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Review article

Thiomers: potential excipients for non-invasive peptide delivery systems ☆

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Abstract

In recent years thiolated polymers or so-called thiomers have appeared as a promising alternative in the arena of non-invasive peptide delivery. Thiomers are generated by the immobilisation of thiol-bearing ligands to mucoadhesive polymeric excipients. By formation of disulfide bonds with mucus glycoproteins, the mucoadhesive properties of these polymers are improved up to 130-fold. Due to formation of inter- and intramolecular disulfide bonds within the thiomer itself, dosage forms such as tablets or microparticles display strong cohesive properties resulting in comparatively higher stability, prolonged disintegration times and a more controlled release of the embedded peptide drug. The permeation of peptide drugs through mucosa can be improved by the use of thiolated polymers. Additionally some thiomers exhibit improved inhibitory properties towards peptidases. The efficacy of thiomers in non-invasive peptide delivery could be demonstrated by various in vivo studies. Tablets comprising a thiomer and pegylated insulin, for instance, resulted in a pharmacological efficacy of 7% after oral application to diabetic mice. Furthermore, a pharmacological efficacy of 1.3% was achieved in rats by oral administration of calcitonin tablets comprising a thiomer. Human growth hormone in a thiomer-gel was applied nasally to rats and led to a bioavailability of 2.75%. In all these studies, formulations comprising the corresponding unmodified polymer had only a marginal or no effect. According to these results drug carrier systems based on thiomers seem to be a promising tool for non-invasive peptide drug delivery.

Keywords: Thiomers; Non-invasive peptide delivery; Mucoadhesion; Enzyme inhibition; Permeation enhancement; Controlled release

1. Introduction

The development of potent formulations for non-invasive peptide delivery represents one of the main challenges in modern pharmaceutical technology. At present most of these extraordinary pharmacological potential therapeutic agents have to be administered via parenteral routes, which are inconvenient because of pain, fear and risks being associated with this type of application. 'Injectable-to-non-invasive-conversions' and in particular 'injectable-to-oral-conversions' are consequently highly in demand. In order to provide a sufficiently high bioavailability with non-invasive peptide delivery systems, however, various hurdles have to be overcome. They include the diffusion barrier (i) being based on the mucus gel layer covering mucosal membranes, which has to be passed by peptides in order to reach the absorption membrane [1], and the enzymatic barrier (ii) being represented by secreted and membrane bound peptidases [2]. Moreover, having reached the absorption membrane in intact form therapeutic peptides have to permeate this membrane barrier (iii) in order to reach the systemic circulation [3]. Pharmaceutical technological attempts to overcome these barriers include the use of enzyme inhibitors [2], permeation enhancers [4]

Abbreviations: Chito-TBA, chitosan-4-thio-butylamidine; CMC-Cys, carboxymethylcellulose-cysteine; GSH, reduced glutathione; hGH, human growth hormone; PAA_{450}, poly(acrylic acid) (MW 450 kDa); PAA_{450}-Cys, poly(acrylic acid)–cysteine (MW 450 kDa); PCP, polycarbophil; PCP-Cys, polycarbophil–cysteine; PEG, poly(ethylene glycol); PTP, protein tyrosine phosphatase.

☆ Dedicated to Prof. H.E. Junginger.

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and multifunctional polymers [5] ideally guaranteeing both enzyme inhibition and permeation enhancement. In case of multifunctional polymers these effects, however, can only take place if a tight contact of the polymer with the mucosa is provided for the whole period of peptide drug release and absorption. Apart from enzyme inhibitory and permeation enhancing properties multifunctional polymers should therefore offer strong mucoadhesive features.

Among this group of multifunctional polymers exhibiting all these mentioned properties, thiolated polymers—designated thiomers—are the most promising for non-invasive peptide delivery. Due to the immobilisation of thiol groups on well-established multifunctional polymers such as poly(acrylates) or chitosans their enzyme inhibitory, permeation enhancing and mucoadhesive properties can be strongly improved [6,7]. Within this review the features of thiomers as well as their advantages and potential for non-invasive peptide delivery are discussed. The summarised data should provide a good starting point for further developments and applications of thiomers in non-invasive peptide delivery.

2. Synthesis of thiomers

2.1. Anionic thiomers

Anionic thiolated polymers generated thus far all exhibit carboxylic acid groups as anionic substructures. These carboxylic acid groups offer the advantage, that sulfhydryl moieties can be easily attached to such polymers via the formation of amide bonds. Appropriate ligands are overall cysteine [7], cysteamine [8] and homocysteine [9]. The formation of amide bonds can be mediated by carbodiimides. An unintended oxidation of thiol groups during synthesis can be avoided by performing the reaction under inert conditions. Alternatively the synthesis can be performed at a pH < 5. At this pH range the concentration of thiolate-anions, representing the reactive form for oxidation of thiol groups, is low, and the formation of disulfide bonds can be almost excluded. In addition, disulfide bonds formed during synthesis can be cleaved thereafter by the addition of reducing agents such as dithiothreitol or $NaBH_4$. The total amount of immobilised, reduced and oxidised thiol groups can be determined by reducing first of all the entire amount of oxidised thiol groups with $NaBH_4$ followed by quantifying the thiol groups with Ellman's reagent. Skipping the reduction process allows the determination of the ratio of oxidised to reduced thiol groups. The chemical structure of anionic thiolated polymers is shown in Fig. 1.

2.2. Cationic thiomers

Cationic thiomers are mainly based on chitosan. The primary amino group at the 2-position of the glucosamine subunits of this polymer is the main target for the immobilisation of thiol groups. As outlined in Fig. 1 sulfhydryl-bearing agents can be covalently attached to this primary amino group via the formation of amide or amidine bonds. In the case of amide bonds the carboxylic acid group of the ligands cysteine and thioglycolic acid react with the primary amino group of chitosan mediated for instance by carbodiimides [11]. The formation of disulfide bonds by air oxidation during the synthesis can be avoided as described above.

In the case of amidine bonds 2-iminothiolane is used as coupling reagent. It offers the advantage of a simple one-step coupling reaction. In addition, the thiol group of the reagent is protected towards oxidation because of the chemical structure of the reagent. The chemical reaction of chitosan with 2-iminothiolane is illustrated in Fig. 2 [12].

3. Features of thiomers

3.1. Mucoadhesive and cohesive properties

Mucoadhesive properties can provide an intimate contact with the mucosa at the site of drug uptake preventing a presystemic metabolism of peptides on the way to the absorption membrane in the gastrointestinal tract. Additionally, the residence time of the delivery system at the site of drug absorption is increased. Moreover, a steep concentration gradient on the absorption membrane representing the driving force for passive drug uptake can be provided.

Anionic polymers feature mucoadhesive properties via hydrogen bonding, van der Waal's interactions and chain entanglement with the mucus [15]—forces stronger than the electrical repulsion caused by electrostatic interactions. In contrast, cationic polymers adhere to the negatively charged mucus mainly due to electrostatic forces [16]. As both anionic and cationic mucoadhesive polymers exhibit a high buffer capacity, a demanded microclimate regarding the pH can be adjusted and maintained over numerous hours within the polymeric network [17]. So far, however, mucoadhesive delivery systems have not reached their full potential due to insufficient mucoadhesive properties of these polymers [18,19]. On the contrary, the strong mucoadhesive properties of thiomers are believed to be based on additional covalent bonds between thiol groups of the thiomer and cysteine-rich subdomains of mucus glycoproteins [20]. This theory was confirmed by findings of mucoadhesion studies, where a higher amount of thiol groups on the polymer resulted in higher mucoadhesive properties [21–23]. As listed in Table 1, the mucoadhesive properties of all polymers thus far tested could be strongly improved by the immobilisation of thiol groups.

Among all anionic thiomers, poly(acrylic acid)-cysteine (450 kDa; PAA_{450}-Cys) offers the highest mucoadhesion as determined by tensile studies. After adjusting this thiolated polymer to pH 3, PAA_{450}-Cys exhibited its strongest

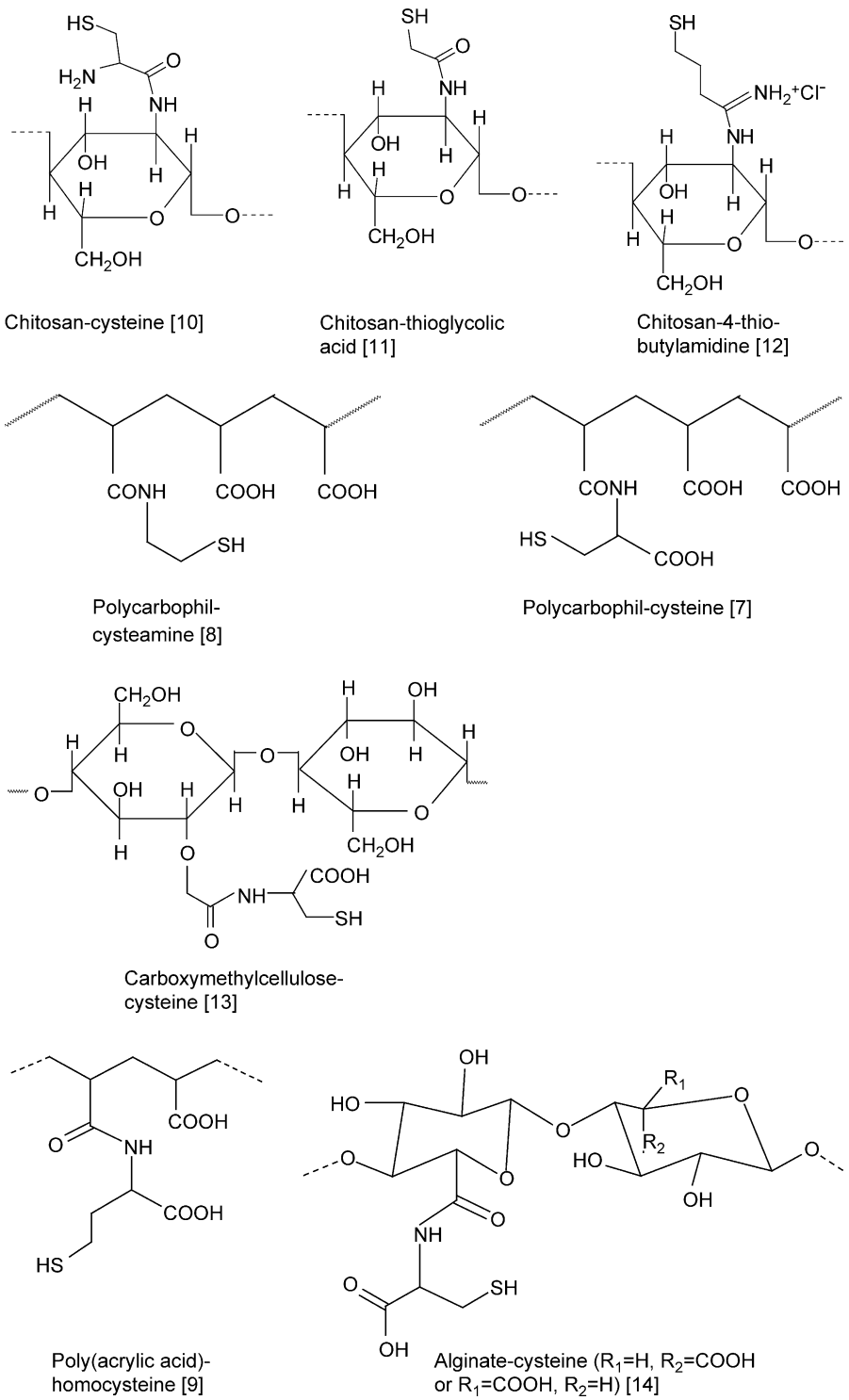

Fig. 1. Structure of thiolated polymers.

mucoadhesion. When the pH of PAA_{450}-Cys was shifted to higher pH levels, mucoadhesion decreased [27]. Polycarbophil-cysteine (PCP-Cys) was more than 2-fold less mucoadhesive than the PAA_{450}–Cys conjugate probably due to less chain flexibility [24]. PAA–Cys conjugates of even lower molecular masses (2, 45, 250 kDa) exhibited minor mucoadhesive properties [24].

The chitosan–4-thio-butylamidine conjugate (400 kDa; chitosan–TBA) showed almost 30-fold improved mucoadhesive properties compared to unmodified chitosan. These findings were confirmed by mucoadhesion studies with the rotating cylinder method, where chitosan-TBA-tablets remained attached to porcine intestinal mucosa 130-fold longer than unmodified chitosan tablets [25]. Also for

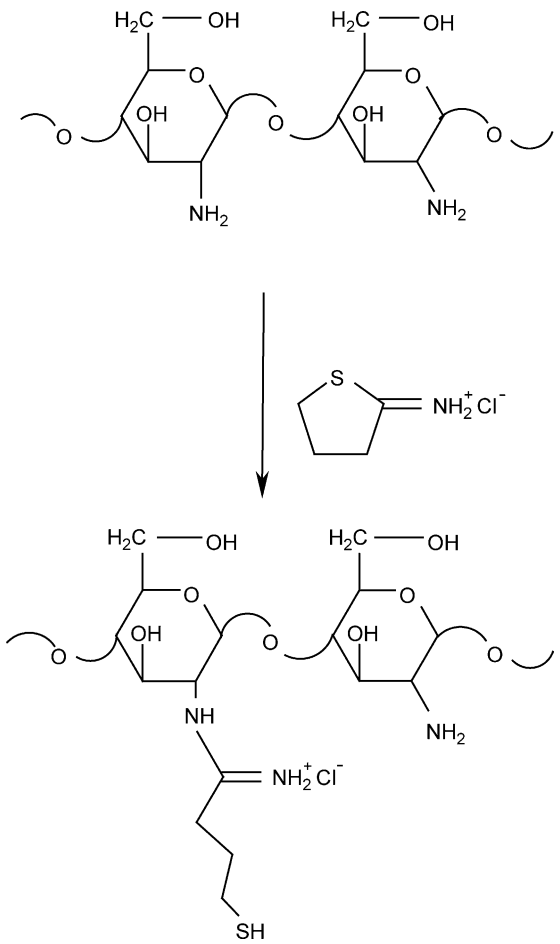

Fig. 2. Presumptive synthetic pathway for the modification of chitosan with 2-iminothiolane [12].

Table 1
Comparison of the mucoadhesive properties of various polymeric excipients

Polymer	Total work of adhesion (μJ); means ± SD ($n = 3–8$)	Reference
Poly(acrylic acid)-cysteine	695 ± 80	[24]
Chitosan-TBA	682 ± 100	[25]
Polycarbophil-cysteine	280 ± 68	[7]
Chitosan-thioglycolic acid	234 ± 0.4	[21]
Polycarbophil-cysteamine	192 ± 14	
Poly(acrylic acid)	171 ± 53	[24]
Sodium carboxymethyl cellulose-cysteine	157 ± 6	[13]
Polycarbophil	110 ± 28	[7]
Sodium carboxymethylcellulose	108 ± 17	[13]
Sodium alginate-cysteine	102 ± 36	[14]
Poly(methacrylic acid)-cysteine/starch	90 ± 15	[26]
Poly(methacrylic acid)/starch	28 ± 3	[26]
Sodium alginate	26 ± 1	[14]
Chitosan HCl	23 ± 10	[21]

Mucoadhesion studies were performed via tensile tests.

chitosan-TBA the pH of the polymer turned out to be crucial. While conjugates adjusted to pH 3 displayed the strongest mucoadhesion, the mucoadhesive properties of the same polymer were comparatively lower when the pH was adjusted to pH > 5 [25]. An explanation for this effect can be given by the pH-dependent reactivity of thiol groups. At pH values above 5, thiol groups become more reactive leading to the formation of disulfide bonds already within the polymeric network itself before reacting with disulfide and/or thiol substructures of the mucus. Thiomers of lower pH are less reactive. Their thiol groups become only reactive when interpenetrating the mucus gel layer exhibiting a pH 5–7. Consequently thiomers of a comparatively lower pH probably form disulfide bonds with the mucus gel layer. Besides the covalent bonds between thiomers and mucus explained above, the strong mucoadhesion of chitosan-TBA (400 kDa) seems to be based on additional ionic interactions between the cationic amidine substructure of the chitosan–TBA conjugate and anionic substructures within the mucus layer. Chitosan–TBA conjugates with higher (600 kDa) or lower (150 kDa) molecular mass showed comparatively lower mucoadhesion [22].

Covalent bonds are believed to be formed not only between thiomer and mucus, but also within the thiomer itself. This theory was confirmed by the decrease in free thiol groups within thiomers resulting in an increase in viscosity [7,14]. Inter- and intramolecular disulfide bonds improve the cohesive properties of the thiolated polymer compared to the unmodified polymer. Due to improved cohesive properties the disintegration time of PAA_{450}-Cys tablets could be more than 70-fold prolonged compared to the unmodified polymer [24].

Although thiomers show strongly improved mucoadhesive properties, the adhesion of delivery systems being based on such polymers is nevertheless limited by the natural mucus turnover. The mucus turnover in the human intestine, for instance, was determined to be in the range of 12–24 h [28]. Consequently, at least within this time period, the adhesion of the delivery system will fail.

3.2. Enzyme inhibitory properties

Presystemic metabolism of peptide and protein drugs in particular in the GI-tract can be regarded as one of the main reasons for their limited bioavailability after non-invasive administration. Therefore, numerous research groups have focused their interest on the development of drug delivery systems providing a protective effect towards secreted as well as membrane-bound enzymes. Two major strategies have thereby been pursued: the incorporation of low molecular mass enzyme inhibitors and the use of polymers exhibiting enzyme inhibitory properties.

Thiomers are promising candidates within the group of enzyme inhibiting polymers. The inhibitory properties of poly(acrylates) on intestinal proteases were first reported by

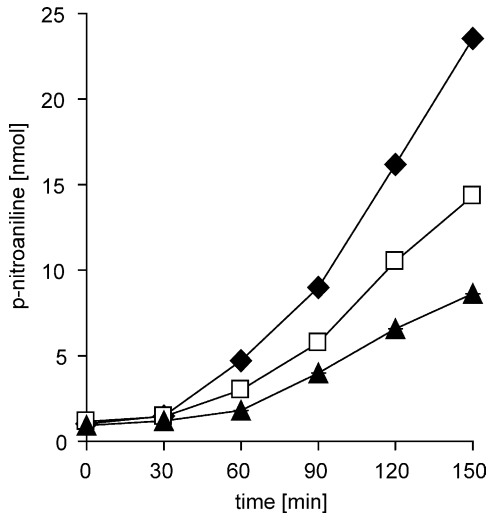

Fig. 3. Time-course of the formation of *p*-nitroaniline from leu-*p*-nitroanilide by aminopeptidases present on intact buccal mucosa during incubation without polymer (◆); with 0.25% (m/v) polycarbophil (□); with 0.25% (m/v) polycarbophil–cysteine-conjugate (▲). Each point represents the mean (± SD; $n = 3$). (Adapted from Langoth et al. [34]).

Hutton et al. [29]. They found a strong reduction of albumin degradation by a mixture of proteases in the presence of carbomer 934P. A subsequent study by Lueßen et al. [30] showed that PCP and carbomer 934P were potent inhibitors of the proteolytic enzymes trypsin, α-chymotrypsin and carboxypeptidase A. As a result of the covalent attachment of cysteine to PCP, the inhibitory effect of the polymer towards carboxypeptidase A, carboxypeptidase B and chymotrypsin could be significantly improved [31]. PCP-Cys also had a significantly greater inhibitory effect than unmodified PCP on the activity of isolated aminopeptidase N and aminopeptidase N present on intact intestinal mucosa [32].

The inhibitory effect of thiolated polymers was also tested on intact vaginal mucosa [33] as well as on buccal mucosa [34]. As shown in Fig. 3, both thiolated and unmodified PCP significantly inhibited the hydrolysis of the synthetic substrate leu-*p*-nitroanilide by aminopeptidases present on the buccal mucosa. Thiolated PCP was thereby significantly more effective than the unmodified polymer.

The strongly improved enzyme inhibitory properties of PCP-Cys in comparison to unmodified PCP can be explained by the inhibitory effect of L-cysteine itself towards carboxypeptidase A, carboxypeptidase B and aminopeptidase N due to the binding of the Zn^{2+} ion from the enzyme structure [31,32].

Compared to delivery systems based on the co-administration of enzyme inhibitors, thiomers offer the advantage that the inhibitory effect can be concentrated and localised on the delivery system. Hence, systemic toxic side effects as well as feedback regulations leading to an increased enzymatic activity can be avoided.

3.3. Permeation enhancing properties

In order to improve the bioavailability of peptide or protein drugs administered via mucosal routes permeation enhancers often have to be added to the delivery system. Generally, two types of permeation enhancers are in use: low molecular mass permeation enhancers such as sodium salicylate or medium-chain glycerides [4,35] and polymers displaying permeation enhancing properties.

The influence of thiomers on the permeation of hydrophilic model compounds and peptide drugs across freshly excised bovine nasal mucosa [36], rabbit cornea [37] as well as intestinal mucosa was evaluated in Ussing type chambers. Various thiomers such as PCP-Cys [38], PAA_{450}-Cys [39], CMC-Cys [40], chitosan-cysteine [10] and chitosan-4-thiobutylamidine [41] showed a strong permeation enhancing effect (Table 2). This effect could be further improved due to the addition of the permeation mediator glutathione [42]. As shown in Fig. 4, the transport of rhodamine 123 across freshly excised small intestinal mucosa was significantly improved compared to unmodified chitosan utilising 0.5% chitosan–TBA conjugate combined with 5% glutathione [25]. Results of in vitro permeation studies could be confirmed by various in vivo studies as described below.

The likely mechanism being responsible for the permeation enhancing effect of the thiomer/glutathione system

Table 2
Permeation enhancing properties of various thiomers in comparison to the corresponding unmodified polymers tested on freshly excised intestinal mucosa of guinea pigs

Permeation enhancer	Test compound	Apparent permeability coefficient [$P_{app} \times 10^{-6}$ (cm/s)]	Enhancement ratio (P_{app} thiomer/ P_{app} unmodified control polymer)	Ref.
PCP-Cys	Na-Flu	5.27 ± 0.11	1.57	[38]
PCP-Cys	bac-FITC	2.69 ± 0.09	1.37	[38]
PCP-Cys	insulin-FITC	2.50 ± 0.15	1.35	[38]
PCP-Cys/GSH	Na-Flu	14.64 ± 0.93	2.93	[42]
PCP-Cys/GSH	bac-FITC	9.94 ± 0.82	2.06	[42]
PCP-Cys/GSH	LMWH	0.39 ± 0.02	2.2	[43]
PCP-Cys	LMWH	0.19 ± 0.04	1.1	[43]
PCP-Cys/GSH	hGH-FITC	n.a.	3	[36]
PAA_{450}-Cys	Na-Flu	8.38 ± 0.24	1.29	[39]
PAA_{450}-Cys/GSH	Na-Flu	9.65 ± 0.38	1.48	[39]
PAA-HC/GSH	Na-Flu	5.9 ± 1.97	2.4	[9]
CMC-Cys	Na-Flu	12.92 ± 0.41	1.8	[40]
CMC-Cys	bac-FITC	5.58 ± 0.54	1.32	[40]
CMC-Cys	ins-FITC	5.55 ± 0.65	1.31	[40]
Chitosan-Cys	bac-FITC	n.a.	signif.	[10]
Chitosan-TBA/GSH	rhodamine	3.0 ± 1.2	3.6	[25]
Chitosan-TBA	rhodamine	1.5 ± 0.7	1.8	[25]

bac-FITC, flucorescein-isothiocyanate labelled bacithracin; ins-FITC, flucorescein-isothiocyanate labelled insulin; LMWH, low molecular weight heparin; PAA-HC, poly(acrylic acid)-homocysteine; Na-Flu, sodium fluoresceine; rhodamine, rhodamine 123.

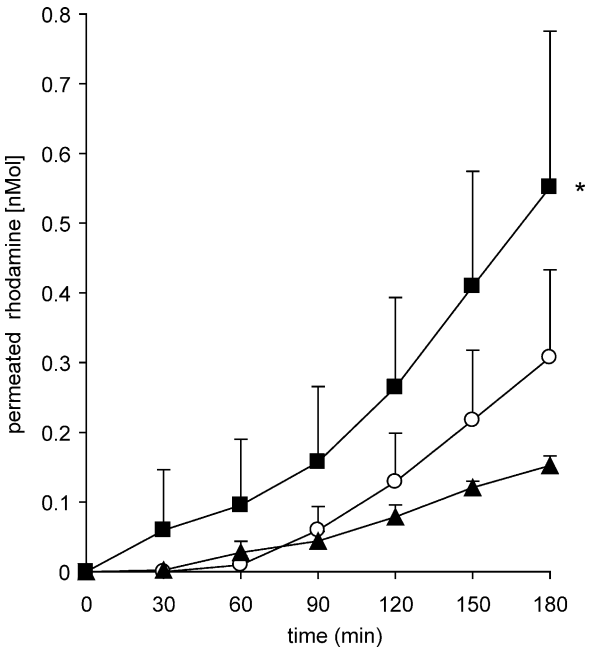

Fig. 4. Permeation enhancing effect of 0.5% (m/v) chitosan–TBA conjugate with 5% (m/v) GSH (■), of 0.5% (m/v) chitosan–TBA (○) and of 0.5% (m/v) unmodified chitosan (▲) on the permeation of rhodamine 123 across freshly excised small intestinal mucosa. Indicated values are means of at least three experiments ±SD. * Differs from unmodified chitosan $P < 0.05$. (Adapted from Bernkop-Schnürch et al. [25]).

has been ascribed to be based on the inhibition of the enzyme protein tyrosine phosphatase (PTP). PTP is able to dephosphorylate tyrosine residues of occludin, which is believed to play an essential role in the opening process of the tight junctions. This dephosphorylation results in the closing of the tight junctions, leading consequently to a decreased permeation of hydrophilic macromolecules. According to this theory, the inhibition of PTP by reduced glutathione will lead to a phosphorylation and an opening of the tight junctions. However, the inhibitory effect of glutathione is limited as it is rapidly oxidised on the cell surface [44]. The presence of the thiolated polymer is therefore essential, as it prevents the oxidation of glutathione on the surface of the mucosa.

Highly advantageous properties of the thiomer/glutathione permeation enhancing system include its comparatively prolonged effect and its low toxicity. In contrast to low molecular mass permeation enhancers thiomers will not be absorbed from the mucosal tissue due to their high molecular mass [45] and therefore can act for a longer time period while systemic side effects can be excluded. Native glutathione is present on the apical side of the mucosa in a relatively high concentration and is involved in detoxification processes [46].

As the mechanism being responsible for the permeation enhancing effect of thiomers seems to be completely different to that of well-established low molecular mass permeation enhancers used in non-invasive peptide delivery systems, a combination seems promising. Guggi et al. for instance, could already demonstrate that by the combined use of a thiomer with a low molecular mass permeation enhancer, a synergistic permeation enhancing effect for the paracellular drug uptake could be achieved [47]. Consequently the efficacy of non-invasive peptide delivery systems comprising a thiomer should be further improved by the addition of certain low molecular mass permeation enhancers, of which release can be controlled by the thiomeric carrier matrix.

3.4. Thiomers as matrices for controlled drug release

A controlled peptide drug release out of the delivery system represents a prerequisite for an increased absorption rate and an enhanced bioavailability. The cohesion and stability of the delivery system over the intended period of peptide drug liberation is in most cases a prerequisite in order to achieve controlled release.

Thiolated polymers display excellent cohesive properties. Matrix tablets of PAA_{450}-Cys and PCP-Cys, for instance, were stable for more than 48 h in simulated intestinal fluid without any observable erosion [24]. The thiol functions on the polymeric backbone of thiomers enable them not only to form disulfide bridges with mucus glycoproteins, but also to form inter- as well as intramolecular disulfide bonds. This cross-linking of the polymer chains results in the high stability of drug carrier systems based on thiomers.

Thiolated polymers were demonstrated to guarantee a controlled drug release by using model drugs such as fluorescence-labelled insulin [48]. Release studies of fluorescence-labelled insulin showed that an almost zero-order release kinetic can be provided by the use of thiolated PCP as carrier matrix. Thiol/disulfide exchange reactions between insulin and the thiolated polymer could thereby be excluded [48]. The reason for this sustained release is the cross-linking within the matrix tablet, which provides a tightened three-dimensional polymeric network leading to a more controlled release. Apart from a sustained peptide drug release over numerous hours a rapid drug release can also be guaranteed, in particular when peptide drugs are incorporated in thiomer microparticles [49].

3.5. Stability

3.5.1. Stability of thiomers

Because of the sensitivity of thiol groups towards oxidation, the chemical stability of thiomers has already been investigated in detail. PCP-Cys and chitosan-TGA were tested both as representative anionic and cationic candidates, respectively. The polymers were tested in the form of freeze-dried powders and matrix-tablets. Polymers were stored for a period of 6 months at four different storage conditions, namely at −20 °C (56% relative humidity; RH), at 4 °C (53% RH), at 20 °C (70% RH), and at 22 °C

(25% RH). Samples were taken after 6 months to determine the formation of disulfide bonds and the remaining thiol groups on the polymer. When the PCP–Cys and chitosan–TGA conjugate were stored in the form of a powder, a decrease in free thiol groups was observed only after storage at 20 °C and 70% RH. Both polymers were found to be stable under all storage conditions when compressed into matrix-tablets [50].

3.5.2. Stability of peptides incorporated in to thiomers

Another aspect of stability focuses on the stability of the therapeutic peptide being incorporated in a thiomeric carrier matrix. As most peptide drugs bear thiol and/or disulfide bonds in their chemical structure, thiol/disulfide exchange reactions with thiomers cannot be excluded a priori. Studies investigating such peptide–thiomer interactions, however, revealed that they take place only to a very limited extent. Moreover, for many therapeutic peptides such interactions can be excluded completely. Although generalisations must always be viewed with great caution, thiol/disulfide exchange reactions do not seem to take place if at least one of following demands are fulfilled:

- solid delivery systems with no or comparatively low water content are generated
- the pH of the thiomers is below 5 leading to a marginal ratio of thiolate anions, which are the functional groups being responsible for thiol/disulfide interactions and oxidation processes
- the thiol/disulfide moieties of the therapeutic peptide being embedded in an anionic thiomer are neighboured by non-ionic or anionic amino acids [31]
- the thiol/disulfide moieties of the therapeutic peptide being embedded in a cationic thiomer are neighboured by non-ionic or cationic amino acids [31]

Evidence for these theories is not only provided by various in vitro studies [31] but also by biofeedback studies in different animal species with different peptide drugs demonstrating that these therapeutic agents do not loose their efficacy having been embedded in a thiomer [51,52].

4. Peptide delivery systems based on thiomers

4.1. Tablets

Approximately 40% of all dosage forms are tablets. Due to their convenient route of administration and their long-lasting shelf life patient compliance is very high. Depending on the drug carrier matrix and the auxiliary agents used, peptide liberation can be adjusted to delay or prolong release. If polymers are used as drug carrier matrices for tablets, the polymer forms a gel after contact with the liquids of mucosal membranes. In order to guarantee a swelling of orally given tablets on the intestinal mucosa, tablets can be enteric coated [52] or in the case of stomach targeted delivery systems, coating with trigylcerides was shown to be sufficient to provide a swelling of the dosage form once it reached the stomach [51]. In addition, thiolated poly(methacrylic acid)/starch compositions were shown to swell only at pH > 5 even without an enteric coating [26]. The thickness of the gel layer controls on the one hand the diffusion of the peptide out of the polymer-matrix and hinders on the other hand the diffusion of peptidases into the swollen polymeric network. This swollen polymeric network is much less effective, if it disintegrates before the peptide diffuses out of it. Therefore only polymers with strong cohesive properties used as peptide drug carrier systems can guarantee a diffusion controlled release. In the gastrointestinal tract, however, mucoadhesion of polymer tablets is thought to be limited mainly because of peristalsis.

4.2. Microparticles

Microparticles based on poly(acrylic acid) or chitosan lack strong cohesive properties. Consequently they disintegrate rapidly and cannot control the release of the embedded peptide drug. Chitosan microparticles can be stabilised by addition of multivalent anions, but as a consequence mucoadhesion decreases. The use of multifunctional polymers like PAA_{450}-Cys for microparticle preparation led to particles with highly improved cohesive properties [53]. They are stabilised by the formation of intramolecular disulfide bonds within the microparticles during the preparation process. Consequently a controlled drug release out of such microparticles can be achieved. The release of the peptide drug can be prolonged by the addition of hydrophobic excipients like Eudragit RS® to the polymer [49]. Disintegration studies showed a stability of these thiomeric microparticles over 24 h, whereas particles comprising unmodified poly(acrylic acid) disintegrated within minutes.

Microparticles display per se a prolonged residence time on mucosal membranes compared to single-unit dosage forms [54]. This residence time on mucosal membranes is even further improved when they exhibit mucoadhesive properties. Due to the immobilisation of thiol groups on microparticles the mucoadhesive properties are additionally improved. PAA_{450}-Cys microparticles, for instance, were almost 15-times more mucoadhesive on the intestinal mucosa than unmodified polymer particles [49].

4.3. In situ gelling formulations

Polymers displaying in situ gelling properties have been described to stabilise themselves once applied as liquid or semisolid formulations at the site of drug delivery. This in situ gelation combines the advantages of a solution, being easy to administer for the patient with the favourable properties of a gel, which displays limited clearance and increased mucoadhesiveness.

Several concepts for in situ gelling systems have been described so far. The sol–gel transition can be induced by a shift in pH (i) [55], in temperature (ii) [56] or in electrolyte concentration (iii) [57]. Thiolated polymers represent a new type of in situ gelling polymers [23,58]. At physiological pH values, sufficiently high amounts of negative thiolate anions are present within the polymer representing the active form for oxidation. This oxidation leads to the formation of inter- and intramolecular disulfide bonds being responsible for an increase in viscosity. The in situ-gelling properties of deacetylated gellan gum, for instance, which shows a strong increase in viscosity in the presence of electrolytes [59] could be significantly improved by the immobilisation of thiol groups [60]. Furthermore, PAA-Cys, chitosan-TGA and chitosan-TBA have shown excellent in-situ gelling properties, with a clear correlation between the total amount of polymer-linked thiol groups and the increase in viscosity of the formed gel [12,61].

5. In vivo performance—proof of concept

5.1. Oral peptide delivery

Although it is impossible to differentiate between the impact of certain properties of thiomers in vivo, their overall capability for the oral application of therapeutic peptides could be demonstrated in different in vivo studies [51,52,62,63]. The anionic thiomers PCP-Cys and PAA_{450}-Cys were used as drug carrier matrices for insulin and the cationic chitosan–TBA conjugate served as matrix for salmon calcitonin. The mentioned peptide drugs are commercially available as injectable forms and also as a nasal spray in the case of calcitonin. However, patient compliance for these dosage forms is low due to the inconvenient form of application. In contrast, oral administration would improve patient compliance dramatically, but until now oral bioavailability has been too low to permit therapeutic employment [64]. Therefore the drugs mentioned above were chosen as model candidates to evaluate the potential of thiolated polymers as carrier systems for the peroral administration of peptides.

Marschütz et al. developed insulin tablets based on the thiolated polymer PCP-Cys containing the enzyme inhibitors elastatinal and Bowman–Birk-inhibitor covalently-linked to carboxymethylcellulose. This insulin formulation led to a maximum decrease in blood glucose level of 36% and the effect was maintained for more than 80 h [52].

In another study Caliceti et al. generated insulin tablets with PAA_{450}-Cys as the drug carrier matrix. Insulin was chemically modified with poly(ethylene glycol) (PEG) in order to achieve a higher stability towards elastase. After oral administration to diabetic mice these tablets led to a decrease of the blood glucose level by almost 60% and the effect lasted for 20 h. Orally given pegylated insulin in solution did not show an effect at all. The pharmacological efficacy of this oral formulation was calculated to be 7% versus s.c. injection. Results of this study are shown in Fig. 5 [62].

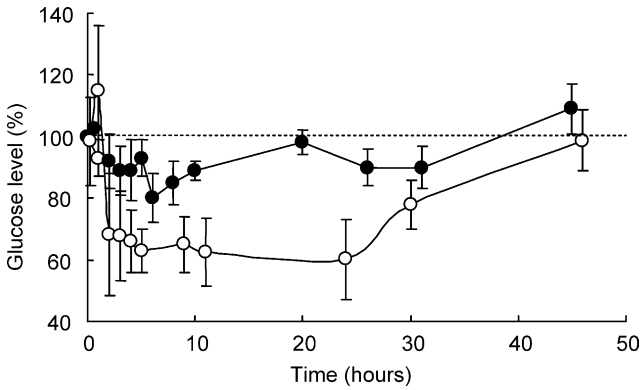

Fig. 5. Decrease in blood glucose level in diabetic mice after oral administration of pegylated insulin loaded tablets (○) and pegylated insulin in solution (●). Each point represents the mean ± SD of 10 experiments. (Adapted from Caliceti et al. [62]).

Guggi et al. evaluated chitosan–TBA conjugate tablets comprising calcitonin. Small intestine targeted tablets and stomach targeted formulations were tested. The drug delivery systems based on chitosan–TBA-conjugate contained salmon calcitonin, optionally the permeation mediator, reduced glutathione, and different enzyme inhibitors to avoid enzymatic degradation. Chitosan–BBI and chitosan–elastatinal conjugate were added to enteric coated delivery systems targeted to the small intestine. The stomach targeted calcitonin delivery system comprised a chitosan–pepstatin A conjugate to inhibit pepsinic degradation of the protein. The different calcitonin delivery systems were orally administered to rats and the plasma calcium level as pharmacological response was determined. The oral application of calcitonin in ascorbic acid solution and control tablets based on unmodified chitosan resulted in no significant effect. In contrast, calcitonin in chitosan–TBA conjugate tablets led to a more than 5% decrease of the plasma calcium level. Thiolated chitosan tablets comprising reduced glutathione displayed a significantly higher pharmacological efficacy compared to chitosan-TBA tablets lacking this permeation mediator. The strongest effect was achieved with the stomach targeted system. The calcium level decreased by more than 10% and the effect lasted for more than 12 h [51].

5.2. Nasal peptide delivery

The nasal route represents an attractive alternative to parenteral delivery for an increasing number of therapeutic peptides such as calcitonin, insulin, desmopressin, buserelin and octreotide. However, bioavailabilities of nasally administered peptides often do not exceed 1% due to low membrane permeability, a short local residence time at the site of absorption and a high metabolic turnover in

the nasal epithelium [65]. The three major strategies to increase the bioavailability of intranasally administered peptide drugs are (i) the use of permeation enhancers, (ii) incorporation of enzyme inhibitors and (iii) increasing local drug residence time using mucoadhesive polymers [66].

Thiomers seem to be capable of combining most of these strategies. Therefore, the suitability of thiomers as multifunctional vehicles for systemic nasal peptide delivery was evaluated in vivo. As model drug, human growth hormone (hGH), a protein drug of 191 amino acids (22 kDa) was utilised, which is used to treat short stature in children due to growth hormone deficiency, Turner's syndrome or chronic renal failure. Currently, hGH has to be administered by daily injections, which are difficult and painful resulting in low patient acceptance [67]. A nasal delivery system for hGH would therefore be highly appreciable.

For the in vivo study, an aqueous nasal gel formulation was developed consisting of PCP-Cys, glutathione and hGH in a final concentration of 0.3, 0.5 and 0.6% (m/v), respectively. As controls a 0.3% (m/v) PCP gel and physiological saline were prepared containing the same amount of hGH. These formulations were administered to conscious rats ($n = 4–5$) and the hGH plasma level was monitored via ELISA as a function of time. As shown in Fig. 6, the PCP-Cys/glutathione/hGH nasal gel delivery system resulted in a significantly higher hGH plasma concentration compared to both controls with an absolute bioavailability of 2.75 ± 0.37%. Furthermore, in contrast to the controls the thiomer gel delivery system was able to prolong the efficacy of hGH.

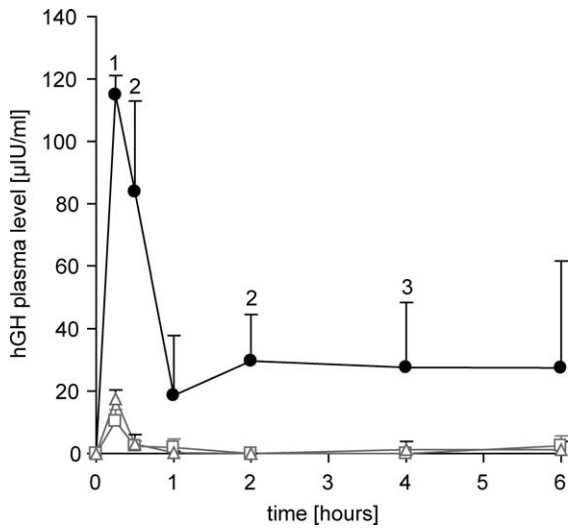

Fig. 6. Plasma concentration-time profiles following intranasal administration of hGH incorporated in PCP-Cys/GSH (●), unmodified PCP (□) and physiological saline (Δ). Data represent the mean ± SD of $n = 4–5$ for all delivery systems. [1] Differs from unmodified PCP control gel $P < 0.0001$; [2] differs from control $P < 0.01$; [3] differs from control $P < 0.05$. (Adapted from Leitner et al. [36]).

6. Conclusion

The immobilisation of thiol-bearing compounds on polymeric excipients such as poly(acrylates) and chitosans leads to a significant improvement in their mucoadhesive, enzyme inhibitory and permeation enhancing properties. As the cohesive properties are also strongly improved because of a cross-linking process via disulfide bond formation within the polymeric network, a mainly diffusion-controlled sustained release of thiomer embedded peptide drugs can be guaranteed. In comparison to non-invasive peptide delivery systems comprising unthiolated multifunctional polymers, the efficacy of delivery systems comprising the corresponding thiolated version is therefore significantly higher. A 'proof of concept' could meanwhile be provided in various animal species, for various non-invasive routes of application using various therapeutic peptides embedded in different types of thiomers. According to these results, thiomers seem to represent a promising new generation of multifunctional polymers for non-invasive peptide delivery.

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