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Thiolated polymers: synthesis and in vitro evaluation of polymer–cysteamine conjugates

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Abstract

The purpose of the present study was to synthesize and characterize novel thiolated polymers. Mediated by a carbodiimide cysteamine was covalently linked to sodium carboxymethylcellulose (CMC) and polycarbophil (PCP). The resulting CMC–cysteamine conjugates displayed 77.9 ± 6.7 and 365.1 ± 8.7 μmol thiol groups per gram of polymer, whereas the PCP–cysteamine conjugates showed 26.3 ± 1.9 and 122.7 ± 3.8 μmol thiol groups per gram of polymer (mean $\pm$ S.D.; $n = 3$). In aqueous solutions above pH 5.0 both modified polymers were capable of forming inter- and/or intra-molecular disulfide bonds. The reaction velocity of this oxidation process was accelerated with a decrease in the proton concentration. The oxidation proceeded more rapidly within thiolated CMC than within thiolated PCP. Permeation studies carried out in Ussing-type chambers with freshly excised intestinal mucosa from guinea pigs utilizing sodium fluorescein as model drug for the paracellular uptake revealed an enhancement ratio ($R = P_{app}$ (conjugate)/P_{app} (control)) of 1.15 and 1.41 (mean $\pm$ S.D.; $n = 3$) for the higher thiolated CMC–cysteamine (0.5%; m/v) and PCP–cysteamine conjugate (1.0%; m/v), respectively. The decrease in the transepithelial electrical resistance values was in good correlation with the enhancement ratios. Due to a high crosslinking tendency by the formation of disulfide bonds stabilizing drug carrier systems based on thiolated polymers and a permeation enhancing effect, CMC– and PCP–cysteamine conjugates represent promising excipients for the development of novel drug delivery systems. © 2001 Published by Elsevier Science B.V.

Keywords: Carboxymethylcellulose–cysteamine conjugate; Crosslinking; Permeation enhancement; Polycarbophil–cysteamine conjugate; Thiomers

1. Introduction

Within the last years thiolated polymers—or so-called thiomers—have proven to be a promising new class of polymeric excipients in drug delivery. In contrast to traditionally used mucoadhesive polymers, which adhere to the mucus by non-covalent bonds, such as hydrogen bonds and

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ionic interactions (Peppas and Mikos, 1990), thiomers are capable of forming covalent bonds leading to *improved mucoadhesive properties*. The underlying mechanism is based on thiol/disulfide exchange reactions and on an oxidation process between the reactive thiol groups of the mucoadhesive polymer and cysteine-rich subdomains of the mucin glycoproteins (Gum et al., 1992). The formed disulfide bonds are representatives of the bridging structure most commonly encountered in biological systems, providing covalent adhesion of thiomers to the mucus layer. For example, polycarbophil (PCP)–cysteine conjugates and chitosan–thioglycolic acid conjugates display on freshly excised intestinal mucosa greater than two- and four-times higher mucoadhesiveness compared to the corresponding unmodified polymers, respectively (Bernkop-Schnürch et al., 1999a; Kast and Bernkop-Schnürch, 2001). Additionally, thiolated polymers show *improved cohesive properties*. A simple pH-dependent oxidation process results in an inter- and/or intra-chain disulfide bond formation within the polymeric network. This crosslinking takes place during the swelling process in aqueous media leading to a substantial stabilization of e.g. matrix tablets comprising a thiomer (Bernkop-Schnürch et al., 2000; Clausen and Bernkop-Schnürch, 2001a). Furthermore, thiolated polymers display *enzyme inhibitory capabilities*, which render them useful in non-invasive peptide delivery. This is believed to be due to polymer conjugates complexing the divalent metal ions, such as zinc ions from the enzyme structure. Metalloproteases, such as aminopeptidase N often responsible for the degradation of therapeutic peptides on mucosal membranes, can be inhibited by thiomers (Bernkop-Schnürch and Thaler, 2000). Recently, a *permeation enhancing effect* was demonstrated for various thiolated polymers, such as PCP–cysteine conjugates and carboxymethylcellulose (CMC)–cysteine conjugates (Clausen and Bernkop-Schnürch, 2000, 2001b). The efficacy of thiomers has meanwhile also been verified in vivo. A significant reduction in the glucose level of diabetic mice was achieved by the oral administration of insulin microtablets based on a PCP–cysteine conjugate (Marschütz et al., 2000).

In order to expand the knowledge of thiolated polymers, it was the aim of this study to generate new types of thiomers. The sulfydryl compound cysteamine was chosen as ligand for CMC and PCP, as it can be easily attached to these anionic mucoadhesive polymers displaying numerous carboxylic acid groups. In contrast to the previously used ligand cysteine, cysteamine does not exhibit a carboxylic acid group, which should exclude undesired side reactions, such as the formation of Cys–Cys amide bonds or the formation of ester bonds with the hydroxyl groups of CMC (Bernkop-Schnürch and Steininger, 2000). The synthesis of CMC–cysteamine conjugates and PCP–cysteamine conjugates followed by the in vitro characterization provides valuable information regarding structure/function relations. Obtained results should help to find more connections between different types of thiomers representing a good basis for optimizing their features.

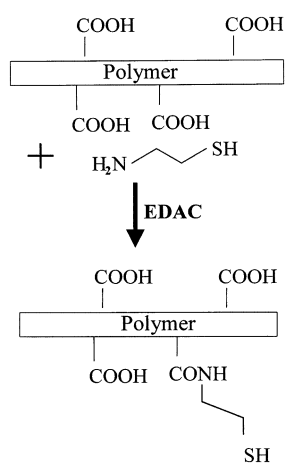

Fig. 1. Synthesis scheme for the generation of polymer–cysteamine conjugates; polymer – NaCMC or PCP; EDAC – 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride.

Table 1
Synthesis of polymer–cysteamine conjugates; carboxylic acid moieties of CMC and PCP were activated for 30 min with EDAC in aqueous solutions; after the addition of cysteamine, reaction mixtures were incubated at pH 4–5 and room temperature for 3 h

Polymer–cysteamine conjugate	Polymer/demineralized water (g/ml)	EDAC (final conc., mM)	Added cysteamine HCl (g)	Thiol groups (μmol/g polymer); mean ± S.D., $n = 3$
CMC–cysteamine conjugate 78	2/200	50	0.25	77.9 ± 6.7
CMC–cysteamine conjugate 365	2/200	50	0.5	365.1 ± 8.7
CMC–cysteamine control	2/200	–	0.25–0.5	0
PCP–cysteamine conjugate 26	1/200	50	0.083	26.3 ± 1.9
PCP–cysteamine conjugate 123	1/200	50	0.125	122.7 ± 3.8
PCP–cysteamine control	1/200	–	0.083–0.125	0

2. Materials and methods

2.1. Synthesis of polymer–cysteamine conjugates

As shown in Fig. 1, the covalent attachment of cysteamine to sodium carboxymethylcellulose (NaCMC) and PCP, respectively, was achieved by the formation of amide bonds between the primary amino group of the sulfydryl compound and a carboxylic acid group of the polymer. Two grams of NaCMC (average molecular weight 1000 kDa; Kwizda, Vienna, Austria) and 1 g of sodium hydroxide neutralized PCP (molecular weight > 700 kDa; Noveon AA1, BF Goodrich, Brecksville, OH) were each hydrated in 200 ml of demineralized water. The carboxylic acid moieties of the polymers were activated for 30 min by the addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC; Sigma, St. Louis, MO) in a final concentration of 50 mM. Cysteamine hydrochloride (Sigma) was added in amounts as listed in Table 1 and the pH was adjusted within the range of 4–5. Reaction mixtures were incubated for 3 h under permanent stirring at room temperature. The resulting polymer–cysteamine conjugates were isolated in the dark by dialyzing at 10 °C against 1 mM HCl, two times against the same medium but containing 1% NaCl and then exhaustively against 1 mM HCl. Control samples were prepared and isolated in exactly the same way as polymer–cysteamine conjugates but EDAC was omitted during the coupling reaction. All samples were lyophilized by drying frozen aqueous polymer solutions at − 30 °C and 0.01 mbar (Christ Beta 1-8K; Osterode am Harz, Germany). Polymer–cysteamine conjugates and controls were stored at 4 °C until further use.

2.2. Determination of the thiol group content

The concentration of thiol groups on the polymer–cysteamine conjugates was determined by iodometric titration (1.00 mM iodine; indicator: starch) at pH 3.0, as described previously (Bernkop-Schnürch and Steininger, 2000).

2.3. Determination of remaining primary amino groups

The quantification of remaining primary amino groups on the polymer–cysteamine conjugates and controls were achieved by utilizing 2,4,6-trinitrobenzenesulfonic acid (TNBS; Sigma) reagent. Samples (5.0 mg) of each conjugate and control were hydrated in 690 μl demineralized water and incubated with 300 μl of 4% $NaHCO_3$ and 10 μl of 5% TNBS at 37 °C for 2 h. The absorbance was measured at 450 nm (Lambda 16; Perkin–Elmer,

Vienna, Austria). The amount of unconjugated primary amino groups was calculated using standard curves obtained by the amino-group determination of a series of corresponding polymer solutions with increasing amounts of cysteamine.

2.4. Disulfide bond formation within the polymer conjugates

Polymer–cysteamine conjugates 1.0% (m/v) displaying different amounts of covalently attached thiol groups were hydrated in either 100 mM acetate buffer pH 5.0, 100 mM phosphate buffer pH 6.0, or 100 mM phosphate buffer pH 7.0, and incubated at 37 °C under vigorous shaking. In order to reduce the viscosity of PCP–cysteamine conjugates, all buffer solutions contained 0.9% NaCl. At predetermined time points, 500-μl samples were withdrawn and transferred to 50 μl of 1 M HCl, in order to quench any further oxidation. The amount of remaining thiol groups was determined by titration as described above.

2.5. Sulfydryl-binding studies

Sulfydryl-binding studies were carried out by the same analytical method as used for other thiomers in order to achieve comparable results (Bernkop-Schnürch and Thaler, 2000). Preliminary investigations, however, revealed that the CMC–cysteamine conjugates cannot be separated from unbound cysteine by centrifugation, as no pellet of the polymer is formed. Moreover, other techniques, such as dialysis or precipitation did not lead to reproducible results. Hence, sulfydryl-binding studies were exclusively performed with a PCP–cysteamine conjugate. Briefly, 5 mg of the PCP–cysteamine conjugate 123 (Table 1) and the unmodified polymer were each hydrated in 1 ml of 0.9% NaCl. After the addition of 1 mg of L-cysteine hydrochloride monohydrate to each sample, the pH was adjusted to either 5.0, 6.0, 7.0, or 8.0 with 1 M NaOH. Reaction mixtures were incubated for predetermined time periods at 37 °C while shaking. After the addition of 10 mg of NaCl, samples were centrifuged for 5 min at $17\,000 \times g$ and the supernatants containing unbound cysteine discarded. The remaining pellets were diluted 1:8 with 2.9% NaCl, centrifuged (5 min; $17\,000 \times g$) and the supernatant removed. This purification step was repeated four times followed by the addition of saline (0.9%) in order to obtain a final volume of 690 μl. After the addition of 300 μl of 4% $NaHCO_3$ and 10 μl of 5% TNBS samples were incubated at 37 °C for 2 h and absorbance was measured at 450 nm (Lambda 16; Perkin–Elmer). The amount of polymer attached cysteine was calculated from a standard curve, which was obtained from samples containing unmodified PCP and increasing amounts of cysteine.

2.6. Permeation studies

Permeation studies with sodium fluorescein were carried out in Ussing-type chambers displaying a permeation area of 0.64 cm^2. Small intestinal mucosa from the upper part of the ileum of guinea pig was excised immediately after sacrifice. Without stripping off the underlying muscle layer permeation studies were started within 10 min of sacrifice. All experiments were preformed at least in triplicate in an atmosphere of 95% O_2 and 5% CO_2 at 37 °C. The donor and acceptor compartments of the Ussing-type chamber were both filled with 1 ml of the incubation medium containing 250 mM NaCl, 2.6 mM $MgSO_4$, 10.0 mM KCl, 40.0 mM glucose and 50 mM $NaHCO_3$, buffered with 40 mM *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethane-sulfonic acid) (HEPES; Sigma) pH 7.0. After 15–20 min of preincubation, the solution in the donor chamber was replaced by the same medium but containing 0.001% (m/v) sodium fluorescein dispersed in 1.0% (m/v) NaCMC, 1.0% (m/v) CMC–cysteamine conjugate 365, 0.5% (m/v) NaPCP, or 0.5% (m/v) PCP–cysteamine conjugate 123, respectively. Samples of 200 μl were withdrawn from the acceptor chamber every 30 min over a 3-h incubation period. Samples were immediately replaced by the same medium at 37 °C. The permeation of fluorescein was determined using a fluorimeter (SLT; Spectra Fluor, Tecan, Austria). Cumulative corrections were made for the previously withdrawn samples in determining the total amount permeated. After all experiments, the viability of the tissue was verified with trypan blue dye as described previously (Uchiyama et al., 1998).

2.7. Measurement of the transepithelial electrical resistance

The influence of the applied polymers on the transepithelial electrical resistance (TEER) of the intestinal mucosa was monitored by a Millicell® ERS meter (Millipore Corp., Bedford, MA) connected to a pair of side by side electrodes. Measurements were performed every 5 min before applying the polymer and then every 30 min over a 3-h incubation period.

2.8. Data analysis

All statistical data analyses were performed using the Student's t-test with $P < 0.05$ as the minimal level of significance unless indicated otherwise.

The apparent permeability coefficients (P_{app}) for sodium fluorescein were calculated according to the following equation:

$$P_{app} = Q/Act,$$

where P_{app} is the apparent permeability coefficient (cm/s), Q is the total amount permeated throughout the incubation time (μg), A is the diffusion area of the Ussing-type chamber (cm^2), c is the initial concentration of the marker in the donor compartment ($\mu g/cm^3$), and t is the total time of the experiment (s).

Transport enhancement ratios (R) were calculated from P_{app} values by

$$R = P_{app}\,(\text{conjugate})/P_{app}\,(\text{control}).$$

3. Results and discussion

3.1. Chemical characterization of polymer–cysteamine conjugates

The degree of modification of CMC and PCP was quantified by iodometric titration. Polymers being prepared and purified in exactly the same way as the conjugates, but omitting EDAC during the synthesis reaction, exhibited no reducing properties, whereas CMC–cysteamine as well as PCP–cysteamine conjugates displayed numerous reducing thiol groups. Analyses of polymer–cysteamine conjugates and controls for primary amino groups showed that the unconjugated cysteamine was completely removed after the purification. As both polymer–cysteamine conjugates display numerous thiol moieties and no primary amino groups, strong evidence for the formation of amide bonds between the polymers and cysteamine is provided. Both polymer–cysteamine conjugates remained stable after lyophilization as well as in aqueous solutions of pH 5.0 and lower. The freeze-dried thiomers had the appearance of a white odorless powder. The amount of covalently attached thiol groups on CMC and PCP is shown in Table 1. Although PCP displays at least 2.6-fold more carboxylic acid groups per gram polymer than CMC, which are essential for the covalent attachment of cysteamine (Fig. 1), the thiol group content of the PCP–cystamine conjugate 123 was only 1.6-fold higher than that of the CMC–cysteamine conjugate 78, prepared under comparable reaction conditions.

This is in contrast to previous studies focusing on the synthesis of polymer–cysteine conjugates. The coupling reaction was thereby also mediated by a carbodiimide using cysteine without a carboxyl protective group, which resulted in a 10-fold higher amount of cysteine attached to CMC compared to PCP (Bernkop-Schnürch et al., 2000). A reason for this observation might be the additional formation of ester bonds between the hydroxyl groups of CMC and the carboxylic acid group of cysteine which can be excluded in the case of cysteamine. Because of the formation of ester bonds the pH raised continually during the coupling reaction of CMC–cysteine conjugates (from 3–4 up to pH 6–7), whereas it remained constant at the coupling reaction of CMC–cysteamine conjugates. As a result of this side reaction, CMC–cysteine conjugates showed also primary amino groups, which reduce the mucoadhesive properties of such conjugates as it is well established that anionic and cationic moieties on the same polymer compensate their adhesive features (Bernkop-Schnürch and Krajicek, 1998). In contrast, CMC–cysteamine conjugates do not exhibit this drawback which renders them to be a promising new development in this research field.

3.2. Formation of disulfide bonds

Matrix tablet and microparticle delivery systems containing thiolated hydrophilic polymers have been shown to be stabilized under physiological conditions by the crosslinking via the formation of disulfide bonds (Bernkop-Schnürch et al., 2000). Depending on the pH and on the type of thiomer, the thiol groups are oxidized forming inter- and intra-molecular disulfide bonds. Thereby, the molecular weight of the polymer increases which can be monitored by a time-dependent increase in viscosity (Bernkop-Schnürch et al., 1999a). At pH 5.0, the thiol groups of CMC–cysteamine conjugates and PCP–cysteamine conjugates remained stable towards oxidation, whereas at pH 6.0 and 7.0 significant formation of disulfide bridges was observed. The results of this study are shown in Figs. 2–4. The formation of disulfide bonds above a pH 5.0 can be explained by the pK_a values of the CMC– and PCP–cysteamine conjugate calculated by the ACD-Software (Toronto, Canada) to be 9.7. Raising the pH of the medium from 5.0 to 6.0 and 7.0 lead to a 10- and 100-fold higher concentration in negative thiolate anions, $-S^-$, respectively, representing the reactive form in oxidation (Snyder, 1987). Comparing the formation of disulfide bonds between the PCP–cysteamine conjugate 123 (Fig. 4) and the CMC–cysteamine conjugate 78 (Fig. 2) revealed at pH 6.0 a more rapid oxidation of the latter one. Greater disulfide bond formation for CMC–cysteamine conjugates may be due to the relatively lower density of carboxylic acid groups on the CMC–cysteamine conjugate, leading to a lower repulsive effect between the polymer chains and in turn allowing greater interaction between thiol groups to form disulfide bonds. Comparing the formation of disulfide bonds between the two PCP–cysteamine

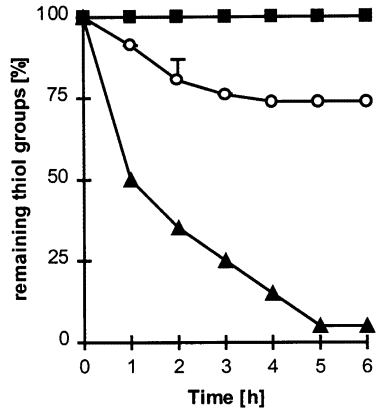

Fig. 3. Decrease in thiol groups of 1.0% (m/v) PCP–cysteamine conjugate 26 at pH 5.0 (■), pH 6.0 (○), and pH 7.0 (▲). Indicated values are mean ± S.D. of at least three experiments.

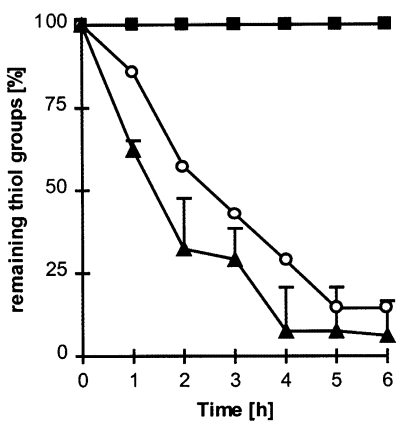

Fig. 2. Decrease in thiol groups of 1.0% (m/v) NaCMC–cysteamine conjugate 78 at pH 5.0 (■), pH 6.0 (○), and pH 7.0 (▲). Indicated values are mean ± S.D. of at least three experiments.

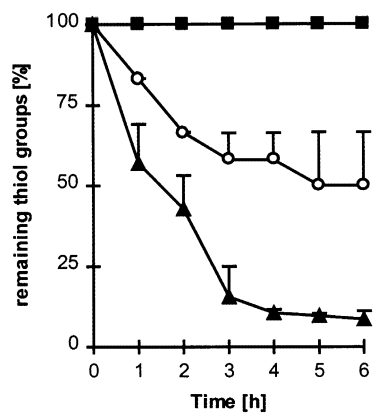

Fig. 4. Decrease in thiol groups of 1.0% (m/v) PCP–cysteamine conjugate 123 at pH 5.0 (■), pH 6.0 (○), and pH 7.0 (▲). Indicated values are mean ± S.D. of at least three experiments.

conjugates displaying different amounts of covalently attached thiol groups (Figs. 3 and 4), revealed a faster oxidation of the conjugate with the higher covalent attachment of cysteamine. Previous studies carried out with CMC– and PCP–cysteine conjugates demonstrated a more rapid oxidation at higher polymer conjugate concentrations (Bernkop-Schnürch et al., 2000). A high thiol concentration either gained by a high amount of covalently attached sulfydryl groups or by a high thiomer concentration seems to lead to the same effect. A reason for this observation might be the fact that at higher thiol concentrations comparatively more thiol groups come close to each other thereby forming disulfide bonds more rapidly. According to these results, the formation of disulfide bonds within particulate delivery systems or matrix tablets comprising high concentrations of a thiomer with many thiol groups should take place quite rapidly during the swelling process in aqueous media and should contribute to their stability as well as viscoelasticity. For example, matrix tablets based on PCP disintegrate within 2 h, whereas tablets comprising a PCP–cysteine conjugate remain stable for days (Bernkop-Schnürch et al., 2000). A similar effect can be expected for the conjugates described here. They might, therefore, be promising excipients as mucoadhesive drug carrier matrices which should exhibit high stability, such as sustained release buccal delivery systems (Junginger et al., 1999) or orally given microspheres (Delgado et al., 1999).

3.3. Cysteine-binding studies

The improved mucoadhesion of thiolated polymers is based on formation of covalent bonds to cysteine-rich subdomains of mucus glycoproteins (Bernkop-Schnürch et al., 1999a). The capability of thiomers to bind cysteine is, therefore, a key parameter for their mucoadhesive properties. In order to investigate this feature, sulfydryl-binding studies were carried out with the PCP–cysteamine conjugate 123 at pH 5.0, 6.0, 7.0 and 8.0 covering the physiological relevant pH range. Fig. 5 shows, that the binding of cysteine to the thiolated polymer is dependent upon length of incubation with

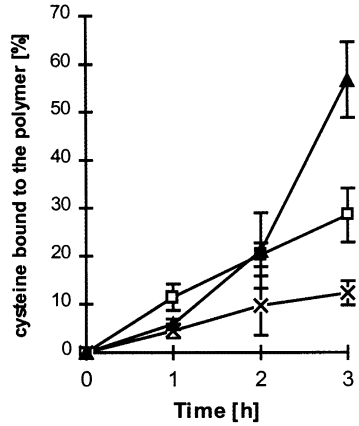

Fig. 5. Cysteine-binding studies; 0.5% (m/v) of the PCP–cysteamine conjugate 123 and 0.1% (m/v) of L-cysteine were incubated at pH 6.0 (×), pH 7.0 (□), and pH 8.0 (▲) at 37 °C; indicated values are given in percent of the theoretical maximum of cysteine which can be bound to the polymer (mean ± S.D.; $n = 3$).

the sulfydryl compound and the pH of the media. At pH 8.0, cysteine is bound to more than 50% of all sulfydryl groups of the thiolated polymer within 3 h. While at pH below 8, the amount of covalently bound cysteine was significantly lower. The more the pH decreases, the less of cysteine is bound to the thiolated polymer. The effect was monitored till pH 5.0 where cysteine was not bound at all to the polymer. This pH dependence of the binding process can be explained by the concentration of negative thiolate anions, $-S^-$, which increases at higher pH being essential for the formation of disulfide bonds.

Regarding the pH on various mucus layers, such as on the gastrointestinal, buccal, and vaginal mucosa, it is generally more in the acidic range, where the binding capability of the polymer–cysteamine conjugates is poor. Anionic polymers, however, are known to display a high buffer capacity. Previous studies, for instance, demonstrated that carbomer can buffer even a simulated gastric fluid over several hours (Bernkop-Schnürch and Gilge, 2000). According to this, a relatively higher pH can be provided on the interface between the thiolated polymer and the mucus layer, which should favor the formation of disulfide bonds in this area.

Comparing the cysteine-binding capability of the PCP–cysteamine conjugate with that of PCP–cysteine (Bernkop-Schnürch and Thaler, 2000) revealed an almost twice as high binding efficacy of the latter one. A reason for this observation might be the fact that conjugated cysteamine in contrast to conjugated cysteine does not display a carboxylic acid group being located in the immediate neighborhood to the sulfydryl moiety. As opposite charges being closely located to thiol groups promote the formation of disulfide bonds (Snyder et al., 1981, 1983), unconjugated free cysteine displaying a cationic substructure seems to be less rapidly bound to PCP–cysteamine lacking a negatively charged group close to its thiol moiety.

3.4. Effect of the polymers on sodium fluorescein permeation

The influence of both polymer–cysteamine conjugates on the cumulative transport of sodium fluorescein—widely used as marker for the paracellular way of absorption (Sakai et al., 1997)—was plotted as cumulative transport over a time period of 180 min. Results of this study as shown

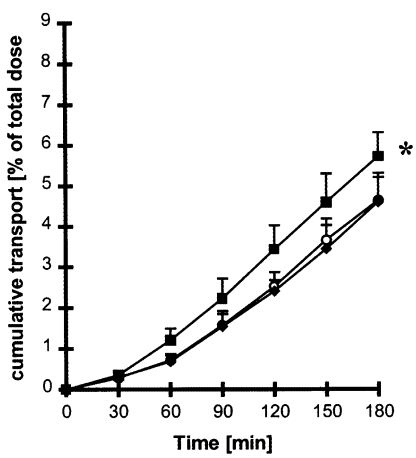

Fig. 6. Permeation enhancing effect of 1.0% (m/v) CMC–cysteamine conjugate 365 (■) in comparison to 1.0% (m/v) unmodified CMC (○), and without any polymer (◆). The transport of sodium fluorescein across small intestinal mucosa from guinea pigs was evaluated at 37 °C in Ussing-type chambers. Indicated values are mean ± S.D. of at least three experiments. (*) Differs from control without polymer, $P < 0.1$.

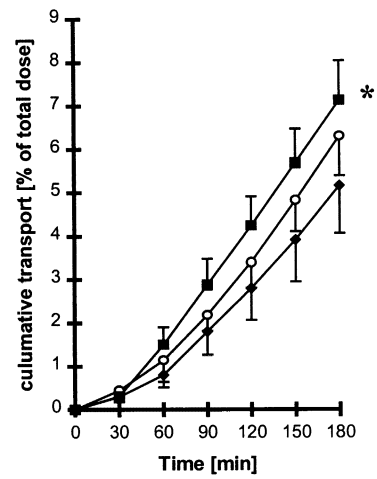

Fig. 7. Permeation enhancing effect of 0.5% (m/v) PCP–cysteamine conjugate 123 (■) in comparison to 0.5% (m/v) unmodified PCP (○), and without any polymer (◆). The transport of sodium fluorescein across small intestinal mucosa from guinea pigs was evaluated at 37 °C in Ussing-type chambers. Indicated values are mean ± S.D. of at least three experiments. (*) Differs from control without polymer, $P < 0.1$.

in Figs. 6 and 7 demonstrated an increased transport of sodium fluorescein across the intestinal mucosa due to the addition of the CMC–cysteamine conjugate or the PCP–cysteamine conjugate. The permeation enhancing effect of an unmodified poly(acrylate) as shown in Fig. 7 for PCP, has also been reported previously by Borchard et al. (1996) and Luessen et al. (1996). The transport enhancement ratios of all tested polymers are listed in Table 2. These results are in good correlation with permeation studies carried out with CMC– and PCP–cysteine conjugates also displaying a permeation enhancing effect (Clausen and Bernkop-Schnürch, 2000, 2001b). In the case of polymer–cysteamine conjugates, the lacking carboxylic acid group in the immediate neighborhood to the thiol moiety seems, therefore, to have no crucial influence on this effect. Further evidence for this theory is given by the permeation enhancing effect of chitosan–cysteine conjugates, which do not exhibit a negatively charged group being closely located to the thiol group as well (Bernkop-Schnürch et al., 1999b). According to these considerations, the permeation

Table 2
Comparison of the apparent permeability coefficient (P_{app}) of 1.0% (m/v) CMC–cysteamine conjugate and 0.5% (m/v) PCP–cysteamine conjugate for sodium fluorescein across the intestinal mucosa of guinea pigs

Polymer	Apparent permeability coefficient ($P_{app} \times 10^{-6}$ (cm/s)), mean ± S.D., $n = 3$	Enhancement ratio (P_{app} (conjugate)/P_{app} (control))
Without polymer	7.37 ± 0.61	1
NaCMC	6.73 ± 0.85	0.91
CMC–cystamine conj. 365	8.50 ± 0.42	1.15
NaPCP	9.39 ± 1.23	1.28
PCP–cystamine conj. 123	10.34 ± 1.32	1.41

enhancing effect of thiolated polymers is primarily based on the thiol groups irrespective of the surrounding functional groups. This knowledge should contribute to reveal the underlying mechanism, which might be based on the inhibition of the thiol-dependent protein tyrosine phosphatase being involved in the closing process of the tight junctions (Collares-Buzato et al., 1998; Staddon et al., 1995). Following this theory, the permeation enhancing effect of thiolated polymers must be based on an opening of the tight junctions. TEER measurements demonstrated that both thiomers cause a significant decrease of the resistance compared to the control without any polymer. After an incubation period of 180 min with 1.0% CMC–cysteamine conjugate 365 or 0.5% PCP–cysteamine conjugate 123, the TEER of the small intestinal mucosa decreased by 15.2 ± 5.6% and 20.8 ± 2.0% (mean ± S.D.; $n = 3$), respectively. This significant decrease in TEER caused by the addition of the thiomers is in agreement with the results obtained with other thiolated polymers (Clausen and Bernkop-Schnürch, 2000, 2001b).

In summary, the features of CMC– and PCP–cysteamine conjugates are in good agreement with so far generated thiomers supporting their unique properties in the case of improved cohesive properties and permeation enhancement. In particular, the CMC–cysteamine conjugates seem to represent a promising new type of thiomer as they do not exhibit disturbing covalently attached primary amino groups, such as it is the case for CMC–cysteine conjugates. CMC– and PCP–cysteamine conjugates might be helpful for drug delivery systems for which a high in situ crosslinking tendency in order to stabilize the polymeric carrier matrix is essential and/or a permeation enhancing effect for the paracellular uptake is favorable.

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