Synthesis of Macromolecular Prodrugs of Procaine, Histamine and Isoniazid

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The attachment of various drugs bearing $-NH_2$ groups to poly-α,β-aspartic acid as a biodegradable carrier afforded in good yields macromolecular prodrugs which were characterized with respect to composition and drug load by spectroscopic and analytical methods. *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in an aqueous medium proved to be useful in the attachment reaction. Isoniazid, procaine and histamine were covalently coupled as pendant groups onto poly-α,β-aspartic acid *via* an amide bond. In principle, controlled release of the aforementioned drugs can be achieved by biodegradation of the polymer or by cleavage of covalently bound polymer–drug conjugates.

Keywords macromolecular prodrug; procaine; histamine; isoniazid; poly-α,β-aspartic acid; pendant-chains system

More effective drug therapy should be achievable by appropriately controlling the rate of release, the duration of release and the site of release of the drug. During the past few years, there has been a growing interest in controlled release of drugs by polymeric systems[1,2]; these systems may be assigned to two general categories based on the mechanism of release. In the first, the drug is physically dissolved, adsorbed, entrapped, or dispersed in a polymeric matrix. In the second category, the drug is chemically bound to the polymer either as a structural unit along the backbone or as a pendant group. In principle, drugs are released from these systems by chemically or biologically induced cleavages of the covalently bound conjugates. The most common cleavage reaction is hydrolysis induced by water in the molecular environment.[3]

Two different synthetic routes have been employed in the preparation of polymers that contain drugs as pendant substituents. In the first, the drug is converted to a polymerizable derivative that is subsequently polymerized to afford the macromolecular combination. Drugs have also been chemically bound to preformed synthetic or naturally occurring polymers; the method involves the reaction of a drug or drug derivative with a polymer containing functional groups. Drugs have been attached to preformed polymers to yield systems with pendant esters, amides, carbamates, or acetals. Polymeric release systems can be applied to maintain constant levels of the drug in a particular body compartment for extended periods of time. Using these systems, peak concentrations of the drug can be avoided and a decrease in systemic toxicity can be achieved.[1,2]

In preceding papers we have described the synthesis, purification, characterization and *in vitro* transfer of high-molecular-weight prodrugs of isoniazid and drugs bearing carboxylic groups using polysuccinimide (PSI) and α,β-poly(*N*-hydroxyethyl)-D,L-aspartamide as biodegradable carriers.[4,5] We now turn our attention to poly-α,β-aspartic acid (**1**) (PAA).

Aspartic acid can be incorporated into polymers through peptide bonds or in the form of a cyclic imide. Various applications of polyaspartic acid derivatives have been suggested: as blood plasma expanders, as models in developing protein immobilization techniques on insoluble carriers, or as drug carriers.[6] N-Substituted polyaspartamides appeared of particular interest.

The objective of the present investigation was to examine the attachment of various drugs bearing $-NH_2$ groups with different degrees of basicity with **1** as a biodegradable carrier. The obtained high-molecular-weight prodrugs were characterized with respect to composition and drug load by spectroscopic and analytical methods.

Experimental

Infrared (IR) spectra were recorded on a Perkin-Elmer 1720 infrared Fourier-transform spectrophotometer as nujol mulls and ultraviolet (UV) spectra on a Kontron UVICON model 860 spectrophotometer for aqueous solutions. Elemental analyses were performed by the Kurt-Eder service (Geneva, Switzerland). The products were quantitatively dried before analysis under reduced pressure (10^{-3} mmHg) at room temperature for 48 h on P_2O_5. Intrinsic viscosities, $[\eta]$, were determined by measuring reduced viscosities in the range of 2—10 mg/ml (Ubbelhode viscometer; outflow time, 100—200 s; T = 25 °C) and extrapolating the concentration to zero. Polysuccinimide was dissolved in 0.1 M LiCl in dimethylformamide (DMF) ($M_r = 30200$ according to the Mark-Houwink relationship, $[\eta] = 1.32 \times 10^{-2} M_r^{0.76} = 33.7$ ml/g),[7] and **1** in 0.2 M NaCl in water, pH 7.3, $[\eta] = 22.43$ ml/g. All chemicals used were commercial products. Solvents were distilled before use. Polysuccinimide was prepared by polycondensation of DL-aspartic acid in the presence of phosphoric acid at 180 °C according to the method of Neri *et al.* in almost quantitative yield.[8]

Poly-α,β-aspartic Acid (1) Polysuccinimide (3 g, 10^{-4} mol) was dissolved in DMF (30 ml), and an equivalent amount of 2 N NaOH was added dropwise. The addition time did not exceed 24 h, during which time the temperature must be maintained in the range of 25—30 °C and the pH value between 9 and 10.[9] The mixture was then treated with 1 N HCl and the pH was lowered to 4.5. The resulting mixture was dialyzed exhaustively against 0.1 M HCl and then against flowing water using Visking dialysis tubing (18/32″) with a molecular weight cut-off of 12000—14000. After dialysis, the solvent was evaporated off *in vacuo* on a bath warmed at 40 °C. The resulting material was further purified by repeated dissolutions in water and reprecipitations with acetone. Analytical and spectral data were in agreement with those of the literature.[9,10] IR ν_{max} cm^{-1}: 1530, 1660, 1730; no imide absorption at 1720 cm^{-1} was observed. *Anal.* Calcd for $C_4H_5NO_3 \cdot 0.6H_2O$: C, 38.15; H, 4.92; N, 11.12. Found: C, 38.43; H, 5.13; N, 10.78.

General Procedure for Covalent Linkage of Drugs onto Poly-α,β-aspartic Acid Poly-α,β-aspartic acid (**1**) (0.50 g) and the appropriate drug (2×10^{-3} mol) were dissolved in water (about 20 ml) at room temperature, and finely powered *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (0.9 g, 4.69×10^{-3} mol) was gently added with stirring. The resulting reaction mixture was stirred for 48 h, after which time the excess EDC was destroyed by portionwise addition of sodium acetate. The solution was then diluted with water (30 ml) and dialyzed exhaustively against deionized water for 100 h using Visking dialysis tubing (18/32″) with a molecular weight cut-off of 12000—14000; further purification was accomplished by ultrafiltration. Analytical and spectral data are listed below.

Polyaspartic Acid–Benzylamine Conjugate (3) Yield 97% based on starting material **1**. UV λ_{max} nm: 267 (sh), 254. IR ν_{max} cm^{-1}: 1530, 1660, 1730. *Anal.* Calcd for $C_{6.59}H_{7.59}N_{1.37}O_{2.63}$ (37% substitution): C, 53.45; H, 5.13; N, 12.96. Found: C, 52.97; H, 5.18; N, 13.14. The substitution percent was confirmed by UV spectrophotometry. The content of linked benzylamine (**2a**) was evaluated by comparison of $E_{256}^{1\%}$ of the pure amine ($E_{256}^{1\%} = 19.05$) with that of **3** ($E_{256}^{1\%} = 7.81$).

Polyaspartic Acid–Isoniazid Conjugate (4) Yield 97% based on starting **1**. UV λ_{max} nm: 265. IR ν_{max} cm^{-1}: 1650, 1708, 1730. *Anal.* Calcd for $C_{6.82}H_{7.35}N_{2.41}O_3$ (47% substitution): C, 47.88; H, 4.30; N, 19.73. Found: C, 47.60; H, 4.61; N, 20.01. The substitution percent was confirmed by UV spectrophotometry. The drug load was established by comparison of $E^{1\%}_{262}$ of pure isoniazid (**2b**) with that of **4**: 312.2 and 144.26, respectively.

Polyaspartic Acid–Procaine Conjugate (5) Yield 95% based on **1**. UV λ_{max} nm: 273. IR ν_{max} cm^{-1}: 1650, 1708, 1730. *Anal.* Calcd for $C_{8.29}H_{10.94}N_{1.66}O_{3.33}$ (33% substitution): C, 53.21; H, 5.86; N, 12.43. Found: C, 52.87; H, 6.03; N, 12.61. UV spectrophotometry was not suitable to evaluate the amount of the linked drug owing to the marked difference in the absorptions of procaine (**2c**) and the adduct **5**.

Polyaspartic Acid–Histamine Conjugate (6) Yield 93% based on starting material **1**. IR ν_{max} cm^{-1}: 1650, 1705, 1730. *Anal.* Calcd for $C_{5.55}H_{7.17}N_{1.93}O_{2.69}$ (31% substitution): C, 46.30; H, 4.98; N, 18.79. Found: C, 46.51; H, 5.01; N, 19.01. The drug load in the adduct could not be established by UV measurements because both histamine (**2d**) and the adduct absorbed in the same range as **1**. All compounds are believed to be microheterogeneous with respect to molecular weight and degree of substitution, but each behaves as a single on entity in dialysis and ultrafiltration.

Results and Discussion

We have concentrated on the use of **1** as a drug-carrier because this polymer contains sufficient reactive groups for derivatization. After partial derivatization the drug–polymer adducts will still be water-soluble owing to residual carboxylate groups and, in the quantities required for use as a drug-carrier, **1** is not expected to be toxic.

The product of the thermal polycondensation of aspartic acid, the so-called anhydropolyaspartic acid or polysuccinimide, is readily converted to **1** by alkaline hydrolysis. During the reaction hydrolytic ring opening proceeds at both carbonyl groups and a polymer containing both α- and β-peptide bonds may be obtained.[10,11] The polymer conformation is affected by the relative amounts of the two types of bonds; a large amount of α bonds gives a highly α-helical polymer. The ratio between α and β bonds, that affects the conformational behavior, can be modified by controlling the pH of the medium in which the hydrolysis of polysuccinimide is accomplished. Compound **1** undergoes a conformational induced by changes of its degree of ionization α^*. When α^* decreases, the relative proportion of α-helix conformation increases. Poly-α,β-aspartic acid is completely α-helical at $\alpha^* = O$.[12]

The attachment reactions of drugs to the polymer were carried out in aqueous solution. Compound **1** containing only α-peptide bonds was sparingly soluble in water (solubilization may be increased by adjusting the pH with 2 N NaOH), whereas a polymer containing a large amount of β-peptide bonds was very soluble. Therefore, to obtain a polymer with a suitable amount of β-peptide bonds, it was necessary to maintain the pH of hydrolysis of polysuccinimide in the range of 9 to 10.[9,11]

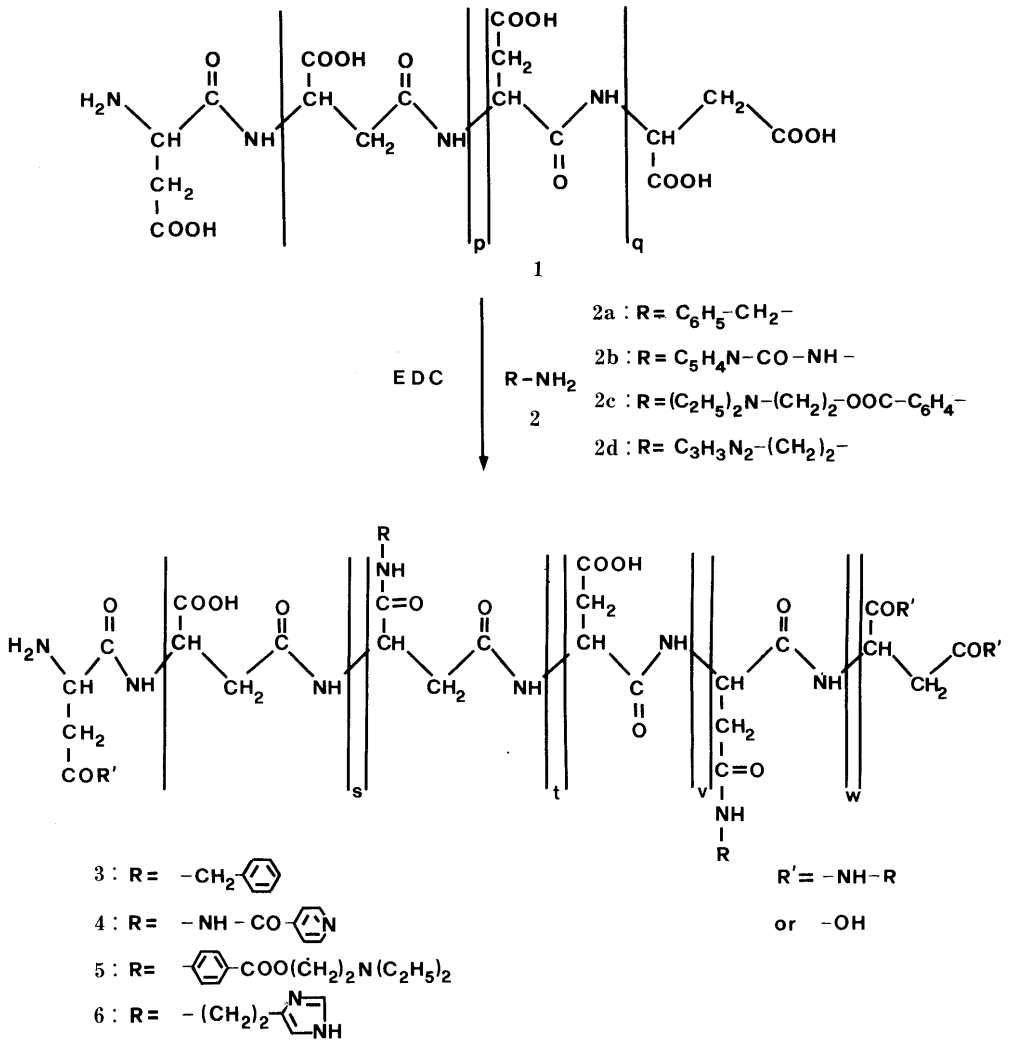

Chart 1

In our efforts to attach drugs bearing $-NH_2$ groups directly onto **1**, we found that the majority of the well established techniques of peptide synthesis[13] were not suitable. The use of either *N,N*-carbonyl diimidazole (CDI) or ethyl chloroformate was unsuccessful and a rapid decrease in viscosity and poor recovery of **1** after dialysis were observed as a consequence of isomerization phenomena or chain scission due to the overactivation of lateral carboxylic groups of **1**. CDI behaved similarly in attempts to couple drugs onto polyglutamic acid.[14] The preactivation of lateral carboxylic acid groups in **1** by EDC in an aqueous medium proved to be very successful. EDC allowed the reaction between nucleophilic drugs and **1** before the latter underwent isomerization or degradation. The reaction was successful only when EDC was added as the last component of the reaction mixture. The adduct formation is depicted in Chart 1.

The ability of poly-α,β-aspartic acid to covalently bind small molecules *via* an amide bond was verified using benzylamine (**2a**) as a model compound; **2a** was used to establish the general reaction conditions. The attachment was carried out in aqueous solution with the use of EDC, and characterization of the obtained adduct **3** was done by spectrophotometry and elemental analysis.

By the same method we were able to attach isoniazid (**2b**) procaine (**2c**) and histamine (**2d**) to **1**. As above, the covalent conjugate drug–polymer bonding was evidenced by IR and UV spectrophotometry and elemental analysis.

The UV absorptions of the amide poly-α,β-aspartic acid–isoniazid adduct (**4**) in aqueous solution showed a λ_{max} bathochromic shift on going from the drug (λ_{max} 262 nm) to the adduct (λ_{max} 265 nm). It has been reported that isoniazid does not exhibit UV absorption shifts until pH 12; however, its acetyl derivative undergoes a λ_{max} shift value in a pH 10 solution.[15] Our adduct **4** showed the λ_{max} value of 305 nm in a buffered pH 10 solution.[5] The adduct **5** showed at λ_{max} hypsochromic shift on going from **2c** (λ_{max} 290 nm) to **5** (λ_{max} 273 nm) in aqueous solution. The same shift value was observed in the UV absorption of the *N*-acetylprocaine. Analogous behavior was seen with the adduct **6**, for which a hypsochromic shift of 2 nm was observed compared to **2d**.

The IR absorptions provided additional chemical evidence for the generation of covalent conjugate drug–polymer bonds. The IR spectra of the adducts showed typical bands due to amide C=O groups and the absorptions of strongly polar –NH, as reported in the experimental section.

The amount of drugs linked to the polymer was determined by elemental analyses and/or UV spectrophotometry. The content was evaluated by using the corresponding free drug as the reference compound. Comparison of the $E^{1\%}_{max}$ value in aqueous solution of the linked drug with that of the reference compound allowed calculation of the load of the active substance in the macromolecular prodrug.

The drug load in the polymer showed that some of the carboxylic groups of **1** were not involved in the derivatization reaction and remained as free pendant groups. This is an important result; in fact the rate of hydrolysis of an amide linkage in a polymer is strongly dependent on the groups surrounding it. Hydrophilic neighboring groups enhance hydrolysis while hydrophobic groups can slow or even prevent the reaction. There is also evidence that in the case of enzyme-catalyzed hydrolysis the hydrophilicity of the system is more important than the bonding energy in determining the overall rate of release.[16]

Kinetic experiments under controlled conditions of pH, temperature and molecular weight are in progress to confirm these hypotheses.

Acknowledgement We thank the Ministero della Pubblica Istruzione for financial support.

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