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Drug delivery from nonpeptidic α-amino acid containing polyamides

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Summary

New nonpeptidic α-amino acid-based polyamides were used for drug delivery. Benzocaine was covalently linked via a spacer to N-protected poly(L-cystyl-L-cystine) and to poly(adipoyl-L-lysine). Its release by α-chymotrypsin attack was followed by UV. It was nearly quantitative within two days with L-cystine-based polyamide. Protected forms of these polyamides were used as matrices. Release of benzocaine entrapped in polyamide from (L-cystine) was controlled by diffusion. When entrapped in polyamide from (L-lysine) it was controlled by diffusion and swelling.

Introduction

During the past two decades, researchers associated with pharmaceutical industries have shown an increasing interest for the synthesis of polymers for biomedical applications. For example, many studies have been devoted to the well-known bioresorbable poly(L-lactide) and its copolymers, often used for sutures, screws etc...

A rapidly increasing number of papers are now concerning the pharmaceutical uses of polymers: drug delivery, which includes controlled release systems (the rate is predetermined) and targeted delivery systems (the site of action is predetermined). A few recent papers review the different aspects of drug delivery (1-4). For controlled release systems, the drug is usually mixed into a polymeric matrix or dispersed into a reservoir closed by a polymeric membrane. In actively targeted delivery systems, drug has to be covalently bound to the backbone and released on the site, generally by enzymatic cleavage. This implies the synthesis of a soluble macromolecular drug conjugate.

A wide range of degradable and nondegradable polymeric backbones were investigated. Among them, we can note, for example, some polysaccharides (5), some methacrylamide-based copolymers (6), and polymers from trifunctional α-amino acids: poly(L-glutamic acid) (7) and poly(L-lysine) (8).

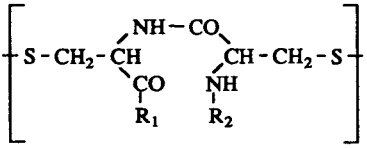
In previous papers, we reported the synthesis of nonpeptidic α-amino acid-based polyamides: poly(L-cystyl-L-cystine) (9-10), poly(adipoyl-L-lysine) (11-12) and the first results of their biodegradation (12).

In the present article, we give the results obtained with these polymers used as drug conjugates or as matrices.

Experimental

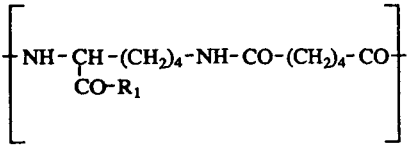
Materials: carbonochloridic acid 1-methylethenylester (isopropenyloxycarbonyl chloride) was obtained from Janssen, α-chymotrypsin (40-60 U/mg) and benzocaine were provided by Aldrich, and used as received. Poly(L-cystyl-L-cystine) and its

protected forms (PCC I and PCC II) (9-10), poly(adipoyl-L-lysine benzyl ester) PAL I and its deprotected form PAL II (11-12) were obtained as previously described.



PCC I $R_1 = C_6H_5\text{-}CH_2\text{-}O$, $R_2 = C_6H_5\text{-}CH_2\text{-}O\text{-}CO$

PCC II $R_1 = OH$, $R_2 = C_6H_5\text{-}CH_2\text{-}O\text{-}CO$

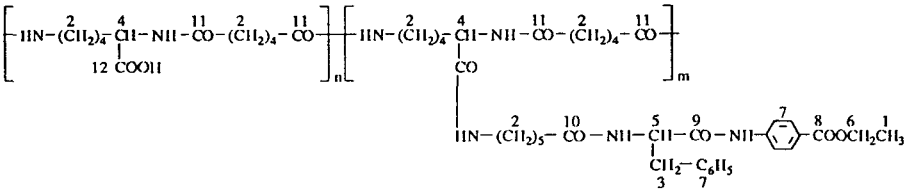


PAL I $R_1 = C_6H_5\text{-}CH_2\text{-}O$

PAL II $R_1 = OH$

Spacer-drug compound, ω-aminocaproyl-L-phenylalanyl-benzocaine (SD) was prepared by the peptidic solution method, using classical protections and deprotections: benzocaine was first coupled to L-phenylalanine (by the mixed anhydride method), the resulting product was then coupled to ω-aminocaproic acid (by the active ester method).

Synthesis of drug conjugates. Similar procedures were applied to PCC II and PAL II. Typically, 500 mg ($1{,}95.10^{-3}$ mol) of PAL II were dissolved in a mixture of 20 mL of dry and freshly distilled and degassed dimethylformamide (DMF) and 0,37 mL ($2{,}15.10^{-3}$ mol) of diisopropylethylamine (DIEA). The solution was protected from atmospheric moisture and cooled to -5°C. 235 µL ($2{,}15.10^{-3}$ mol) of carbonochloridic acid 1-methylethenylester were slowly added, so that the temperature maintained between -10°C and -5°C. The mixture was allowed to react for 30 min. 5 mL of DMF containing 990 mg ($2{,}15.\ 10^{-3}$ mol) of spacer-benzocaine hydrobromide (SD II, see Scheme 1) and 0,37 mL of DIEA were then slowly added. The temperature was allowed to warm to room temperature. After concentration under vacuum, the conjugate was precipitated with dilute acetic acid (10 mL in 100 mL of water), thoroughly washed, filtered and dried. A degree of fixation of 50% was determined by ^{1}H NMR.



^{1}H NMR (DMSO d6, ref. TMS, ppm): 1,1-2,1 (H1, H2); 2,6-3,1 (H2, H3); 4,1-4,7 (H4, H5, H6); 7,1-8,3 (H7, 4 NH), 10,5 (NH Bzc). ^{13}C NMR (DMSO d6, ref. TMS, ppm): 14 (C1); 23-35 and 40 (C2), 37 (C3); 52 (C4); 55 (C5); 60 (C6); 119-143 (C7); 165 (C8); 171 (C9); 172 (C10,C11); 174 (C12)

Drug release by enzymatic cleavage.
All the samples used for release studies must be previously thoroughly washed with buffer pH 7,4.
Spacer-drug I or conjugate powders (6 mg) were suspended in 5 mL of 0,2M Tris buffer pH 7,4 containing α-chymotrypsin (0,5 mg/mL). Spacer-drug II (6 mg) was dissolved in 5 mL of Tris buffer containing α-chymotrypsin (from 5.10^{-2} mg/mL to 2.10^{-1} mg/mL). All the samples were incubated at 37°C and the release of benzocaine was determined by measuring the optical density at 285 nm.

Drug release from matrix.
Pellets were prepared by the melt-pressing method. 2 mg of benzocaine were uniformly dissolved into 38 mg of polymer matrix (PAL I or PCC I), and melt-pressed at 100°C for

30 min. The noncrystalline pellets were then incubated at 37°C in 5 mL Tris buffer 7,4. Benzocaine release was determined as above.

Results and discussion.

Synthesis of the conjugates.
Morawetz (13) and Kopecek (14) showed that *p*-nitraniline release from acrylic conjugate by α-chymotrypsin attack was optimal with the spacer ω-aminocaproyl-L-phenylalanyl. So we used this spacer to bind a model drug, benzocaine (*p*-ethyl aminobenzoate, Bzc) to PAL II and PCC II. The synthesis of the spacer can be schematized as follows (Scheme 1):

Z-Phe-OH ——> Z-Phe-Bzc ——> HBr.Phe-Bzc

——>Z-Acp-Phe-Bzc ——> HBr.Acp-Phe-Bzc
(SD I) **(SD II)**

with Z= benzyloxycarbonyl; Acp= ω-aminocaproyl; Phe= phenylalanyl.

SD II was reacted with carboxylic groups of PAL II and PCC II, by the mixed anhydride method. The coupling agent used was carbonochloridic acid 1-methylethenylester (isopropenyloxycarbonyl chloride), $CH_2=C(CH_3)$-O-COCl that provides aceton as a by-product (instead of an alcohol). ^{13}C and ^1H NMR showed the presence of benzocaine in the resulting product. The total absence of benzocaine in similar experiments carried out in the absence of coupling agent gave the evidence of covalent bonding of the spacer-drug compound. Moreover, these two conjugates were found to be insoluble in Tris buffer, when the starting materials were soluble. ^1H NMR spectra allowed the determination of benzocaine content by comparing benzocaine NH peak (10,5 ppm) to backbone peaks (CH for example). The degrees of fixation were 25% for PCC II and 50% for PAL II. Lower contents could be obtained by varying the amount of SD II during the synthesis. When bound benzocaine/free carboxylic groups ratio was lower than 0,1, solubilization was obtained with PAL II and PCC II.

Enzymatic benzocaine release from conjugates.
It was studied in heterogeneous medium by α-chymotrypsin attack in Tris buffer 7,4 at 37°C. The progress of release was followed by measuring the optical density of the solution at 285 nm. Preliminary tests carried out with spacer-drug compounds SD I and SD II showed that α-chymotrypsin was quite able to release benzocaine, even in heterogeneous medium. However, release was very slow for hydrophobic SD I: 6% were released within two months. For soluble SD II, the release was much faster, and could be followed by UV. The maximum of absorption shifted from 270 nm for SD II to 285 nm when benzocaine was totally cleaved. The reaction was complete after 15 min when α-chymotrypsin concentration was 0,2 mg/mL and 3 hours for 0,05 mg/mL. This reaction rate clearly shows that substrate-enzyme complex can easily be formed. Moreover, α-chymotrypsin was shown to degrade neither PCC II nor PAL II.

Benzocaine release from PCC II conjugate.
Release was carried out in heterogeneous medium, on powdered conjugate whose benzocaine degree was 25%. The results are given on Fig. 1. After a burst due to the easy attack of the surface particles, the release carried on more slowly and was nearly complete after two days. This high rate compared to the one obtained with SD I can be explained by a higher hydrophilicity of the conjugate, due to the presence of free carboxylic groups. At the end of the reaction, the polymer bearing spacer side groups remained insoluble, while its homologous only bearing free carboxylic side groups was soluble. This clearly means that the bond between the backbone and the hydrophobic spacer was not cleaved.

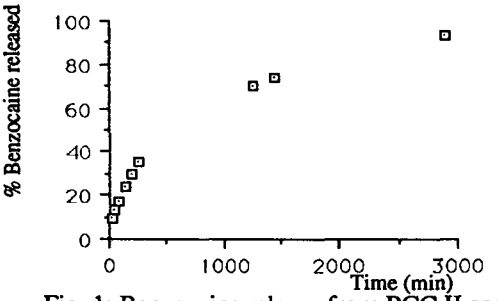


Fig. 1: Benzocaine release from PCC II conjugate

Benzocaine release from PAL II conjugate.
Release was studied in heterogeneous medium from a conjugate whose benzocaine degree was 50% in the conditions used for PCC II conjugate, but the results were quite different. After a higher initial burst (26% after 15 min), the conjugate progressively dissolved. 33% remained insoluble after 24 hours, and dissolution was total after 96 hours: the inherent viscosity of the enzymatic solution (0,63 dL/g after 2 weeks) showed the presence of a non-degraded polymer in solution. While releasing benzocaine, α-chymotrypsin attack gave raise to new solubilizing carboxylic groups. This result confirms the higher hydrophilicity and solubility of PAL compared to PCC: a poly(adipoyl-L-lysine) bearing 50% benzyl ester groups was soluble in Tris buffer 7,4. Conjugates from PCC II and PAL II with low degrees of fixation (<5%) are soluble in Tris buffer, and the preceding results show that they could be suitable as drug conjugates.

Benzocaine release from PCC I matrix.
In time-controlled drug delivery systems, drug is entrapped in a polymeric matrix. Its release can be controlled by the solvent (swelling), by diffusion or by erosion of the matrix. We studied the release rate of benzocaine entrapped in a matrix of totally protected poly(L-cystyl-L-cystine). In a previous paper (12), we showed that this polymer was resistant to Tris buffer and to enzymes, probably because of its high hydrophobicity. The experiments were carried out on melt-pressed pellets containing 5% benzocaine, at 37°C in Tris buffer pH 7,4. The results are shown on Fig. 2. 87% of benzocaine were released after 7 weeks. For a diffusion-controlled system, the release rate may be expressed by Higuchi formula (15), deriving from Fick's first law. In this formula, the amount of substance released is proportional to the square root of time. On Fig. 3, we plotted the amount of released benzocaine versus $t^{1/2}$. The straight line obtained showed that release was only due to diffusion.

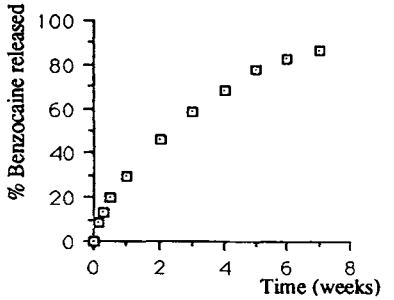


Fig. 2: Benzocaine released from PCC I matrix

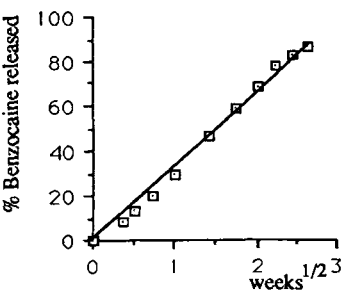


Fig. 3: Benzocaine released versus $t^{1/2}$

Benzocaine release from PAL I matrix.
Release of benzocaine from poly(adipoyl-L-lysine benzyl ester) PAL I was tested in the same conditions. The results obtained (Fig. 4) are quite different. After an important burst during the first week, the release rate decreased for the next three weeks, and then strongly increased. After 8 weeks, 95% of benzocaine were released. No dissolution of the matrix was noted confirming our preliminary experiments: this system was not bioerodible. The aspect of the curve could be explained by the noncrystalline structure and by the relatively hydrophilic character of PAL I compared to PCC I. The diffusion of benzocaine located next to the surface gave raise to pores and made the access of solvent easier. With PAL I matrix, the release was controlled by diffusion and by swelling. A visual observation of the pellet at the end of the experiment confirmed this interpretation.

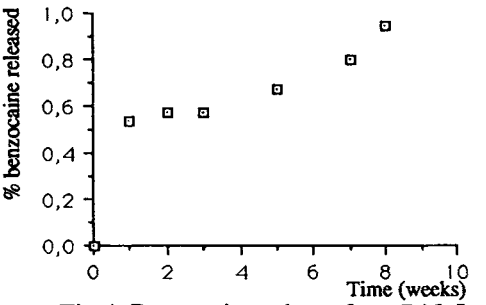


Fig 4: Benzocaine release from PAL I matrix

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