Synthesis of Polymeric Derivatives of Isoniazid: Characterization and *in Vitro* Release from a Water-Soluble Adduct with Polysuccinimide

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Coupling of isoniazid with polysuccinimide afforded a water-insoluble polymeric pro-drug; by reaction with ethanolamine it was chemically transformed in a water-soluble adduct. The *in vitro* release of isoniazid from the drug–polymer adduct was studied by using an artificial stomach wall lipid membrane. The transfer rate constant from simulated gastric juice to simulated plasma was defined and compared with that of an equivalent dose of pure drug.

Keywords isoniazid pro-drug; polymeric pro-drug; macromolecular carrier; polysuccinimide–isoniazid adduct; isoniazid

Controlled release formulations are desirable in the treatment of chronic diseases to keep the drug blood levels relatively constant for a longer period of time. A reduction of the frequency of administration not only enhances the patient compliance, but also maintains the minimal effective concentration without exceeding the maximal tolerated one which, very often, leads to an increase of unwanted side effects. In recent years the synthesis of polymer–drug adducts has received increasing attention from numerous workers. Covalent linking of a low-molecular-weight drugs to a polymer alters the body distribution, often results in delayed release into the body fluids and prolongs the duration of action. By this technique, side effects of drugs, due to hyperdosage, are minimized while ensuring adequate drug delivery to target cells or tissues.

We are interested in systems in which drugs are linked to natural or synthetic polymers. In a preceding paper from these laboratories we described the synthesis, purification and characterization of α,β-poly(*N*-hydroxyethyl)-DL-aspartamide and some of its adducts with drugs bearing carboxylic groups.[1] Our attention has now turned to isoniazid (**1**).

The use of isoniazid as an antitubercular agent is well known.[2] This drug, however, exhibits toxicity characterized by peripheral and optic neuritis, central nervous system stimulation and hepatitis on repeated dosing. Isoniazid can inhibit the microsomal mixed function oxidase activity, leading to a reduction of the clearance and an elevation of the plasma level of coadministered drugs and to a drug intoxication in patients.[3] It is claimed that isoniazid derivatives as hydrazones or 1,1-methylene bis (2-isonicotinyl hydrazine) or glucosamyl derivatives show reduced immediate toxicity and are better tolerated.[4] This is due to a gradual hydrolysis *in vivo* which affords the free drug. It is hoped that covalently bonding the antitubercular agent to a polymeric carrier, for slow release *in vivo*, would overcome the toxicity problems.

In this paper we describe the attachment of isoniazid to polysuccinimide (**2**) as a macromolecular carrier. Coupling of the drug by a covalent linkage afforded a water-insoluble adduct (**3**). We then looked for a suitable chemical transformation to obtain solubility, since the water solubility of a drug–polymer adduct is significant in pharmaceutical formulation.[5] We also studied the *in vitro* transfer of isoniazid from the drug-polymer adduct using an artificial stomach wall lipid membrane. The transfer rate of the adduct from simulated gastric juice to simulated plasma was defined and compared with that of an equivalent dose of pure isoniazid.

Experimental

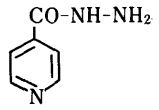
Viscosity measurements were carried out at 25 °C using an Ubbelohde viscometer with polysuccinimide in a 0.1 M dimethylformamide (DMF) solution of LiCl in the concentration range of 2—10 mg/ml. Weight-average molecular weight of polymer was determined by applying the Mark–Houwink equation[6] $[\eta] = 1.32 \times 10^{-2} \times M^{0.76} = 33.7$ ml/g, and a value of molecular weight (M) = 30200 was obtained. Ultraviolet (UV) spectra in aqueous solutions were recorded with an Hitachi Perkin Elmer model 200 spectrophotometer and infrared (IR) spectra on an JASCO IR-810 spectrophotometer as Nujol mulls. *In vitro* release experiments were carried out on a Sartorius SM16750 apparatus (Sartorius Membranfilter GmbH, Gottingen). Elemental analyses (C, H, N) were performed by the Kurt Eder Service (Geneva, Switzerland). The products were quantitatively dried before analysis (10^{-3} mmHg, room temperature, P_2O_5, 48 h). DL-Aspartic acid, isoniazid, DMF and ethanolamine were purchased from FLUKA (Switzerland).

Methods Polysuccinimide was prepared by polycondensation of DL-aspartic acid in the presence of H_3PO_4 at 180 °C according to Neri *et al.*[7] in almost quantitative yield.

Synthesis of Isoniazid–Polysuccinimide Water Insoluble Adduct (3) Polysuccinimide (2 g, 6.62×10^{-5} mol) was gently added to a warm solution (60 ± 0.1 °C) of isoniazid in 20 ml of DMF. The reaction mixture was stirred, for 40 h at 60 °C under reduced pressure, and the solvent was evaporated off *in vacuo*. The resulting water-insoluble gelatinous material was dissolved in 15 ml of DMF and poured into a non-solvent such as *n*-butanol (150 ml). The resulting solid material was repeatedly dissolved in DMF (15 ml) and reprecipitated in water (100 ml) until the disappearance of isoniazid UV absorption in the aqueous mother liquor. The pure adduct **3** was filtered off and dried; yield 1.94 g, 97% based on polysuccinimide.

Synthesis of Isoniazid–Polysuccinimide Water Soluble Adduct (4) Ethanolamine (4 ml, 4.08 g, 6.68×10^{-2} mol) was added dropwise to a stirred solution of **3** in DMF (3 g in 20 min of solvent) over a period of about four minutes. The reaction temperature was maintained at 25—30 °C on a water bath. The reaction mixture was set aside at room temperature for 2 h, after which time it was diluted with 10 ml of DMF and slowly poured into *n*-butanol (200 ml) with constant stirring. The resulting water soluble solid material was filtered off, collected, dissolved in 30 ml of water and purified by exhaustive dialysis against deionized water using Visking dialysis tubing 18/32'' with a molecular weight cutoff of 12000—14000. The purified solution was evaporated at 40 °C at reduced pressure and dried *in vacuo* on H_2SO_4 at room temperature. The pure adduct **4** (2.88 g) was obtained in a yield of 96% based on starting material **3**. *Anal.* Calcd for $C_{66}H_{100}N_{23}O_3$ (related to 15% of substitution): C, 46.75; H, 5.90; N, 19.00. Found: C, 46.44; H, 6.17; N, 18.88.

***In Vitro* Release** The release of isoniazid from the polymeric adduct was evaluated by using the Sartorius absorption simulator equipped with an artificial gastric juice (buffer pH 1.1 solution of hydrochloric acid, sodium chloride and glycine, phase I) and a simulated plasma (buffer pH 7.5 solution of disodium and monopotassium phosphate, phase II). As an artificial lipoid membrane, the M1 gastric barrier supported by an RS type Sartorius membrane filter (Kit SM 15701) was used. The temperature of the solutions (phase I and phase II) was maintained during experiments at 39 + 1 °C in order to obtain 37.5 + 1.5 °C in the diffusion chamber. The fluids were sampled (3 ml) at suitable intervals and concentration of



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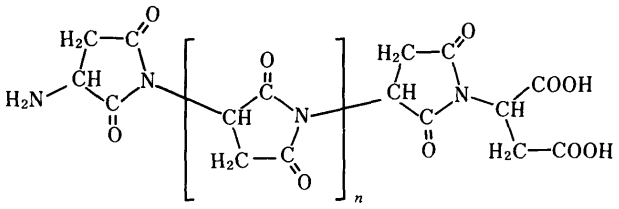

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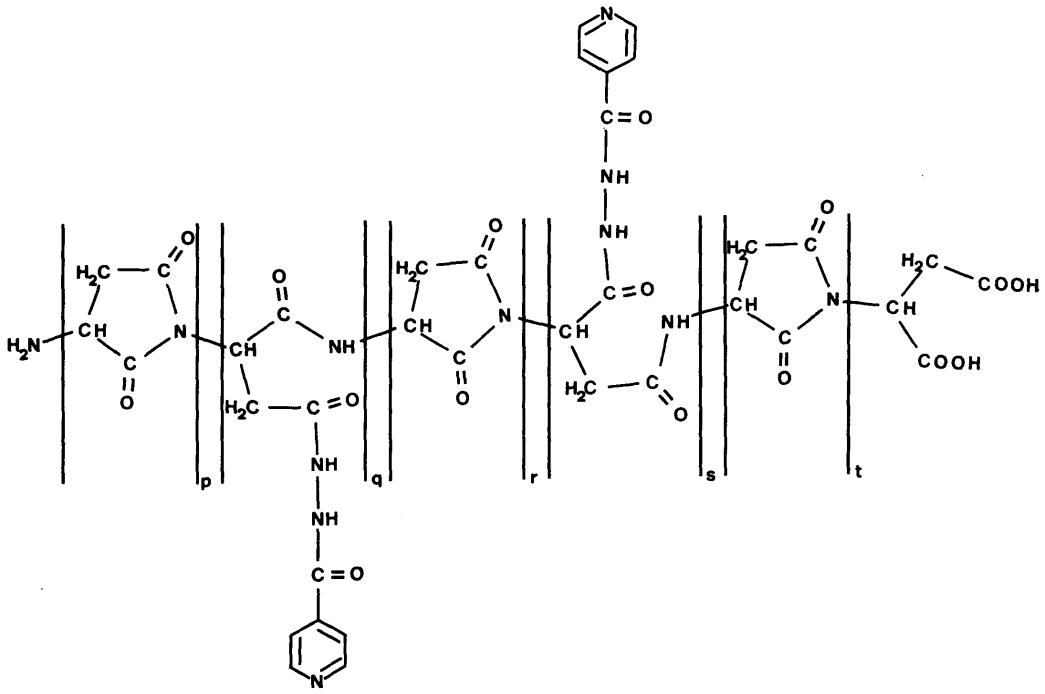

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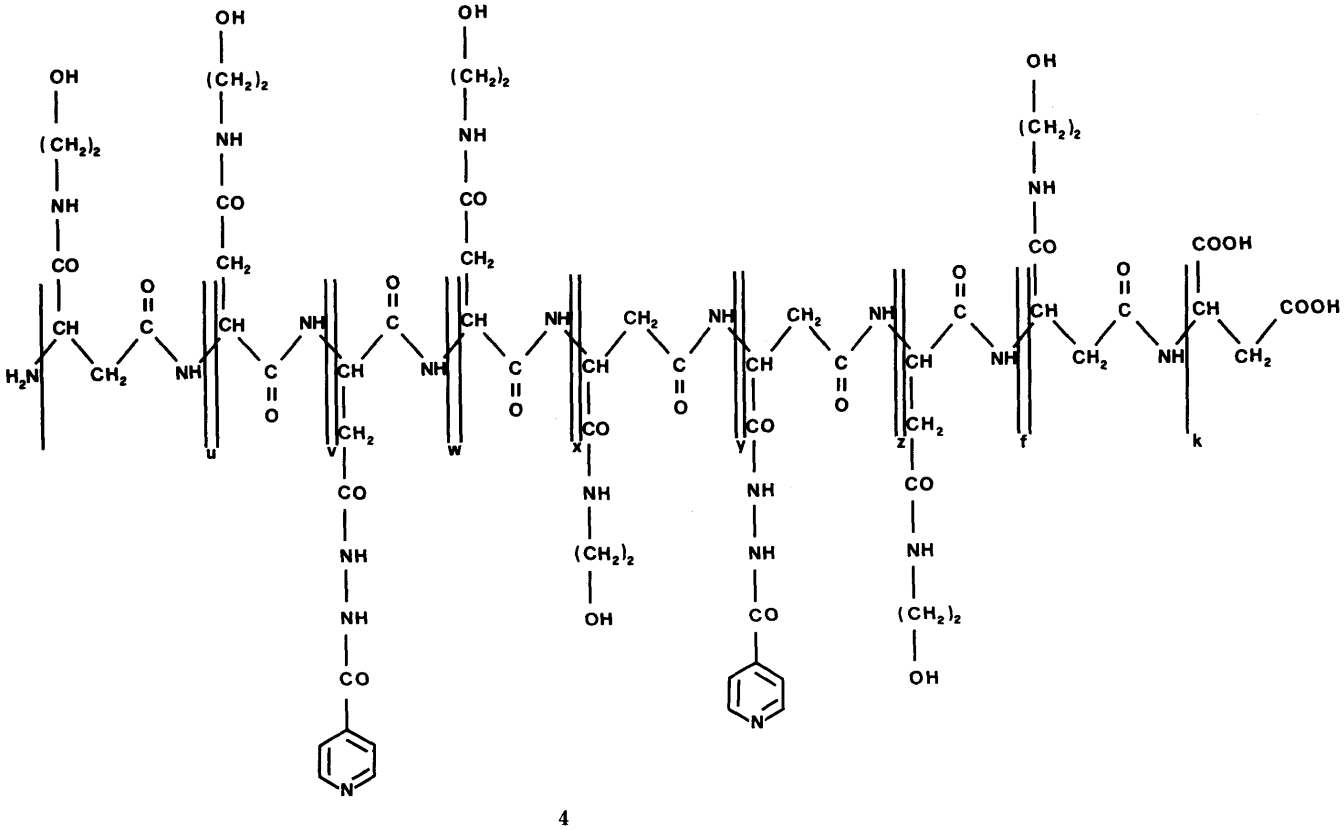

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Fig. 1

isoniazid was measured by UV spectrophotometry at 262 nm to determine the diffused drug in phase II and the undiffused drug in phase I. The amount of isoniazid–polymer adduct, **4**, used was equivalent to 50 mg of the pure drug. Analogously, diffusion of the pure isoniazid was obtained by dissolving 50 mg of the substance in phase I. Because the concentrations are affected by volume variation, the amount of diffused drug in phase II was corrected according to Stricker's equation.[8] Every experiment was repeated five times; the average deviation was ±5%.

Results and Discussion

An example of the use of a drug-polymer adduct to reduce toxic symptoms (gastrointestinal or renal toxicity in antimycobacterial chemotherapy of pulmonary turberculosis) is the use of hinconstarch, equimolar isoniazid and *p*-aminobenzaldehyde thiosemicarbazone with oxidized starch.[9]

Poly(DL-succinimide) (**2**), a reactive polyimide readily prepared by polycondensation of DL-aspartic acid, appeared to be a suitable polymer for covalently bonding isoniazid to a carrier. Coupling of isoniazid (**1**) with **2** was carried out in DMF under reduced pressure for 40 h at 60 °C. The ability of macromolecules to bind small molecules is greatly dependent on the structure of the polymer, mainly the secondary and tertiary structures.[10] The coupling reaction rate of **2** is not a simple function of the basicity of hydrazine[11] or amine[6] derivatives; *i.e.* ethanolamine reacts very quickly, whereas the reaction of comparatively basic amines (histamine) is unexpectedly slow. These results are consistent with our experimental conditions used to synthesize the adduct **3** (Fig. 1).

The obtained polymer–isoniazid adduct was water-insoluble; to improve the solubility the adduct was treated with 1-amino-2-hydroxyethane at 25—30 °C. The part of polysuccinimide unlinked with isoniazid reacts to produce in the adduct a moiety which enhances the water-solubility. It was possible to isolate a product very similar to α,β-poly(*N*-hydroxyethyl)aspartamide which is a water-soluble polymer that is non toxic and non antigenic in animal experiments[5]; it may be also biodegradable in living systems.[12] The presence of a covalent conjugate drug-polymer bond was evidenced by UV and IR analysis. In particular, the UV absorption in aqueous solution showed a λ_{max} bathochromic shift in going from the pure drug (λ_{max} 262 nm) to the adduct (λ_{max} 265 nm). It has been reported that isoniazid does not exhibit absorption shifts until pH 12, though its acetyl derivative undergoes a λ_{max} shift value in a pH 10 solution.[13] Adduct **4** showed, in a buffered pH 10 solution the λ_{max} value 305 nm.

The load of the drug in the adduct **4** was determined by UV spectroscopy and elemental analysis; it was about 15%. The content was evaluated using isoniazid as a model compound ($E^{1\%}_{262}=312.2$ in aqueous solution); **4** exhibited $E^{1\%}_{262}=44.71$ in aqueous solution. The IR spectrum of **4** showed a broad amide carbonyl band centered at 1650 cm^{-1}. Furthermore the carbonyl band of polysuccinimide centered at 1720 cm^{-1}, due to C=O stretching, was not observed in the IR spectrum of the isoniazid–polymer adduct.

The movement of a drug through a lipid membrane is, generally, a first order process. The rate constant from the donor phase to the acceptor (K_d) can be easily determined during the first period of diffusion (up to a diffusion rate of 20% of the equilibrium concentration) since the increase in concentration of the drug in the acceptor compartment has a linear relationship with time. The diffusion rate constant from simulated gastric juice to simulated plasma of isoniazid from the drug–polymer adduct **4**, K_d, was calculated by simply plotting the percent diffused drug against time according to Stricker's method[8,14]; it was 4.1×10^{-6} cm·min^{-1}. In the same way, the diffusion rate constant of the pure isoniazid was calculated; it was $K_d=4.2\times10^{-5}$ cm·min^{-1}. The comparison of the K_d values showed a delay of the transfer of isoniazid from the adduct with respect to the pure drug. This was due to the gradual hydrolysis of the polysuccinimide–isoniazid amide bond in the simulated gastric environment.

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