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Synthesis and biodegradation of copolyesters derived from glutamic acid

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Summary

Copolyesters of glutamic acid hydrochloride and 1,2-ethane diol, 1,3-butane diol, 1,4-butane diol, 1,6-hexane diol and glycerol have been synthesised and characterised by number average molecular weights, I.R. spectra and elemental analysis. Microbial degradation of the co-polyesters has been studied using the fungus Aspergillus niger and the bacterium E.coli. All the polymer samples are degraded by these microorganisms. The less polar copolyesters are more readily degraded by the fungus while the bacterium more efficiently degrades the polymers having a higher proportion of α-NH_2 group. The facile attachment of a suitable drug through the free amine groups of the copolyesters has been illustrated to indicate their possible use as carrier polymers for drugs.

Introduction

A brisk of research activities is going on presently on the development of biodegradable polymers for specialized applications(1-5) such as controlled release drug delivery systems, insecticide and pesticide carriers, as well as non-toxic surgical implant materials. Some of these synthetic polymers have an inherent self destruct mechanism to undergo slow enzymatic degradation releasing the impregnated material at desired rates. Moreover, most of the commercially employed polymers are virtually resistant(6) to microbial attack and their disposal is becoming a problem and represents a significant loss of natural resources. It is desirable, therefore, to develop novel commercially viable polymers specifically designed to degrade under controlled biological conditions. Since many naturally occurring substrates degradable by microorganisms contain α-amino acids, we have undertaken preparation of biodegradable synthetic polymers from α-amino dicarboxylic acids and diols. This paper reports the synthesis of such copolyesters from glutamic acid and some diols(also glycerol) and their degradation by micro organisms and illustrates the attachment of a suitable drug to one of the copolyesters to indicate their possible use as carrier polymers for drugs.

Experimental part

Materials : Glutamic acid, 1,2-ethane diol, 1,3-butane diol, 1,4-butane diol, 1,6-hexane diol, glycerol and p-toluene sulfonic acid (catalyst) were analytical reagent grade materials from either E. Merck or Fluka.

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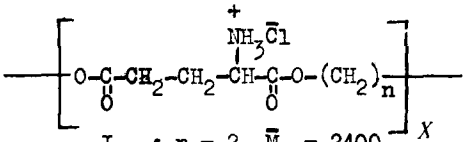
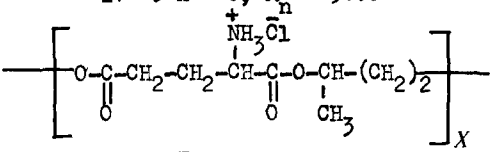
Synthesis : Equimolecular amounts (0.5 mol) of glutamic acid hydrochloride (prepared by passing HCl gas into a dil HCl solution of the acid) and 1,2-ethane diol together with the catalyst p-toluene sulfonic acid (approximately 0.8% of the total weight) were taken in a 250 ml RB flask which was connected to a Dean Stark apparatus for eliminating water aziotropically with benzene. The reaction mixture was heated at 190-200°C under nitrogen atmosphere for 10-12 hrs. When elimination of water subsided, the reaction mixture was heated for an additional 4 hrs under the same condition. The resulting crude polymer was dissolved in dry ethanol and precipitated by dry benzene. After vacuum drying at 45°C poly (glutamic acid-1,2-ethane diol) I was isolated as a sticky material in 83% yield. Repetition of the reaction with 1,3-butane diol, 1,4-butane diol and 1,6-hexane diol gave the polyesters II (85% yield), III (88% yield) and IV (86% yield) respectively as rubbery materials. Stoichiometric amounts of glutamic acid hydrochloride (0.75 mol) and glycerol (0.5 mol) gave a solid polymer V in 82% yield. All of the copolyesters were purified by repeated precipitation from ethanol by benzene and vacuum dried at 45°C and finally stored in a vacuum dessiccator. The expected structures of the polymers would be as shown below.



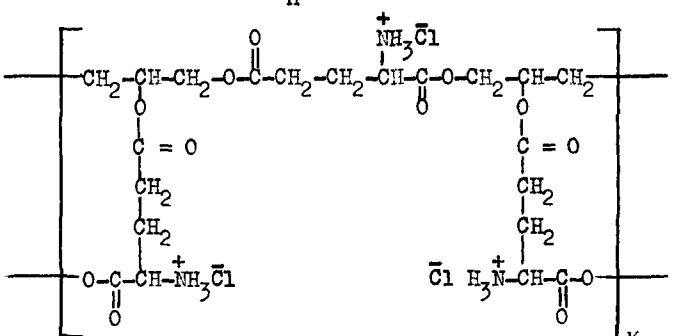
I : n = 2, $\bar{M}_n$ = 2400

III : n = 4, $\bar{M}_n$ = 3200

IV : n = 6, $\bar{M}_n$ = 3600



II : $\bar{M}_n$ = 3000



V : $\bar{M}_n$ = 4800

Characterization : The number average molecular weight ($\bar{M}_n$) of the copolyester samples I-V was determined by a Knauer vapour pressure osmometer and their IR spectra were recorded on mull using a Perkin Elmer IR spectrophotometer. Elemental analysis was carried out for C,H and N.

Fungal degradation : The fungal degradation of the polymers was studied using the fungus Aspergillus niger. Spore suspension was prepared according to a standard method in microbiology. The basal salt solution was prepared by modifying Czapex Dox Broth(7) with Na_2HPO_4(9.8 g) and NaH_2PO_4(7.0 g) per litre. The polyester samples were used as the sole source of carbon and were taken (0.10 g each) in 250 ml Pyrex conical flasks in triplicate along with an empty flask (blank control) for each sample. To each flask including the control ones 12 ml basal salt solution was added and the polymer suspensions were appropriately autoclaved. All the flasks including the control ones were then inoculated with 1 ml of spore suspension aseptically. Triplicate samples and controls were stored loosely capped with cotton plug at 31-34°C for 14 days. The fungi grown in each of the flasks were then collected by filtration, dried in a vacuum oven at 40°C and weighed.

Bacterial degradation : Bacterial degradation of the polyester samples was followed using the bacterium E. coli. A high phosphate mineral salt medium (K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $MgSO_4 \cdot 7H_2O$, 0.102 g; $(NH_4)_2SO_4$, 1.0 g; sodium citrate, 0.47 g; made upto 1 litre) and the bacterial inoculum were prepared following standard methods and polymer samples were used as the only source of carbon. Each of the polymer samples (0.15 g) was taken in 100 ml Pyrex conical flasks in quadruplicate and the polyester suspensions were appropriately autoclaved. Three samples of each set were inoculated with 1 ml of bacterial inoculum aseptically and incubated at 37°C for 45 hrs. The growth of the bacteria in the inoculated flasks of each set was monitored by a turbidimetric method(8) in which measurement was made of the intensity of transmitted light attenuated by scattering by the dispersed bacteria in the medium. Absorbance of the medium at a standard wave length, 440 nm, was recorded taking the uninoculated sample of the corresponding set as reference.

Drug attachment : To an alcoholic solution of a copolyester (IV) alcoholic KOH (~ N/10) was added dropwise with constant stirring to phenolphthalein end point, when the polymer with pendent free amine groups precipitated. A COOH group bearing drug, benzoic acid, was converted into its acid chloride (benzoyl chloride), and added dropwise in excess (based on amine) to the free amine group bearing copolyester in pyridine suepension. Formation of a clear solution from the suspension indicated the complete conversion into the corresponding amide. The pyridine solution was then added dropwise to a large excess of distilled water when the polymer with benzoyl amide side chains precipitated which was collected by decantation. The drug bound copolyester was then purified by dissolving in solvent ether and washing the solution several times with distilled water. Finally the solvent was evaporated off and the drug bound copolyester was collected.

Results and Discussion

Synthesis : Synthesis of the polyesters has already been described. In about 3-4 hrs after the start of the reaction the polymers start separating as lumps of solids which continue to grow in size with time upto 10-12 hrs. To achieve high molecular weight products the reaction is continued for an additional 4 hrs in each case.

Characterization : The polyesters I-V have been characterized by their molecular weights, elemental analysis and IR spectra. The number average molecular weights have been shown against their respective structures.

Elemental analysis : Found for (I) : C.39.05%; H. 5.91%; N. 6.93%. Calculated for ($C_7H_{12}O_4NCl$) : C. 40.09%, H. 5.72%; N. 6.68%. Found for (II) : C. 45.08%; H. 7.09%; N. 6.19%. Calculated for ($C_9H_{16}O_4NCl$): C.45.47%; H. 6.73%; N. 5.89%. Found for (III) : C. 45.82%; H. 6.46%; N. 6.12%. Calculated for ($C_9H_{16}O_4NCl$) : C. 45.47%; H. 6.73%; N. 5.89%. Found for (IV) : C. 49.23%; H. 7.16%; N. 5.70%. Calculated for ($C_{11}H_{20}O_4NCl$) : C. 49.71%; H. 7.53%; N. 5.27%. Found for (V) : C. 39.31%; H. 5.69%; N. 6.94%. Calculated for ($C_{21}H_{34}O_{12}N_3Cl_3$) : C. 40.22%; H. 5.43%; N. 6.70%.

In the IR spectra of the copolyesters I-V, the C=O stretching frequency shifted from 1710 cm^{-1} to 1735 cm^{-1} and a sharp band due to $C-\overset{+}{N}H_3$ appeared at 2920 cm^{-1} in place of a broad superimposed band in the range 2400-3500 cm^{-1} due to $C-\overset{+}{N}H_3$ and C-OH in the original unreacted acid. These indicate the formation of ester bonds. In view of the results of elemental analysis and IR spectra the product structures I-V are confirmed.

Degradation by Aspergillus niger : All the polyesters I-V are capable of supporting fungal growth. Table-I summarises the results of fungal degradation. The growth of the fungi in the polymer suspensions increases in the order IV < III < V < II < I. In the series I,III and IV, when the distance between the successive ester bonds is small, the pendent ammonium ions are close together and the whole polyester chain takes on a more polar character. This may lead to some deactivation of the ester bonds towards enzyme hydrolysis. This proposition is substantiated by the fact that II and V in which the successive ester bonds are separated by three carbon atoms support the growth of the fungi almost to the same extent, and the amounts of fungi grown lie in between those in I and III where the successive ester bonds are separated respectively by two and four carbon atoms.

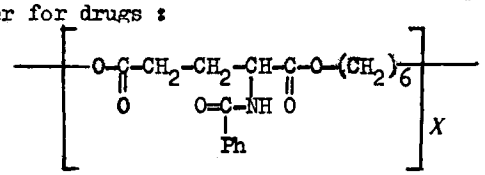
Table I. Degradation of the copolyesters by Aspergillus niger and E. coli

Substrate	Growth of Aspergillus niger after 14 days(g)	Growth of E.coli after 45 hrs in terms of absorbance of the medium at 440 nm
I	0.0142	0.571
II	0.0152	0.888
III	0.0224	0.142
IV	0.0338	0.062
V	0.0155	0.131

E. coli Degradation : The results of bacterial degradation are also included in Table I. It shows that all the copolyesters I-V support the growth of the bacterium E. coli. The results indicate that the variations in the amounts of bacterial growth (measured by a turbidimetric method in terms of the absorbance of the medium at 440 nm) in the samples are probably due to their different $\alpha-NH_2$ group concentration. Thus in the series I,III and IV in which nitrogen contents (from $\alpha-NH_2$ group) are 6.68%, 5.89% and 5.27% respectively, the amounts of bacterial growth as seen from the absorbance values are in the order I < III < IV. The growth of the bacteria is maximum in II, although its nitrogen content is less than that of I. The pendent methyl groups

adjacent to the ester bonds in II probably activate the ester bonds towards enzymatic hydrolysis. The growth of the bacteria in the medium containing the polyester V is also reasonable in view of its high nitrogen content.

Attachment of benzoic acid to copolyester IV : As an illustration of drug attachment to the amino acid-diol copolyesters, a readily available COOH bearing drug, benzoic acid known for its antifunal activity, was acetylated and condensed with the free amine groups of the copolyester IV to form the carrier polymer for drugs :



The above structure was confirmed by I.R. data and elemental analysis. I.R. : amide C=O strech, 1690 cm^{-1}; ester C=O strech, 1735 cm^{-1}; amide N-H bend, 1600 cm^{-1}; aromatic C=C ring strech, 1425 cm^{-1}; out of plane aromatic C=C ring bend 690, 670 cm^{-1}; out of plane aromatic C-H bend 710, 780 cm^{-1}. Anal. calcd. for $(C_{18}H_{23}O_5N)_X$: C.64.86%, H. 6.90%, N. 4.20%. Found : C. 64.76%, H. 6.87%, N. 4.14%.

The copolyesters from glutamic acid hydrochloride and diols (also glycerol) thus fall into the class of biodegradable polymers and are capable of attaching suitable drugs through the free amine groups. Attachment of drugs through amide bonds that are expected to suffer hydrolysis in the body would enable slow release of the drug with increased duration of activity (10,11).

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