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Oral peptide delivery: in-vitro evaluation of thiolated alginate/poly(acrylic acid) microparticles

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Abstract

The purpose of this study was to develop an oral thiomer-based microparticulate delivery system for insulin by ionic gelation. The microparticulate matrix consisted of either poly(acrylic acid)-cysteine (PAA-Cys) and alginate-cysteine (Alg-Cys) or the corresponding unmodified polymers (PAA, Alg). Two different viscosities of alginates were provided for the study, low and medium. Three different types of microparticles were prepared via ionic gelation with calcium (Alg, AlgPAA and AlgPAA-Cys) and their different properties evaluated in-vitro (particle size and shape, drug loading and release profile, swelling and stability). The mean particle size of all formulations ranged from 400 to 600 μm, revealing the lowest for thiolated microparticles. SEM micrographs showed different morphological profiles for the three different types of microparticles. Encapsulation efficiency of insulin increased within the following rank order: Alg (15%) < AlgPAA (40%) < AlgPAA-Cys (65%). Alginate and AlgPAA microparticles displayed a burst release after 30 min, whereas the thiolated particles achieved a controlled release of insulin over 3 h. The swelling ratio was pH dependent: in simulated intestinal fluid microparticles exhibited a much higher water uptake compared with simulated gastric fluid. Due to the formation of intraparticulate disulfide bonds during the preparation process, thiolated particles revealed a higher stability. It was also observed that the viscosity of the two alginates used had no influence on the properties of the particles. According to these results AlgPAA-Cys microparticles obtained by ionic gelation and stabilized via disulfide bonds might be an alternative tool for the oral administration of therapeutic peptides.

Introduction

Due to the obvious advantages of non-injectable and in particular oral drug delivery (such as easy and painless administration leading to improved patient compliance), this route of administration represents by far the most convenient one. However, oral application of therapeutic peptides and proteins such as insulin, which is the most widely used protein drug, is highly compromised by various barriers encountered within the gastrointestinal (GI) tract (Lee 1988). Besides the diffusion barrier of the mucus layer covering gastric and intestinal epithelia as well as the mucosal membrane itself, the enzymatic barrier caused by luminal secreted and membrane-bound proteases has to be overcome (Woodley 1994; Bernkop-Schnürch & Fragner 1996). The use of multifunctional polymers exhibiting enzyme inhibitory, permeation enhancing and mucoadhesive properties represents a common strategy to reach this goal. Among such non-invasive delivery systems, *thio*lated poly*mers* (thiomers) have attracted considerable attention concerning their inherent capability to overcome the aforementioned barriers following oral administration to gain sufficient bioavailability (Bernkop-Schnürch et al 2003a). Due to the immobilization of reactive thiol moieties on the polymeric backbone, thiomers are believed to form disulfide bonds with cysteine-rich subdomains of mucin glycoproteins displaying comparatively higher mucoadhesive properties. Additionally they show strongly improved enzyme inhibiting and permeation enhancing effects in comparison with the unmodified polymers (Bernkop-Schnürch & Greimel 2005).

With regards to oral drug delivery, it has been demonstrated that the use of multi-dose particulate delivery systems leads to a decelerated gastrointestinal transit time compared with single-unit dosage forms (Coupe et al 1991). Recently, microparticles based on thiolated poly(acrylic acid), which had been prepared by an emulsification solvent evaporation technique, revealed relatively high mucoadhesive properties leading to a prolonged residence

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time on excised porcine intestinal mucosa in-vitro (Bernkop-Schnürch et al 2003b). Another common way to prepare microparticles is by ionic gelation, of which alginate is the most frequently described and used polysaccharide for protein encapsulation. Besides the advantage of simple practicability, this method provides mild preparation conditions, such as low temperature and avoidance of organic solvents to prevent peptide degradation during the preparation process (Leonard et al 2004).

To benefit from both the use of thiolated microparticulate delivery systems and this simple preparation method, it was the aim of this study to develop microparticles comprising thiolated alginate (Alg-Cys) and thiolated poly(acrylic acid) (PAA-Cys). Furthermore, a sustained release of incorporated insulin out of the particles should be achieved. Resulting particles were evaluated in-vitro and compared concerning their particle size and superficial morphology, encapsulation efficiency and drug release profile, as well as their water uptake capacity and stability properties.

Materials and Methods

Materials

Sodium alginate with low viscosity (~250 cP) and medium viscosity (~3500 cP), poly(acrylic acid) (average molecular mass: 450 kDa, PAA_{450}), L-cysteine hydrochloride anhydrous, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent), calcium chloride dihydrate, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) and insulin (from bovine pancreas) were obtained from Sigma, Vienna, Austria. Acetonitrile and trifluoroacetic acid used for HPLC analysis were purchased from Acros Organics, Belgium. All other chemicals were of reagent grade and obtained from Sigma, Vienna, Austria.

Synthesis and characterization of thiomers

The thiomer conjugates used in this study (alginate-cysteine (Alg-Cys) and poly(acrylic acid)$_{450}$-cysteine (PAA-Cys)) were synthesized according to Bernkop-Schnürch et al (2001a) and Marschütz & Bernkop-Schnürch (2002). The chemical structure of both anionic thiomers is illustrated in Figure 1. In brief, the covalent attachment of L-cysteine to neutralized alginate and PAA_{450} was achieved by the formation of amide bonds between the primary amino group of L-cysteine and the carboxylic acid group of the polymers. Therefore, the carboxylic acid moieties of the polymers were activated for conjugation by the addition of EDAC in a final concentration of 50 mM. After the addition of L-cysteine to the aqueous polymeric solutions in a weight ratio of 1:1 (polymer:amino acid), reaction mixtures were incubated at pH 6 for 3 h at room temperature under stirring. The resulting thiolated conjugates were isolated by exhaustive dialysis in the dark at 10°C to avoid oxidation of the thiol moieties and subsequent lyophilization by drying frozen aqueous polymer solutions at −30°C at 0.01 mbar (Benchtop 2K, VirTis, NY). The polymer-cysteine conjugates were stored at 4°C until further use.

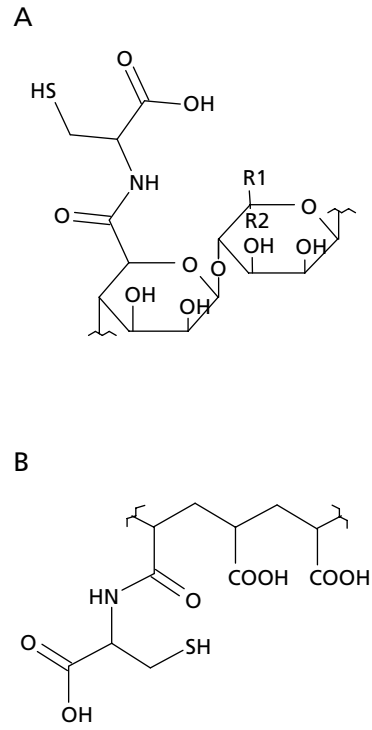

Figure 1 Chemical structure of thiomers: (**A**) alginate-cysteine; $R_1 = H$, $R_2 = COOH$ or $R_1 = COOH$, $R_2 = H$ and (**B**) poly(acrylic acid)$_{450}$-cysteine.

Neutralized polymers (alginate and PAA_{450}) prepared and isolated in the same way as the thiomer conjugates, but omitting EDAC during the coupling reaction, served as corresponding unmodified polymers within the following studies.

Preparation of microparticles

All microparticles evaluated within this study were prepared by ionic gelation of the employed polymers and thiomers, respectively, with calcium chloride. Three different types of insulin-loaded microparticles, each based on low viscous as well as on medium viscous alginate, were prepared: alginate (Alg), alginate/poly(acrylic acid) (AlgPAA) and alginate-cysteine/poly(acrylic acid)-cysteine (AlgPAA-Cys). Each formulation was prepared in triplicate. The final composition of the various microparticles is listed in Table 1.

Table 1 Composition of insulin-loaded microparticles based on alginate (Alg), alginate/poly(acrylic acid) (AlgPAA) and alginate-cysteine/poly(acrylic acid)-cysteine (AlgPAA-Cys). Each type of microparticle was prepared with low or medium viscous alginate, respectively, by ionic gelation

	Alg	AlgPAA	AlgPAA-Cys
Alginate/Alginate-Cys (%)	85	42.5	42.5
PAA_{450}/PAA_{450}-Cys (%)	–	42.5	42.5
Insulin (%)	15	15	15

Alginate microparticles (Alg)

Distilled water (1 mL) was added to 2 mL 2% (w/v) aqueous alginate solution (low and medium viscous, respectively). To this solution, 0.2 mL insulin solution (35.3 mg mL^{-1}) was added and the mixture was vortexed thoroughly. This solution was dropped through a 27G needle (Sterican, B. Braun, Germany) into a 0.1 M $CaCl_2$ solution, which was continuously stirred. The droplets, which formed gel beads instantaneously, were cured in the $CaCl_2$ solution for an additional 12 h. After separation microparticles were washed three times with distilled water before air-drying at room temperature for 24 h.

Alginate/poly(acrylic acid) microparticles (AlgPAA)

A 2% (w/v) aqueous alginate solution (low and medium viscous, respectively; 1 mL) was added to 2 mL 1% (w/v) aqueous solution of neutralized poly(acrylic acid)$_{450}$. To this solution 0.2 mL insulin solution (35.3 mg mL^{-1}) was added and the mixture was vortexed thoroughly. The resulting solution was dropped through a 27G needle (Sterican, B. Braun, Germany) into a 0.1 M $CaCl_2$ solution, which was stirred continuously. After the microparticles formed, curing and drying procedures were carried out as described above.

Alginate-cysteine/poly(acrylic acid)-cysteine microparticles (AlgPAA-Cys)

Microparticles based on thiolated alginate (Alg-Cys) and PAA_{450} (PAA-Cys) were prepared in the same way as the corresponding particles based on unmodified polymers. Instead of alginate and poly(acrylic acid) the thiolated conjugates alginate-cysteine and PAA_{450}-cysteine were used. Curing and drying procedures were carried out as described above.

Determination of the thiol/disulfide content

The amount of free thiol groups immobilized on the thiomer backbone and within the resulting particles, i.e. the degree of modification, was determined photometrically with Ellman's reagent using a microplate reader (Fluostar Galaxy, BMG Labtechnologies, Offenburg, Germany). The total amount of sulfhydryl groups fixed on the polymer, represented by the summation of free thiol groups and of oxidized thiol moieties available in the form of disulfide bonds was quantified after reduction with $NaBH_4$ (Habeeb 1973).

Characterization of microparticles

Particle size determination

The sizes of 50 microparticles of each formulation were measured with a micrometer (FRITSCH Particlesizer, Idar-Oberstein, Germany) and the mean particle size was determined.

Scanning electron microscopy (SEM) analysis

The morphological structure of all microparticle samples was examined by a scanning electron microscope (JSM 53IOLV, Joel, Japan) at 25 kV without coating.

Determination of the drug loading

The encapsulation efficiency of insulin was analysed by an indirect method after separation of the microparticles from the aqueous calcium chloride solution containing the non-incorporated insulin. The amount of free insulin in the supernatant was quantified by a reversed phase HPLC method as described below. The drug loading was calculated as the difference between the total amount of insulin added and that remaining in the supernatant and calculated according to the equation:

$$\text{Encapsulation efficiency } (\%) = (\text{Dt}-\text{Ds}/\text{Dt}) \times 100 \quad (1)$$

where Ds is the amount of the drug in the supernatant and Dt the total (initial) amount. Concentrations were calculated by interpolation from a standard curve.

HPLC conditions

Analysis of peptide samples by reverse-phase HPLC (RP-HPLC) was conducted using a LaChrom Elite system (Merck/Hitachi, Japan). Samples were cooled at 10°C and eluted from a Nucleosil 100−5 C18 column (250×4 mm), maintained at 40°C, with mobile phase A: water, 0.1% TFA, B: acetonitrile, 0.1% TFA, and a flow rate of 1 mL min^{-1}. A linear gradient was applied from 80% A to 30% A over 15 min. Detection was performed by a diode array detector (DAD) at 220 nm.

Drug release studies

The release rate of insulin from various microparticles was analysed in-vitro in simulated intestinal fluid (SIF). Microparticles (1 mg) were placed in an Eppendorf vial containing 1 mL preheated release medium (37°C, 50 mM phosphate buffer pH 6.8, according to Stippler et al (2004)). The assembled vials (n = 3) were closed, placed on an oscillating water bath (GFL 1083) providing a constant temperature of 37°C and incubated for 3 h. At predetermined time points (15, 30, 45, 60, 120, 180 min) 150-μL samples were withdrawn and immediately replaced by an equal volume of preheated release medium. After centrifugation (13 400 rev min^{-1} for 5 min; MiniSpin, Eppendorf, Germany), 100-μL supernatant was transferred into a microtitration plate and the amount of released drug was quantified by RP-HPLC as described above. Concentrations were calculated by interpolation from a standard curve, whereas cumulative corrections were made for the previously removed samples in determining the total amount released.

Evaluation of the swelling behaviour

The water absorbing capacity of various microparticles in simulated gastric fluid (SGF) as well as simulated intestinal fluid (SIF) was determined by a gravimetric method. Therefore, 1 mg each microparticulate formulation was placed in an Eppendorf vial containing 1 mL 0.1 M HCl (adjusted to pH 1.2) and 50 mM phosphate buffer pH 6.8, respectively. The assembled vials (n = 3) were closed and samples were incubated under shaking on an oscillating water bath (GFL 1083) at 37°C. After 1 h, hydrated microparticles were removed from the incubation media and the weight of swollen particles

was determined gravimetrically. The swelling ratio was calculated according to the equation:

$$\text{Swelling ratio} = (Ws - Wd)/Wd \quad (2)$$

where Ws is the weight of swollen particles and Wd the weight of the dry particles.

Disintegration studies

The disintegration behaviour of various microparticles was evaluated visually. In brief, microparticles (1 mg) were placed in an Eppendorf vial containing 1 mL 100 mM EDTA-solution (adjusted to pH 7). The assembled vials (n = 3) were closed and samples were incubated under shaking on an oscillating water bath (GFL 1083) at 37°C for 6 h.

Statistical data analysis

Statistical data analysis was performed with the software SPSS. Depending on the data, the Kruskal–Wallis test, Mann–Whitney test or Friedman test was used. Post-hoc data analyses were performed with the Bonferroni test.

Results

Characterization of the thiomer conjugates

The amount of free thiol groups and disulfide bonds distributed all over the polymeric backbone, e.g. the degree of modification, of various thiomers generated was quantified via Ellman's reagent. The resulting alginate-cysteine conjugates (Alg-Cys), based on either low viscous or medium viscous alginate, both displayed approximately 300 μmol free thiol groups and approximately 50 μmol disulfide bonds per gram polymer, equivalent to a sulfhydryl disulfide ratio of 6:1. Therefore, these two thiolated alginates were comparable with each other concerning their properties provided by the immobilization of thiol moieties on the carbohydrate backbone. The results showed no significant difference ($P > 0.05$) in the post-hoc test. The resulting poly(acrylic acid)$_{450}$-cysteine conjugate (PAA-Cys) revealed a sulfhydryl disulfide ratio of 10:1 displaying approximately 1000 μmol free thiol groups and approximately 100 μmol disulfide bonds per gram polymer. Statistical analyses showed that the PAA-Cys conjugate differed significantly from both Alg-Cys (low viscous, medium viscous) conjugates ($P < 0.05$). The detailed values are summarized in Table 2.

Table 2 Characterization of the thiomers under study, concerning the amount of immobilized thiol groups on the polymeric backbone of alginate-cysteine (Alg-Cys), based on low and medium viscosity, respectively, and poly(acrylic acid)-cysteine (PAA$_{450}$-Cys)

Thiomer	-SH (μmol g^{-1})	-S-S- (μmol g^{-1})	Σ-SH (μmol g^{-1})
Alg-Cys (LV)	287 ± 15	47 ± 14	381 ± 43
Alg-Cys (MV)	328 ± 33	58 ± 5	444 ± 24
PAA$_{450}$-Cys	1001 ± 39	112 ± 7	1224 ± 53

Values are the means ± s.d., n = 3.

Characterization of microparticles

Thiolated anionic microparticles based on alginate-cysteine and poly(acrylic acid)-cysteine containing insulin as active drug were prepared by ionic gelation with calcium chloride. Microparticles based on unmodified alginate or unmodified alginate/poly(acrylic acid) prepared in the same way served as references in these studies.

The microparticles obtained were evaluated and compared concerning their particle size and superficial morphology, their drug load and release profile as well as their swelling and disintegration behaviour, respectively.

Particle size determination

The mean particle size of all microparticulate formulations developed within this study was in a range from 400 to 600 μm. As depicted in Figure 2, thiolated microparticles (AlgPAA-Cys) revealed the lowest particle size and were significantly smaller compared with the corresponding unmodified particles (AlgPAA) when analysing the data with the Kruskal–Wallis test and the corresponding post-hoc analysis. Although both types of alginates led to a similar particle size of the corresponding microparticles, it could be calculated with the Mann Whitney test that there was a significant difference in the particle size when using low viscous and medium viscous alginate ($P < 0.05$). However, the viscosity did not influence the particle size of AlgPAA-Cys ($P > 0.05$).

Scanning electron microscopy (SEM) analysis

Scanning electron microscopy was used to investigate the physical appearance of the microparticles. As illustrated in Figure 3, SEM micrographs revealed different morphological profiles for the three different types of microparticles.

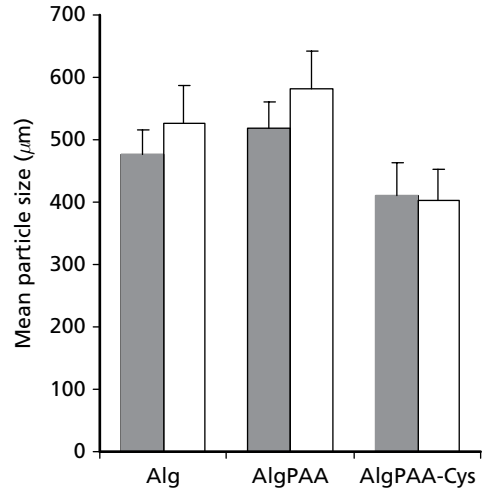

Figure 2 Particle sizes of alginate (Alg), alginate/poly(acrylic acid) (AlgPAA) and alginate-cysteine/poly(acrylic acid)-cysteine (AlgPAA-Cys) microparticles prepared by ionic gelation. Microparticles were based on low viscous (grey bars) or medium viscous (blank bars) alginate, respectively. Significantly different from AlgPAA particles: means ± s.d., n = 3.

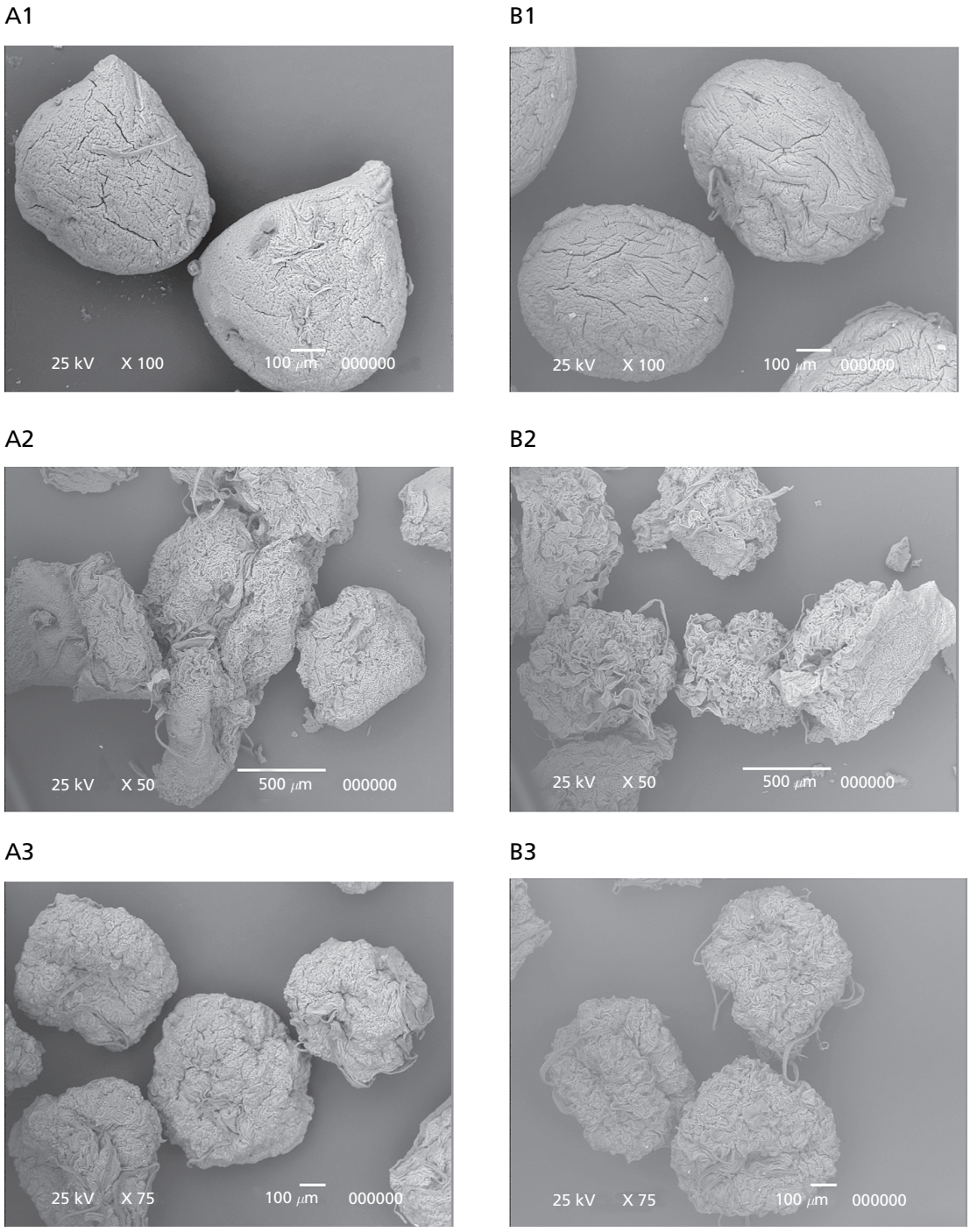

Figure 3 SEM micrographs of microparticles prepared by ionic gelation based on (A) low viscous and (B) medium viscous alginate, respectively: alginate (Alg) (1), alginate/poly(acrylic acid) (AlgPAA) (2) and alginate-cysteine/poly(acrylic acid)-cysteine (AlgPAA-Cys) (3).

Alginate particles showed a scarred surface representing the well known egg-box structure (A1/B1). Particles based on alginate/PAA revealed a 'head of lettuce'-like morphology and were likely to form aggregates (A2/B2). Thiolated microparticles in turn showed a more spherical structure than the corresponding particles based on unmodified polymers, representing a 'ball of wool' shape (A3/B3). Alginate and AlgPAA-Cys microparticles were almost spherical particles, whereas AlgPAA microparticles revealed a rather unsorted structure. Furthermore, no structural varieties could be observed between particles based on low viscous and medium viscous alginate, respectively.

Drug loading studies

The encapsulation efficiency of insulin turned out to be highly dependent on the polymeric matrix used for the various microparticles and increased within the following rank order: Alg < AlgPAA < AlgPAA-Cys, revealing a drug loading of approximately 15%, 40% and 65%, respectively ($P < 0.05$). The detailed values are summarized in Table 3. Microparticles based on medium viscous alginate and alginate-cysteine, respectively, showed no significant higher drug loading compared with particles based on the low viscous carbohydrate.

Release profile of insulin

The release of insulin was determined in-vitro in simulated intestinal fluid providing a pH of 6.8. As it is illustrated in Figure 4, alginate (Alg) microparticles displayed a burst release after 30 min. AlgPAA microparticles demonstrated a decelerated but still comparable release profile. In contrast, thiolated particles (AlgPAA-Cys) showed a controlled release of insulin over 3 h. The viscosity of the alginates employed had no influence on the drug release as particles based on both types, namely low and medium viscous alginate, revealed similar release profiles (Figure 4A and B). After 30 min, a significant difference in the release profile of the thiomer microparticles in comparison with the unmodified microparticles (both low viscous and medium viscous) could be observed ($P < 0.05$; Figure 4A and B).

Swelling behaviour

The swelling ratio of the microparticles was dependent on the pH of the surrounding medium. As illustrated in Figure 5, in simulated gastric fluid at pH 1.2 all microparticulate formulations revealed a moderate swelling ratio of approximately 2, whereas in simulated intestinal fluid at pH 6.8 microparticles exhibited a much higher water uptake. The polymeric matrix underlying the microparticles turned out to have an impact on the water uptake, which decreased within the following rank order: Alg > AlgPAA > AlgPAA-Cys, revealing a swelling ratio of approximately 45, 35 and 20, respectively. Thiolated microparticles exhibited a significant lower water uptake compared with the corresponding particles based on unmodified polymers ($P < 0.05$), but no significant difference between thiomer microparticles based on either low viscous or medium viscous alginate could be observed ($P > 0.05$). With regards to the microparticles based on alginate (Alg), they were broken after 1 h, displaying a gel rather than structured particles.

Table 3 Entrapment efficiency of insulin in various microparticles based on low viscous (LV) and medium viscous (MV) alginate, respectively

Microparticles	Entrapment efficiency (%)	
	LV	MV
Alg	11.92 ± 0.50	14.45 ± 0.28
AlgPAA	37.88 ± 0.19	41.31 ± 0.48
AlgPAA-Cys	64.43 ± 0.15	66.83 ± 0.05

Values are the means ± s.d. of three experiments.

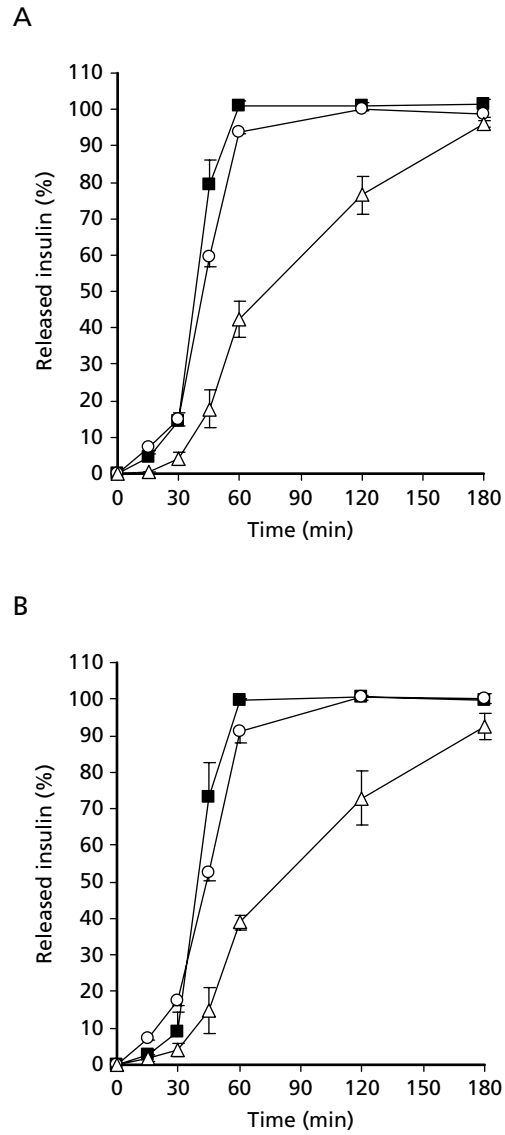

Figure 4 Release profile of insulin from microparticles based on (A) low viscous and (B) medium viscous alginate, respectively: alginate (Alg, ■), alginate/poly(acrylic acid) (AlgPAA, ○), and alginate-cysteine/poly(acrylic acid)-cysteine (AlgPAA-Cys, △). The release study was performed in 50 mM phosphate buffer pH 6.8 at 37°C. Indicated values are the means of three experiments ± s.d.

Degree of cross-linking within the microparticles

During the preparation process the thiomers used formed inter- and intramolecular disulfide bonds. Thiolated microparticles revealed that 85.3 ± 2.1% and 82.2 ± 4.9% of free thiol groups within the particles were oxidized referring to particles based on low viscous and medium viscous alginate, respectively. Therefore, an increased stability of the resulting microparticles was obtained as demonstrated by disintegration studies.

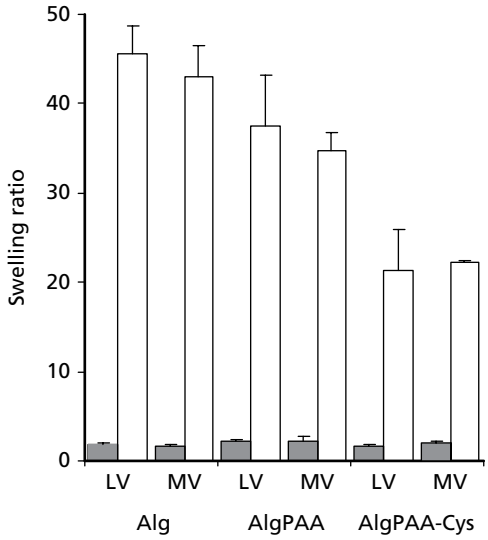

Figure 5 Swelling behaviour of alginate (Alg), alginate/poly(acrylic acid) (AlgPAA) and alginate-cysteine/poly(acrylic acid)-cysteine (AlgPAA-Cys) microparticles incubated either in 0.1 M HCl pH 1.2 (grey bars) or 50 mM phosphate buffer pH 6.8 (blank bars) at 37°C for 1 h. Microparticles were based on low viscous (LV) or medium viscous (MV) alginate. Significantly different from AlgPAA particles: indicated values are the means of three experiments ± s.d.

Disintegration studies

To show the stabilization of thiolated microparticles by the formation of intraparticulate disulfide bonds, stability studies were performed. The sequestration of calcium ions out of the particles as a result of the complexing properties of EDTA led to the disintegration of non-thiolated microparticles (Alg, AlgPAA) within 1 h. In contrast, microparticles based on thiolated polymers (AlgPAA-Cys) remained stable over the whole period of observation (6 h).

Discussion

The use of polysaccharide hydrogel microparticles, intended for various mucosal routes of application and prepared by different preparation methods, represents a common strategy in developing drug delivery systems for therapeutic peptides and proteins (Conti et al 1997; Felt et al 1998; Pereswetoff-Morath 1998; Wee & Gombotz 1998). Ionotropic gelation with calcium chloride, of which alginate is the most frequently described and used polysaccharide for protein encapsulation, offers the advantage of simple manufacturing and provides mild preparative conditions to prevent peptide degradation during the preparation process.

However, in the case of oral drug delivery, microparticles based on alginate prepared by this method rapidly disintegrate in the small intestine due to the sequestration of the cross-linking divalent cations by the surrounding milieu, therefore representing a drawback in the design of controlled-release dosage forms (Chan & Heng 2002). To prevent this erosion of alginate particles at higher pH leading to a burst release of the incorporated drug, gel microparticles have to be reinforced. This is achieved by the addition of chitosan in the majority of cases (Gonzalez-Rodriguez et al 2002; Ribeiro et al 2005).

Within this study a peroral microparticulate delivery system for insulin based on a thiomer matrix (alginate-cysteine and poly(acrylic acid)-cysteine) was developed by ionic gelation. The use of these two anionic thiomers led to improved stability and cohesive properties of the prepared microparticles, mainly due to the formation of inter- as well as intrachain disulfide bonds within the particles. These intraparticular disulfide bonds might also be responsible for the smaller size of the thiolated microparticles in comparison with microparticles based on the corresponding unmodified polymers and, furthermore, for the controlled sustained insulin release.

The ionic character of alginate as well as poly(acrylic acid) allowed pH-dependent swelling and disintegration of the microparticles, which also affected the release properties of these formulations. Recently, it was demonstrated that microparticles obtained by an emulsification solvent evaporation method comprising PAA disintegrated within minutes in simulated intestinal fluid, whereas the corresponding thiolated particles remained stable (Krauland & Bernkop-Schnürch 2004). Results obtained within this study were therefore in good correlation, as it was shown that particles based on alginate or alginate/PAA demonstrated a burst release in simulated intestinal fluid, whereas the thiolated particles achieved sustained release of insulin.

Apart from improved cohesive properties providing decelerated disintegration and sustained drug release, thiolated particles should also display permeation enhancing and enzyme inhibiting properties, being highly beneficial in the field of oral peptide delivery. The inhibitory effect of poly(acrylates) on intestinal proteases was first reported by Hutton et al (1990). Thiolated poly(acrylates) display an even pronounced inhibitory effect against carboxypeptidase A and B as well as aminopeptidase N (Bernkop-Schnürch & Thaler 2000; Bernkop-Schnürch et al 2001b). The permeation enhancing effect of PAA could significantly be improved due to the covalent attachment of cysteine to the polymeric backbone (Clausen & Bernkop-Schnürch 2000; Kast & Bernkop-Schnürch 2002).

Regarding the stabilization of alginate microparticles towards the intestinal milieu by the addition of chitosan, it is known that mixtures of anionic and cationic polymers display less mucoadhesive properties and less in-vivo effectiveness than the individual excipients (Luessen et al 1996). Both employed thiomers (alginate-cysteine and poly(acrylic acid)-cysteine) displaying improved mucoadhesive properties in comparison with the unmodified polymers (Bernkop-Schnürch et al 2001a; Marschütz & Bernkop-Schnürch 2002). Since both are anionic thiomers, a rather synergistic effect can be assumed. Furthermore, thiolated particles showed the highest drug loading of the hydrophilic model compound insulin, mainly due to the strongly improved cohesive properties. Even though microparticles based on medium viscous alginate and alginate-cysteine, respectively, showed a significantly higher drug loading compared with particles based on the low viscous polysaccharide, this observation seemed to be negligible concerning an overall characterization of the microparticles, since the obvious differences relating to encapsulation

efficiency were due to the polymeric matrix used (Alg, AlgPAA, AlgPAA-Cys).

Conclusions

Within this study insulin loaded alginate-cysteine/poly(acrylic acid)-cysteine (AlgPAA-Cys) microparticles were prepared and evaluated in-vitro. Thiolated particles showed the highest drug loading and they could achieve the controlled release of insulin over 3 h in simulated intestinal fluid. The particles based on unmodified polymers showed a burst release. Furthermore, due to intraparticulate cross-linking via disulfide bonds during the preparation process, thiolated particles revealed a more favourable disintegration profile. Results also demonstrated that all parameters investigated were highly dependent on the polymeric matrix underlying the various microparticles, whereas the viscosity of the employed alginates (low and medium viscous) had no influence on the particle properties. Therefore, AlgPAA-Cys microparticles seem to be an alternative tool for the oral administration of peptide drugs.

References

Bernkop-Schnürch, A., Fragner, R. (1996) Investigations into the diffusion behaviour of polypeptides in native intestinal mucus with regard to their peroral administration. *Pharm. Sci*. **2**: 361–363

Bernkop-Schnürch, A., Greimel, A. (2005) Thiomers: The next generation of mucoadhesive polymers. *Am. J. Drug Del*. **3**: 141–154

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

Bernkop-Schnürch, A., Kast, C. E., Richter, M. F. (2001a) Improvement in the mucoadhesive properties of alginate by the covalent attachment of cysteine. *J. Control. Release* **71**: 277–285

Bernkop-Schnürch, A., Zarti, H., Walker, G. F. (2001b) Thiolation of polycarbophil enhances its inhibition of soluble and intestinal brush border membrane bound aminopeptidase N. *J. Pharm. Sci*. **90**: 1907–1914

Bernkop-Schnürch, A., Kast, C. E., Guggi, D. (2003a) Permeation enhancing polymers in oral delivery of hydrophilic macromolecules: thiomer/GSH systems. *J. Control. Release* **93**: 95–103

Bernkop-Schnürch, A., Egger, C., Elhassan, I. M., Krauland, A. H. (2003b) Preparation and in vitro characterization of poly (acrylic acid)-cysteine microparticles. *J. Control. Release* **93**: 29–38

Chan, L. W., Heng. P. W. (2002) Effects of aldehydes and methods of cross-linking on properties of calcium alginate microspheres prepared by emulsification. *Biomaterials* **23**: 1319–1326

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

Conti, B., Giunchedi, P., Conte, U. (1997) Cellulose microparticles in drug delivery. *J. Drug Del. Sci. Tech*. **7**: 331–342

Coupe, A. J., Davis, S. S., Wilding, I. R. (1991) Variation in gastrointestinal transit of pharmaceutical dosage forms in healthy subjects. *Pharm. Res*. **8**: 360–364

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

Gonzalez-Rodriguez, M. L., Holgado, M. A., Sanchez-Lafuente, C., Rabasco, A. M., Fini, A. (2002) Alginate/chitosan particulate systems for sodium diclofenac release. *Int. J. Pharm*. **232**: 225–234

Habeeb, A. F. (1973) A sensitive method for localization of disulfide containing peptides in column effluents. *Analyt. Biochem*. **56**: 60–65

Hutton, D. A., Pearson, J. P., Allen, A., Foster, S. N. (1990) Mucolysis of the colonic mucus barrier by faecal proteinases: inhibition by interacting polyacrylate. *Clin. Sci*. **78**: 265–271

Kast, C. E., Bernkop-Schnürch, A. (2002) Influence of the molecular mass on the permeation enhancing effect of different poly(acrylates). *J. Drug Del. Sci. Tech*. **12**: 351–356

Krauland, A. H., Bernkop-Schnürch, A. (2004) Thiomers: development and in vitro evaluation of a peroral microparticulate peptide delivery system. *Eur. J. Pharm. Biopharm*. **57**: 181–187

Lee, V. H. (1988) Enzymatic barriers to peptide and protein absorption. *Crit. Rev. Ther. Drug Carrier Syst*. **5**: 69–97

Leonard, M., Rastello de Boisseson, M., Hubert, P., Dellacherie, E. (2004) Production of microspheres based on hydrophobically associating alginate derivatives by dispersion/gelation in aqueous sodium chloride solutions. *J. Biomed. Mater. Res. Part A*. **68**: 335–342

Luessen, H. L., de Leeuw, B. J., Langemeyer, M. W., de Boer, A. B., Verhoef, J. C., Junginger, H. E. (1996) Mucoadhesive polymers in per oral peptide drug delivery. VI. Carbomer and chitosan improve the intestinal absorption of the peptide drug buserelin in vivo. *Pharm. Res*. **13**: 1668–1672

Marschütz, M. K., Bernkop-Schnürch, A. (2002) Thiolated polymers: self-crosslinking properties of thiolated 450 kDa poly(acrylic acid) and their influence on mucoadhesion. *Eur. J. Pharm. Sci*. **15**: 387–394

Pereswetoff-Morath, L. (1998) Microspheres as nasal drug delivery systems. *Adv. Drug Del. Rev*. **29**: 185–194

Ribeiro, A. J., Silva, C., Ferreira, D., Veiga, F. (2005) Chitosan-reinforced alginate microspheres obtained through the emulsification/internal gelation technique. *Eur. J. Pharm. Sci*. **25**: 31–40

Stippler, E., Kopp, S., Dressman, J. B. (2004) Comparison of US Pharmacopeia Simulated Intestinal Fluid TS (without pancreatin) and Phosphate Standard Buffer pH 6.8, TS of the International Pharmacopoeia with respect to their use in in vitro dissolution testing. *Dissolut. Technol*. **11**: 6–10

Wee, S., Gombotz, W. R. (1998) Protein release from alginate matrices. *Adv. Drug Del. Rev*. **31**: 267–285

Woodley, J. F. (1994) Enzymatic barriers for GI peptide and protein delivery. *Crit. Rev. Ther. Drug Carrier Syst*. **11**: 61–95