhancing effect attributed to its positive charges (Schipper et al., 1997). As the concentration of positive charges is raised by the modification with 2-iminothiolane and because of the immobilized thiol groups, chitosan–TBA conjugates should show strong permeation enhancing features as well. According to this, chitosan–TBA conjugates might be helpful in order to improve the uptake of poorly absorbable drugs.

4. Conclusions

The modification of chitosan with 2-iminothiolane leads to polymers exhibiting excellent in situ gelling properties and strongly improved mucoadhesive properties. In addition, a controlled drug release can be guaranteed out of thiolated chitosan. Because of these features polymer conjugates described herein represent a useful polymeric carrier matrix for delivery systems, which should provide a prolonged residence time of the drug on the mucosa.

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References

Baumann, H., Faust, V., 2001. Concepts for improved regioselective placement of *O*-sulfo, *N*-sulfo, *N*-acetyl and *N*-carboxymethyl groups in chitosan derivatives. Carbohydrate Res. 331, 43–57.

Bernkop-Schnürch, A., 2000. Chitosan and its derivatives: potential excipients for peroral peptide delivery systems. Int. J. Pharm. 194, 1–13.

Bernkop-Schnürch, A., Hopf, T.E., 2001. Synthesis and in vitro evaluation of chitosan–thioglycolic acid conjugates. Sci. Pharm. 69, 109–118.

Bernkop-Schnürch, A., Scerbe-Saiko, A., 1998. Synthesis and in vitro evaluation of chitosan–EDTA-protease-inhibitor conjugates which might be useful in oral delivery of peptides and proteins. Pharm. Res. 15, 263–269.

Bernkop-Schnürch, A., Steininger, S., 2000. Synthesis and characterisation of mucoadhesive thiolated polymers. Int. J. Pharm. 194, 239–247.

Bernkop-Schnürch, A., Thaler, S., 2000. Polycarbophil–cysteine conjugates as platforms for oral (poly)peptide delivery systems. J. Pharm. Sci. 89, 901–909.

Bernkop-Schnürch, A., Krauland, A., Valenta, C., 1998. Development and in vitro evaluation of a drug delivery system based on chitosan–EDTA BBI conjugate. J. Drug Targ. 6, 207–214.

Bernkop-Schnürch, A., Brandt, U.M., Clausen, A.E., 1999. Synthesis and in vitro evaluation of chitosan–cysteine conjugates. Sci. Pharm. 67, 196–208.

Bernkop-Schnürch, A., Walker, G., Zarti, H., 2001. Thiolation of polycarbophil enhances its inhibition of intestinal brush border

membrane bound aminopeptidase. N.J. Pharm. Sci. 90, 1907–1914.

Clausen, A.E., Bernkop-Schnürch, A., 2000. In vitro evaluation of the permeation-enhancing effect of thiolated polycarbophil. J. Pharm. Sci. 89, 1253–1261.

Clausen, A.E., Kast, C.E., Bernkop-Schnürch, A., 2002. The role of glutathione in the permeation enhancing effect of thiolated polymers. Pharm. Res. 19, 602–608.

Felt, O., Buri, P., Gurny, R., 1998. Chitosan: a unique polysaccharide for drug delivery. Drug Dev. Ind. Pharm. 24, 979–993.

Hassan, E.E., Gallo, J.M., 1990. A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. Pharm. Res. 7, 491–495.

Hornof, M.D., Kast, C.E., Bernkop-Schnürch, A., 2002. In vitro evaluation of the viscoelastic behavior of chitosan–thioglycolic acid conjugates. Eur. J. Pharm. Biopharm. 55, 185–190.

Illum, L., 1998. Chitosan and its use as a pharmaceutical excipient. Pharm. Res. 15, 1326–1331.

Illum, L., Farraj, N.F., Davis, S.S., 1994. Chitosan as a novel nasal delivery system for peptide drugs. Pharm. Res. 11, 1186–1189.

Kast, C.E., Bernkop-Schnürch, A., 2001. Thiolated polymers—thiomers: development and in vitro evaluation of chitosan–thioglycolic acid conjugates. Biomaterials 22, 2345–2352.

Lehr, C.-M., Bouwstra, J.A., Schacht, E.H., Junginger, H.E., 1992. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int. J. Pharm. 78, 43–48.

Mortazavi, S.A., Carpenter, B.G., Smart, J.D., 1993. A comparative study on the role played by mucus glycoproteins in the rheological behaviour of the mucoadhesive/mucosal interface. Int. J. Pharm. 94, 195–201.

Paulsson, M., Hägerström, H., Edsman, K., 1999. Rheological studies of the gelation of deacetylated gellan gum (Gelrite) in physiological conditions. Eur. J. Pharm. Sci. 9, 99–105.

Ross-Murphy, S.B., 1995. Structure–property relationships in food biopolymer gels and solutions. J. Rheol. 39, 1451–1463.

Schipper, N.G.M., Olsson, S., Hoogstraate, J.A., deBoer, A.G., Varum, K.M., Artursson, P., 1997. Chitosans as absorption enhancers for poorly absorbable drugs. 2: Mechanism of absorption enhancement. Pharm. Res. 14, 923–929.

Schramm, H.J., Duelffer, T., 1977. Synthesis and application of cleavable and hydrophilic crosslinking reagents. Adv. Exp. Med. Biol. 86A, 197–206.

Snyder, G.H., Cennerazzo, M.J., Karalis, A.J., Field, D., 1981. Electrostatic influence of local cysteine environments on disulfide exchange kinetics. Biochemistry 20, 6509–6519.

Snyder, G.H., Reddy, M.K., Cennerazzo, M.J., Field, D., 1983. Use of local electrostatic environments of cysteines to enhance formation of a desired species in a reversible disulfide exchange reaction. Biochim. Biophys. Acta 749, 219–226.

Thanou, M., Florea, B.I., Langemeyer, M.W., Verhoef, J.C., Junginger, H.E., 2000. *N*-Trimethylated chitosan chloride (TMC) improves the intestinal permeation of the peptide drug buserelin in vitro (Caco-2 cells) and in vivo (rats). Pharm. Res. 17, 27–31.

Thanou, M., Nihot, M.T., Jansen, M., Verhoef, J.C., Junginger, H.E., 2001. Mono-*N*-carboxymethyl chitosan (MCC), a polyampholytic chitosan derivative, enhances the intestinal absorption of low molecular weight heparin across intestinal epithelia in vitro and in vivo. J. Pharm. Sci. 90, 38–46.

Valenta, C., Kast, C.E., Harich, I., Bernkop-Schnürch, A., 2001. Development and in vitro evaluation of a mucoadhesive vaginal delivery system for progesterone. J. Control Rel. 77, 323–332.

Weber, C., Reiss, S., Langer, K., 2000. Preparation of surface modified protein nanoparticles by introduction of sulfhydryl groups. Int. J. Pharm. 211, 67–78.