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Review

Chitosan and its derivatives: potential excipients for peroral peptide delivery systems

Andreas Bernkop-Schnürch *

Institute of Pharmaceutical Technology, Centre of Pharmacy, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

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Abstract

In the 1990s chitosan turned out to be a useful excipient in various pharmaceutical formulations. By modifications of the primary amino group at the 2-position of this poly(β1→4 D-glucosamine), the features of chitosan can even be optimised according to a given task in drug delivery systems. For peroral peptide delivery these tasks focus on overcoming the absorption (I) and enzymatic barrier (II) of the gut. On the one hand, even unmodified chitosan proved to display a permeation enhancing effect for peptide drugs. On the other hand, a protective effect for polymer embedded peptides towards degradation by intestinal peptidases can be achieved by the immobilisation of enzyme inhibitors on the polymer. Whereas serine proteases are inhibited by the covalent attachment of competitive inhibitors such as the Bowman–Birk inhibitor, metallo-peptidases are inhibited by chitosan derivatives displaying complexing properties such as chitosan-EDTA conjugates. In addition, because of the mucoadhesive properties of chitosan and most of its derivatives, a presystemic metabolism of peptides on the way between the dosage form and the absorption membrane can be strongly reduced. Based on these unique features, the co-administration of chitosan and its derivatives leads to a strongly improved bioavailability of many perorally given peptide drugs such as insulin, calcitonin and buserelin. These polymers are therefore useful excipients for the peroral administration of peptide drugs.

Keywords: Chitosan; Chitosan derivatives; Peroral peptide application; Drug delivery systems

1. Introduction

Natural produced polymers such as cellulose, starch, pectine and alginate represent biodegradable and toxicological harmless raw-materials of low costs. They have therefore been used as abundant excipients in various pharmaceutical formulations for many decades. Because of progress in pharmaceutical sciences leading to more and more sophisticated drug delivery systems, however, the features of these polymers became in many cases insufficient, which has intensified the search for

* Tel.: +43-1-313368476; fax: +43-1-31336779.
E-mail address: andreas.bernkop-schnuerch@univie.ac.at (A. Bernkop-Schnürch)

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new, more specific and suitable polymers. A promising strategy in this direction is the chemical modification of natural produced polymers e.g. the development of cellulose derivatives such as methylcellulose or sodium carboxymethylcellulose. Among these natural produced polymers with properties for chemical modifications, in particular chitin has gained considerable attention. The deacetylation of chitin, which can be isolated from insects, crustacea such as crab and shrimp as well as from fungi such as *Aspergillus niger* (Felt et al., 1998), leads to poly(β1→4 D-glucosamine) or so called chitosan. Because of its superior characteristics together with a very save toxicity profile (Arai et al., 1968), chitosan is widely used as pharmaceutical excipient (Illum, 1998). Due to the primary amino group at the 2-position of each polymer-subunit further chemical modifications are easy feasible. By these modifications, the features of chitosan can be optimised according to a given task in delivery systems focusing on specific pharmaceutic-technological challenges.

One of such challenges is the peroral administration of peptide drugs where chitosan and its derivatives have gained considerable interest in recent years. As oral formulations for therapeutic peptides promise the greatest ease of application and a high patient compliance, thereby excluding any risks such as infections caused by non-sterile needles or haemolytic effects, pharmaceutical industry as well as practitioners involved in the health-system are very interested in such delivery systems. The efficacy of oral formulations, however, is harmed by different barriers encountered with the GI-tract. In general, they can be divided into the absorption (Zhou, 1994) and the enzymatic barrier (Woodley, 1994) which are mainly responsible for a very low bioavailability of orally given peptides and proteins. Because of their permeation enhancing effect (I), enzyme inhibitory capabilities (II), and mucoadhesive properties (III), chitosan and its derivatives are able to reduce both barriers, which makes these polymers important excipients for peroral peptide delivery systems. An overview of chitosan and modified chitosans used in such formulations should provide a good starting point for further research and development in this direction.

2. Permeation enhancement

In particular for peptides displaying a molecular size greater than 30 Å, the intestinal membrane becomes an important rate limiting factor for drug absorption (Lee, 1995). In order to reduce this barrier, the use of permeation enhancers seems to be essential for most peroral peptide delivery systems. Generally, such permeation enhancers can be divided into surfactants, fatty acids, salicylates, chelating agents and swellable polymers (Aungst et al., 1996). The latter ones — especially if they are mucoadhesive — offer the advantage of remaining concentrated at the absorption site of the drug. Beside poly(acrylic acid) derivatives and starch, also chitosan is reported to display such permeation enhancing capabilities which has first been shown by Illum et al. (1994). Chitosan is thereby able to enhance the paracellular route of absorption, which is important for the transport of hydrophilic compounds such as peptides and proteins across the membrane. Various studies carried out on Caco-2 cell monolayers demonstrated a significantly decrease in the transepithelial electrical resistance after the addition of chitosan (e.g. Artursson et al., 1994; Borchard et al., 1996; Dodane et al., 1999). The mechanism underlying this permeation enhancing effect seems to be based on the positive charges of the polymer, which interact with the cell membrane resulting in a structural reorganisation of tight junction-associated proteins (Schipper et al., 1997). Schipper et al. (1996) could also demonstrate that the structural properties of chitosan, i.e. molecular mass and degree of deacetylation, dictate absorption enhancing properties and toxicity to a large extent. Chitosans exhibiting a high degree of deacetylation and high molecular mass display the comparatively highest increase in epithelial permeability as illustrated in Fig. 1. As the features of chitosan determining absorption enhancement are not correlated with those determining toxicity, a selection of chitosans with maximal effect on absorption and minimal toxicity seems to be feasible. Due to the addition of chitosan, so far, a significantly increased transport of buserelin, 9-desglycinamide 8-L-arginine vasopressin and insulin in Caco-2 cell monolayers

could be demonstrated (Kotzé et al., 1997; Lueßen et al., 1997). In the presence of the mucus layer, however, this permeation enhancing effect is comparatively lower, as chitosan cannot reach the epithelium because of size limited diffusion and/or competitive charge interactions with mucins (Schipper et al., 1999). Nevertheless, these results obtained on Caco-2 cell monolayers could be confirmed by in vivo studies, showing an enhanced intestinal absorption of the peptide drug buserelin in rats due to the co-administration of chitosan hydrochloride (Lueßen et al., 1996). Results of this study are shown in Fig. 2.

Whereas unmodified chitosan is only soluble in the acid milieu, its solubility can also be guaranteed at pH-values above 7 by the trimethylation of its primary amino groups. Although this novel chitosan derivative as depicted in Fig. 3 showed in vitro weaker permeation enhancing properties than unmodified chitosan (Kotzé et al., 1999), trimethylated chitosan nevertheless might be a useful tool for delivery systems targeting to the large intestine and colon, where a neutral or even basic environment has to be expected (Kotzé et al., 1998).

Recently, our research group could generate a new chitosan derivative displaying improved permeation enhancing properties. Based on a carbodiimide mediated formation of amide bonds, L-cysteine was thereby covalently linked to chitosan. Whereas only 0.8% of fluoresceine isothiocyanate (FITC) labelled bacitracin could pass the intestinal mucosa of guinea pigs within 3 h due to the addition of 0.5% (m/v) chitosan hydrochloride, it was 1.3% of bacitracin-FITC for the corresponding thiolated polymer. In contrast, a mixture of chitosan hydrochloride and unbound cysteine did not display such an improved permeation enhancing effect (Bernkop-Schnürch et al., 1999).

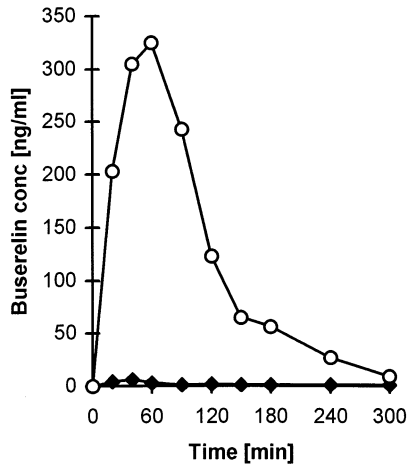

Fig. 2. Serum concentrations after intraduodenal application of buserelin (500 μg/rat). ◆, control (buffer solution pH 6.7); ○, 1.5% (m/v) chitosan hydrochloride. Indicated values are means of at least five experiments (adapted from Lueßen et al., 1996).

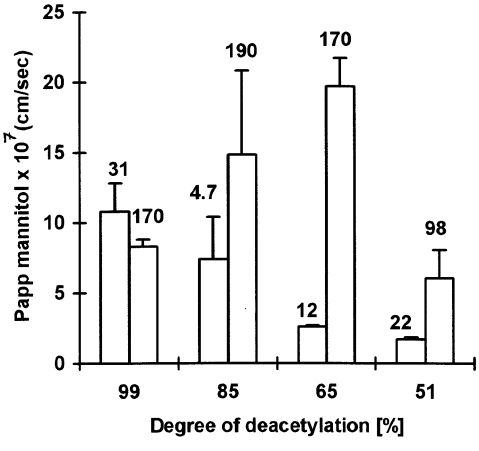

Fig. 1. The mean Papp + S.D. of mannitol across Caco-2 cell monolayers during exposure to 50 μg/ml chitosan. Indicated numbers associated with the bars in the graph show the molecular mass of studied chitosan in kDa (adapted from Schipper et al., 1996).

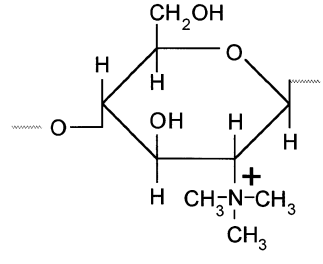

Fig. 3. Structure of chitosantrimethylate.

3. Inhibition of pancreatic serine-proteases

Apart from the absorption barrier, the enzymatic barrier is also responsible for the very poor bioavailability of perorally administered peptide drugs. The pancreatic serine-proteases: trypsin, chymotrypsin and elastase are in many cases responsible for the presystemic metabolism of perorally given (poly)peptide drugs. Ikesue et al. (1993), for instance, demonstrated that insulin is strongly degraded by trypsin, chymotrypsin and elastase, whereas almost no degradation caused by brush border membrane bound enzymes could be observed. Strategies to avoid such a presystemic metabolism include the use of liposomes, micro- and nanoparticles protecting the incorporated peptide drug towards an enzymatic attack in the gut and the use of delivery systems targeting to the colon where the enzymatic activity is comparatively low (Bernkop-Schnürch, 1997). In addition, the use of enzyme inhibitors has also received considerable attention within recent years, as their co-administration leads to a tremendously improved bioavailability of perorally given peptides (e.g. Fujii et al., 1985; Morishita et al., 1992; Langguth et al., 1994; Yamamoto et al., 1994). Co-administrated enzyme inhibitors, however, are strongly diluted in the intestinal fluid, making the use of high amounts of these auxiliary agents necessary, subsequently leading to various systemic toxic side effects (e.g. Melmed et al., 1976; Ge and Morgan, 1993). Recently this problem could be solved by the covalent immobilisation of inhibitors to polymers used as drug carrier matrices, thereby excluding such dilution effects. Among such polymer-inhibitor conjugates, chitosan-inhibitor conjugates have also received considerable attention. In order to make chitosan capable of inhibiting intestinal proteases, various enzyme inhibitors have been covalently linked to this polymer. As a matter of fact that numerous inhibitors of serine proteases exhibit a carbonic acid moiety which is not located in the active site of the inhibitor, these auxiliary agents can be directly bound to chitosan by the formation of amide bonds. Although a spacer is thereby missing which might provide an easier accessibility of the immobilised inhibitor for

Table 1
Chitosan derivatives exhibiting enzyme inhibitory properties[a]

Chitosan derivative	Enzyme(s) inhibited	Reference(s)
Chitosan–antipain	Trypsin (E.C. 3.4.21.4)	(Bernkop-Schnürch et al., 1997a; Bernkop-Schnürch and Scerbe-Saiko, 1998)
Chitosan–chymostatin	Chymotrypsin (E.C. 3.4.21.1)	(Bernkop-Schnürch and Scerbe-Saiko, 1998)
Chitosan–elastatinal	Elastase (E.C. 3.4.21.36)	(Bernkop-Schnürch and Scerbe-Saiko, 1998)
Chitosan–EDTA	Carboxypeptidase A (E.C. 3.4.17.1), Carboxypeptidase B (E.C. 3.4.17.2), Aminopeptidase N (E.C. 3.4.11.2)	(Bernkop-Schnürch et al., 1997b; Bernkop-Schnürch and Krajicek, 1998)
Chitosan–EDTA–antipain	Trypsin, Carboxypeptidase A, Carboxypeptidase B, Aminopeptidase N	(Bernkop-Schnürch and Scerbe-Saiko, 1998)
Chitosan–EDTA–chymostatin	Chymotrypsin, Carboxypeptidase A, Carboxypeptidase B, Aminopeptidase N	(Bernkop-Schnürch and Scerbe-Saiko, 1998)
Chitosan–EDTA–elastatinal	Elastase, Carboxypeptidase A, Carboxypeptidase B, Aminopeptidase N	(Bernkop-Schnürch and Scerbe-Saiko, 1998)
Chitosan–EDTA–BBI	Trypsin, Chymotrypsin, Elastase, Carboxypeptidase A, Aminopeptidase N	(Bernkop-Schnürch and Pasta, 1998)
Chitosan–DTPA	Carboxypeptidase A, Aminopeptidase N	(Bernkop-Schnürch and Freudl, 1999)

[a] BBI: Bowman–Birk inhibitor, EDTA: ethylenediaminetetraacetic acid, DTPA: diethylenetriaminepentaacetic acid.

Table 2
Intestinal brush border membrane bound enzymes and their co-factors (Bernkop-Schnürch, 1998)

Membrane bound peptidases	Co-Factor(s)	Membrane bound peptidases	Co-Factor(s)
Aminopeptidase N (EC 3.4.11.2)	Zinc, cobalt	γ-Glutamyl Transpeptidase (EC 2.3.2.2)	Magnesium
Aminopeptidase A (EC 3.4.11.7)	Zinc, calcium	Peptidyl dipeptidase A (EC 3.4.15.1)	Zinc
Aminopeptidase P (EC 3.4.11.9)	Zinc, manganese	Carboxypeptidase M (EC 3.4.17.12)	Zinc
Aminopeptidase W (EC 3.4.11.16)	Zinc	Carboxypeptidase P (EC 3.4.17.16)	Zinc, mangan
Leucin aminopeptidase (EC 3.4.11.1)	Zinc, magnesium, manganese	Neutral endopeptidase (EC 3.4.24.11)	Zinc
Dipeptidyl peptidase IV (EC 3.4.14.5)	Zinc	Endopeptidase-24.18 (EC 3.4.24.18)	Zinc

the corresponding enzyme, such chitosan-inhibitor conjugates nevertheless turned out to display a strong inhibitory capability (Bernkop-Schnürch et al., 1997a; Bernkop-Schnürch and Scerbe-Saiko, 1998). Table 1 shows so far synthesised and evaluated chitosan-inhibitor conjugates representing useful tools for peroral peptide delivery systems.

4. Inhibition of metallo-peptidases

Besides pancreatic serine proteases, metallo-peptidases represent the second major group of enzymes being responsible for the presystemic metabolism of orally administered therapeutic peptides. Generally, intestinal metallo-peptidases can be divided into the luminally secreted enzymes carboxypeptidase A and B and the brush border membrane bound enzymes as listed in Table 2. Complexing agents are well known to be able to inhibit these peptidases because of the deprivation of the essential divalent cation out of the enzyme structure. Whereas zinc ions represent the co-factor of carboxypeptidase A and B, there are also further metal ions essential for the activity of brush border membrane bound enzymes as shown in Table 2. In order to keep the complexing agent concentrated in the area, where an enzyme inhibition is required, the immobilisation of these auxiliary agents on the drug carrier matrix represents a promising strategy (Bernkop-Schnürch, 1999).

Chitosan itself is well known as an excellent natural metal adsorbent with much higher selectivity than usual commercial chelating resins and high loading capacity (Inoue, 1997). These features of chitosan are considered to be attributable to the following factors:

1. high hydrophilicity of chitosan displaying a large number of hydroxyl groups,
2. large number of primary amino groups with high activity as adsorption sites,
3. flexible structure of polymer chain of chitosan which enables to take suitable configuration for the complexation with metal ions (Inoue et al., 1996).

This complexing capability of chitosan per se, however, turned out to be quite insufficient in order to inhibit metallo-peptidases. Lueßen et al. (1997), for instance, had to realise that the zinc-dependent exopeptidase carboxypeptidase B cannot be inhibited by chitosan. These results led to the development of chemically modified chitosans displaying much higher complexing properties. So far, chitosan-nitrilotriacetic acid (-NTA) (Tikhonov et al., 1996), -ethylenediaminetetraacetic acid (-EDTA) (Bernkop-Schnürch and Krajicek, 1998) and -diethylenetriaminepentaacetic acid (-DTPA) conjugates (Bernkop-Schnürch and Freudl, 1999) have been generated.

4.1. Chitosan–NTA conjugates

In the presence of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide, nitrilotriacetic acid (NTA) can be covalently linked to chitosan thereby forming iminodiacetate residues as shown in Fig. 4. Raising the amount of carbodiimide during the coupling reaction leads to a crosslinking of the polymer chains, as NTA is covalently attached to

more than only one primary amino group of chitosan. Both iminodiacetate and iminomonoacetate residues are able to form 1:1-type complexes with divalent cations (Tikhonov et al., 1996). The pharmaceutical benefit of such complexing polymers, however, has so far not been investigated.

4.2. Chitosan–EDTA conjugates

The search for more effective polymer-complexing agent conjugates led to chitosan–EDTA conjugates. The covalent attachment of this complexing agent to chitosan was achieved by the formation of amide bonds between amino groups of the polymer and carboxyl groups of EDTA mediated by a carbodiimide. EDTA was thereby linked to almost each primary amino group of chitosan (Bernkop-Schnürch and Krajicek, 1998). Due to its strong complexing capability, chitosan–EDTA is a very potent inhibitor of various metallo-peptidases. The degradation of the peptide drug leucine enkephalin on porcine mucosa, for instance, could be 3-fold reduced in the presence of chitosan–EDTA (Bernkop-Schnürch et al., 1997b). Furthermore, its inhibitory activity towards the luminally secreted enzymes carboxypeptidase A and B could be demonstrated (Bernkop-Schnürch and Scerbe-Saiko, 1998). In comparison with other anionogenic polymers such as polycarbophil displaying complexing properties as well, chitosan-EDTA conjugates are to a higher extent compatible with divalent cations e.g. leading only to a coagulation with calcium ions in concentrations higher than 5 mM (Valenta et al., 1998).

4.3. Chitosan–DTPA conjugates

In order to generate chitosan-derivatives exhibiting even higher complexing properties than the chitosan–EDTA conjugate, diethylenetriaminepentaacetic acid (DTPA) — displaying a relative higher association constant towards zinc than EDTA, determined to be $10^{18.6}$ and $10^{16.5}$, respectively — was covalently attached to the polymer as well. Although the concentration of DTPA was two times higher than that of EDTA during the coupling reaction, the amount of covalently bound DTPA was comparatively lower. Whereas the synthesis of chitosan–EDTA conjugates leads to an almost quantitative modification of all primary amino groups (Bernkop-Schnürch and Krajicek, 1998), only $63.8 \pm 5.8\%$ ($n = 3$; $\pm$ SD) of the amino groups could be modified by DTPA. A reason for this observation can be seen in a possible steric hindrance caused by already covalently bound DTPA which might restrict the linkage of another DTPA molecule to the vicinally located primary amino group of the polymer. Accordingly, in contrast to chitosan–EDTA chitosan–DTPA represents a polymer of cationic as well as anionic sub-structures making it easy swellable in the acid and alkaline milieu. The presumptive structure of the chitosan–EDTA and –DTPA conjugates is shown in Fig. 4. Although DTPA displays a higher association constant towards zinc than EDTA, the inhibitory effect of the chitosan–EDTA conjugate towards carboxypeptidase A and aminopeptidase N was more

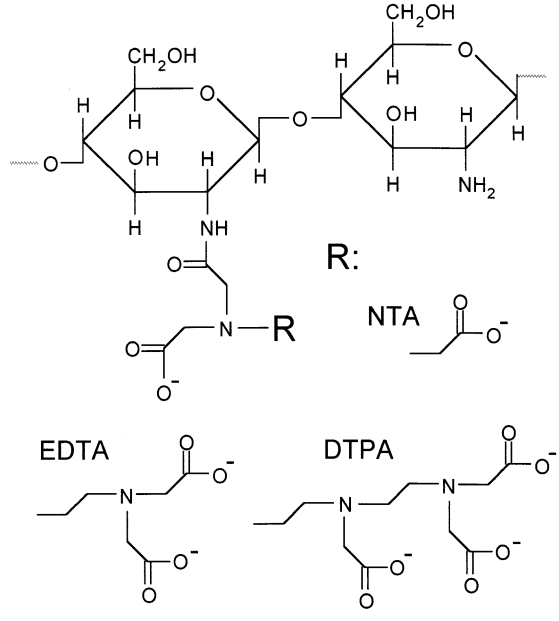

Fig. 4. Chitosan-conjugates with acidic chelate-complex forming agents: NTA: nitrilotriacetic acid; EDTA: ethylenediaminetetraacetic acid; DTPA: diethylenetriaminepentaacetic acid (adapted from Bernkop-Schnürch and Paikl, 1998).

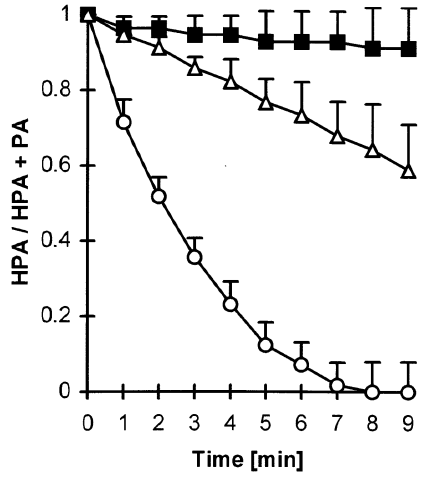

Fig. 5. Comparison of the inhibitory effect of chitosan–DTPA and chitosan–EDTA towards carboxypeptidase A; Hydrolysis of hippuryl-L-phenylalanine (HPA) to L-phenylalanine (PA) and hippuric acid by carboxypeptidase A (0.25 units/ml) in presence of 0.2% chitosan–DTPA (△—△), 0.2% chitosan–EDTA (■—■), and without any chitosan derivative (○—○). Each point represents the mean ± SD of at least three experiments (adapted from Bernkop-Schnürch and Freudl, 1999).

pronounced than that of the chitosan–DTPA conjugate. Results of one of these enzyme inhibition studies are shown in Fig. 5. This lower inhibitory effect could be explained by the fact that DTPA was bound to approximately only each second primary amino group of chitosan, whereas EDTA was bound to almost each amino group of the polymer. Accordingly, on the one hand the binding capacity of the chitosan–DTPA conjugate towards zinc should be lower, and on the other hand the remaining primary amino groups might interfere with the complexation of zinc ions. Generally, an interference of the complexation of zinc ions by other divalent cations — in particular by calcium ions with a determined concentration of 0.4–0.7 mM in the intestinal fluid (Lindahl et al., 1997) — should be negligible, as the association constant of calcium towards DTPA and EDTA, determined to be $10^{10.9}$ and $10^{10.7}$, respectively, is approximately 10^8 and 10^6 times lower than that of zinc.

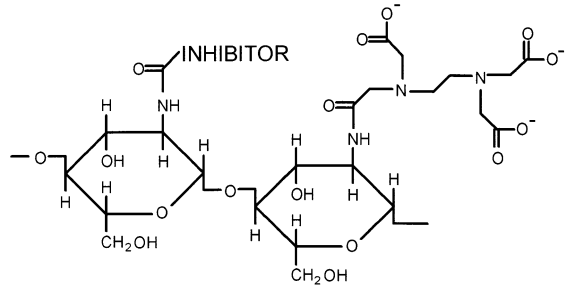

Fig. 6. Presumptive structure of chitosan-inhibitor–EDTA conjugates; covalent attachment of inhibitors (antipain, chymostatin and elastatinal) and the complexing agent (EDTA) was achieved by the formation of an amide bond between a primary amino group of the polymer and a carboxylic acid group of the inhibitor or EDTA (adapted from Bernkop-Schnürch and Scerbe-Saiko, 1998).

5. Combination of different inhibitory effects

The inhibition of serine proteases as well as metallo-peptidases makes the combination of competitive enzyme inhibitors and complexing agents necessary. In order to achieve that goal, the competitive inhibitors antipain, chymostatin and elastatinal have been covalently bound to chitosan. In a second step, EDTA was immobilised to the remaining primary amino groups of the chitosan-inhibitor conjugate leading to polymers as shown in Fig. 6. The former cationogenic polymer became thereby an anionogenic polymer displaying strong mucoadhesive properties. Whereas the polymer immobilised inhibitors provide a protective effect towards pancreatic serine proteases, the immobilised complexing agent guarantees an inhibition of various metallo-peptidases (Bernkop-Schnürch and Scerbe-Saiko, 1998).

In another approach, EDTA was first of all covalently bound to the entire primary amino groups of chitosan. In a second step, the Bowman–Birk inhibitor (Birk, 1985) was immobilised to chitosan–EDTA. Mediated by a carbodiimide, the inhibitor was linked to the polymer by the formation of amide bonds between the primary amino groups of the inhibitor protein and the carboxylic acid groups of the polymer. The resulting chitosan–EDTA BBI conjugate, as depicted in Fig. 7, showed a strong protective effect to-

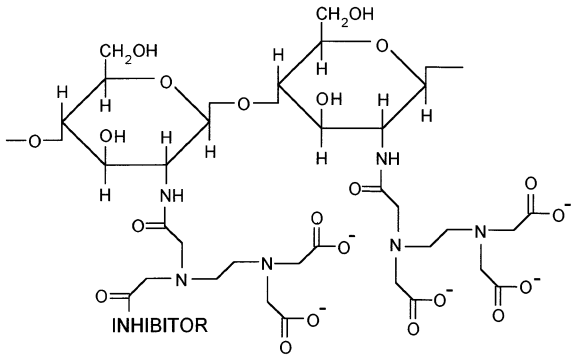

Fig. 7. Presumptive structure of the chitosan–EDTA Bowman–Birk inhibitor (BBI) conjugate; covalent attachment of the Bowman–Birk inhibitor was achieved by the formation of amide bonds between a primary amino group of the protein and a carboxylic acid group of chitosan–EDTA (adapted from Bernkop-Schnürch and Pasta, 1998).

wards trypsin and chymotrypsin. However, the protective effect towards elastase was markedly lower. Due to the high binding affinity of EDTA towards zinc, the zinc-dependent exopeptidases carboxypeptidase A and aminopeptidase N were also inhibited by this polymer-conjugate (Bernkop-Schnürch and Pasta, 1998).

6. Mucoadhesion

Although it has so far been impossible to improve the GI-transit time of pharmaceutical formulations due to the use of mucoadhesive polymers in man (Khosla and Davis, 1987), their benefit could already be verified in various animal models. Especially for peroral peptide delivery systems, from which the therapeutic agent is released in the intestine, the mucoadhesive properties of the dosage form seem to have an important influence on bioavailability. If the delivery system is not adhesive, the released peptide drug will be degraded by luminally secreted proteases on the way between the dosage form and the absorption membrane. Such a presystemic metabolism can be strongly reduced by mucoadhesive formulations providing an intimate contact with the intestinal mucosa. In addition, a somewhat prolonged intestinal transit time and a higher concentration gradient should be provided by mucoadhesive delivery systems (Lehr, 1994). In the 1980s it was believed that (I) strong hydrogen-bonding groups (-OH, -COOH), (II) strong anionic charges, (III) high molecular weight and (IV) sufficient chain flexibility are responsible for mucoadhesion. This theory, however, had to be changed at the beginning 1990s, when Hassan and Gallo (1990) could demonstrate that the positively charged polymer chitosan also displays strong mucoadhesive properties. This phenomena can be explained by electrostatic interactions of the polymer with negatively charged groups such as sialic acid moieties of the mucus layer (Hassan and Gallo, 1990; Lehr et al., 1992). Due to the use of mucoadhesive chitosan-coated liposomes, for instance, the bioavailability of perorally administered insulin could be strongly improved (Takeuchi et al., 1996). Results of this study are shown in Fig. 8.

A further improvement of the mucoadhesive properties of chitosan could be achieved by the covalent attachment of ethylenediaminetetraacetic acid (EDTA). The cationic polymer becomes thereby anionogenic. Results of mucoadhesion studies as shown in Table 3 demonstrate that both the exclusive cationic polymer chitosan and the almost entire anionic polymer chitosan–EDTA

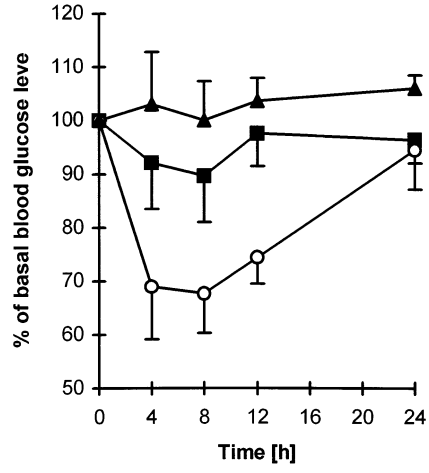

Fig. 8. Change in basal blood glucose level (%) after peroral administration of insulin encapsulated in liposomes. ▲: control without liposomes; ■: uncoated liposomes; ○: chitosan-coated liposomes. Indicated values are means of at least five experiments ± SD (adapted from Takeuchi et al., 1996).

Table 3
Comparison of the mucoadhesive properties of various chitosan derivatives (Bernkop-Schnürch and Freudl, 1999)

Polymer	Maximum detachment force (mN) ± S.D., $n = 4–5$
Chitosan HCl	32.4 ± 14.5
Chitosan–DTPA	3.0 ± 1.3
Chitosan–EDTA	81.7 ± 9.9
Control (no disc)	1.3 ± 0.1

exhibit a significantly higher maximum detachment force than the cationic as well as anionic polymer chitosan–DTPA. On the one hand, mucoadhesion of cationic polymers seems to be based on electrostatic interactions with negatively charged moieties of the mucus (Hassan and Gallo, 1990) and, on the other hand, for anionic polymers such as chitosan–EDTA mucoadhesion can be explained by the hydrogen bond formation of their carboxylic acid groups with the mucus gel layer. The combination of cationic as well as anionic moieties on the same polymer might compensate both effects. These results are in good accordance with earlier investigations demonstrating that the adhesive properties of chitosan–EDTA conjugates are strongly reduced if there are remaining primary amino groups on the polymer conjugate (Bernkop-Schnürch and Krajicek, 1998). Similar observations were made by Lueßen et al. (1996) after the peroral administration of buserelin in rats. Whereas the co-administration of chitosan hydrochloride leads to a strongly increased intestinal buserelin bioavailability, it was much lower using a mixture of the cationic polymer chitosan with the anionic polymer carbomer. Reasons for these results can be seen in reduced mucoadhesive and/or absorption enhancing properties based on a reduced charge density which has to be taken into consideration for the chitosan-DTPA conjugate as well.

7. Delivery systems based on chitosan and its derivatives

The type of the peroral delivery system for peptide drugs mainly relies on the predominant task of the dosage form. Depending on the structure of the therapeutic peptide either the permeation enhancing effect, the protective effect, or the mucoadhesive properties are prevalent. Whereas the permeation enhancing effect for peptides of a molecular size smaller than 30 Å in diameter seems to be less important, it becomes essential for peptides displaying a molecular size above this presumptive 'cut off' of the absorption membrane. For therapeutic peptides, which are rapidly degraded by intestinal enzymes, on the other hand, the protective and mucoadhesive properties of the delivery system are more substantial. The use of chitosan and its derivatives as drug carrier matrix per se (I) might thereby allow the development of formulations which can guarantee the benefit of all these properties. In contrast, if these polymers are used as coating material (II), their mucoadhesive properties become the focus of attention.

7.1. Carrier matrices

As tablets provide an accurate dosage and are easily to manufacture and handle, so far the development of delivery systems based on chitosan and its derivatives focused in many cases on this type of dosage form. The peptide drug can thereby be homogenised with chitosan and/or its derivatives and directly compressed to tablets. Aside from the protective effect of chitosan-inhibitor and/or chitosan-complexing agent conjugates, the dosage form itself has thereby also an important influence on the protection of incorporated therapeutic peptides. Luminally secreted proteases first of all have to penetrate into the polymeric network of the hydrophilic matrix tablet in order to degrade the embedded peptide drug. Hence, matrix tablets based on chitosan or its derivatives, even exhibiting no inhibitory effect, can provide at least a partial protection towards an intestinal enzymatic attack. In order to make use of this effect, the cohesiveness of the polymer with the incorporated therapeutic agent should be as high as possible. A rapid disintegration of the delivery system would certainly lead to a strong increase in the surface area of the dosage form thereby leading to a much higher accessibil-

ity of embedded peptide drugs for intestinal proteases. Strategies to improve the cohesiveness of chitosan and its derivatives include the use of ionic and covalent crosslinkers, which can guarantee a markedly higher mechanical stability of the carrier matrix. However, the crosslinking of mucoadhesive polymers leads to a slower drug release as well as to reduced mucoadhesive properties (Bernkop-Schnürch et al., 1998a). The cohesiveness can therefore not be raised ad libitum by a crosslinking of the polymer. Alternatively, the cohesiveness of these polymers can be raised to some extent by precipitating them in organic solvents and air-drying instead of lyophilisation (Bernkop-Schnürch et al., 1998a).

Even if the dosage form does not disintegrate within several hours, its protective effect for the incorporated peptide drug is still quite limited. The addition of chitosan-inhibitor conjugates, however, can guarantee a much higher protective effect. Insulin, for instance, being incorporated in chitosan–EDTA as drug carrier matrix is almost completely degraded within 4.5 h by an artificial intestinal fluid containing trypsin, chymotrypsin and elastase. On the contrary, more than half of the polypeptide drug remains undegraded if only 10% of chitosan–EDTA are substituted by a chitosan–EDTA BBI conjugate (Bernkop-Schnürch et al., 1998b). Results of this study are shown in Fig. 9.

In another study, the protective effect of dosage forms containing insulin as model drug and chitosan as mucoadhesive carrier matrix towards trypsin was investigated. Results of this study demonstrated, that a sufficient protection of the embedded peptide drug could only be achieved by the addition of a chitosan–antipain conjugate (Bernkop-Schnürch et al., 1997a).

Beside mucoadhesive, permeation enhancing and protective properties, the release behaviour of the dosage form has also a major influence on the bioavailability of the incorporated peptide drug. The release rate of therapeutic peptides from mucoadhesive polymers depends mainly on the molecular size of the drug. According to the calculation of the diffusion coefficient, in which the radius of a molecule indirectly correlates with the diffusion coefficient, smaller peptides will be faster released than larger ones. In order to control their release from chitosan and its derivatives various strategies have so far been pursued. The release of peptide drugs from anionogenic chitosan derivatives such as chitosan–EDTA, for instance, can be controlled by an ionic or covalent crosslinking of the polymer. An ionic crosslinking, on the one hand, can be provided by divalent cationic compounds such as lysine or 1,8-diaminooctane (Bernkop-Schnürch et al., 1998a). On the other hand, a covalent crosslinking can be achieved by a comparatively lower amount of EDTA during the coupling reaction as one EDTA molecule is thereby bound to more than only one amino-group of chitosan (Bernkop-Schnürch and Krajicek, 1998).

Additionally, the release rate of a peptide drug can be reduced or enhanced by raising or lowering the share of the polymer in the delivery system, respectively. In dependence on the peptide drug which should be perorally administered, the release rate of the therapeutic agent can therefore be adjusted in a very simple way by taking these parameters into account.

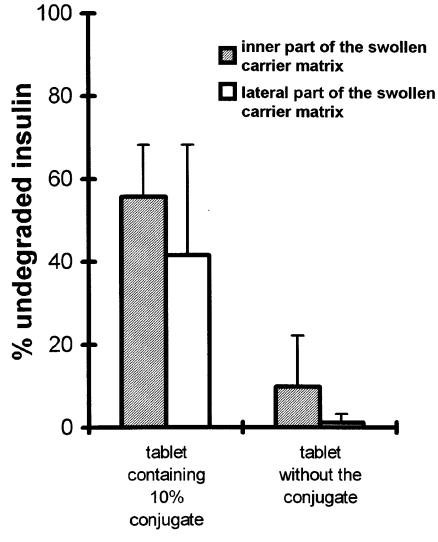

Fig. 9. Amount of remaining undegraded insulin (%) in drug delivery systems without (control) and with 10% chitosan-EDTA BBI conjugate. Tablets were incubated for 4.5 h with trypsin, chymotrypsin and elastase under physiological enzyme concentrations at 37°C (adapted from Bernkop-Schnürch et al., 1998b).

In another approach, chitosan has also been used as carrier matrix in microcapsules. Based on an interfacial crosslinking of chitosan by ascorbyl palmitate in a water/oil dispersion, insulin was thereby incorporated in the polymer. The microcapsules obtained showed an entrapment efficiency approaching 100%, release kinetics approaching zero order and a release rate which could be increased by decreasing the chitosan content in the preparative solution (Aiedeh et al., 1997).

7.2. Coating material

In order to give formulations such as liposomes and microparticles mucoadhesive properties, chitosan is also used as coating material. The high efficacy of chitosan-coated liposomes providing both mucoadhesive properties and a protective effect for therapeutic peptides towards an intestinal enzymatic attack has already been mentioned (Fig. 8). Whereas a coating of liposomes with anionogenic mucoadhesive polymers e.g. poly(-acrylic acid) can only be achieved by the covalent attachment of hydrophobic moieties such as cholesteryl groups on the polymer, chitosan in advantage can be used as coating material without any modifications (Takeuchi et al., 1996).

Aside from a strongly improved bioavailability of insulin due to the chitosan-coating of liposomes containing the peptide drug, the bioavailability of calcitonin could be improved by such a coating as well. In contrast to non-adhesive liposomes, chitosan-coated liposomes led thereby to a significantly decrease in calcium concentration in blood after oral administration in rats (Takeuchi et al., 1999).

8. Conclusions

Since chitosan displays mucoadhesive properties as well as strong permeation enhancing capabilities for hydrophilic compounds together with a very safe toxicity profile, it has been widely investigated over the last few years for peroral peptide delivery systems. To date, it could already been shown by various in vivo studies that due to the co-administration of this polymer the bioavailability of many peptide drugs including insulin, calcitonin and buserelin could be strongly improved. Additionally, these properties can be further improved by simple chemical modifications on its primary amino group. So far, chitosan derivatives exhibiting a better solubility, stronger mucoadhesive capabilities and enzyme inhibitory properties towards luminally secreted proteases and brush border membrane bound peptidases could be generated. These unique features make chitosan and in particular its derivatives to valuable excipients for the peroral administration of peptide drugs.

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