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GLYCOLIDE DEUTERIATION BY HYDROGEN ISOTOPE EXCHANGE USING THE HSCIE METHOD

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SUMMARY

Although poly(α-hydroxy acid)s derived from lactic and glycolic acids (PLAGA) are currently used for many surgical and pharmacological applications, their behaviour and their fate *in vivo* are still a source of concern according to some authors. As part of our attempts to label these polymers and monitor their fate by using radioactivity, investigation was made of the possibility to exchange the protons present in glycolide molecules by hydrogen isotopes using the High-temperature Solid-state Catalytic Isotope Exchange (HSCIE) method which was previously successful in the case of lactide. Accordingly $^{1}_{1}H \rightarrow ^{2}_{1}H$ exchanges were carried out on glycolide in the presence of $Pd/CaCO_3$ at various temperatures and 550 mbar pressure of deuterium. Substitution yields as high as 24% were obtained, depending primarily on temperature. PLAGA polymers with lactyl and glycolyl repeating units labeled with deuterium were synthesized and characterized.

Key words : isotopic exchange, glycolide, lactide, deuterium, labeling, poly(α-hydroxy acid)

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INTRODUCTION

Nowadays, poly(α-hydroxy acid)s (PLAGA) constitute the most currently used family of biocompatible and bioresorbable polymers. Many applications as surgical materials and pharmaceutical formulations have been proposed in the literature and the stage of industrial development was reached in several cases (sutures, devices for bone surgery, drug delivery systems) and was extended recently to packagings as well. The behaviour and the fate of PLAGA polymers in complex living media are still a matter of question and concern. In order to monitor chemicals which are sooner or later absorbed or dispersed in complex media, the best method is radiolabeling, as it is currently done in the case of drugs in pharmacology. Isotopic hydrogen exchange is preferable to the introduction of radioactive nuclides to label a biointeractive molecule because the latter can lead to property modification and thus behaviour modification, especially when specific receptor recognition or metabolism is involved.

This is the reason why we have recently undertaken the prospecting of methods to radiolabel PLAGA polymers. In a first attempt, lactide was successfully labeled with ^{2}H and ^{3}H (1) by using the High-temperature Solid-state Catalytic Isotope Exchange (HSCIE) method (2-8). PLA polymers with high specific radioactivity were synthesized by ring opening polymerization of the radioactive monomers (2).

In the present paper, we wish to report the results of the use of the same method to label glycolide. Isotopic hydrogen exchange was initially investigated using deuterium because of the difficult-to-handle radioactivity of tritium and because it was previously shown for lactide that deuterium is a good compound to model the behavior of tritium. Last but not least, deuterium is less expensive than tritium. The HSCIE method was applied to glycolide with the double aim of labeling glycolide and of making PLAGA copolymers labeled in both glycolyl and lactyl-type units.

EXPERIMENTAL

Glycolide was obtained from Purac (Gorinchem, Netherlands) and was purified by sublimation at 50°C under vacuum (10^{-3} mbar) prior to use. 5% $Pd/CaCO_3$ was purchased from Acros Organics (Geel, Belgium). Commercial THF (Carlo Erba, Milan, Italy) was distilled in the presence of sodium metal prior to use. The deuterium gas was purchased from Euriso-Top (CEA, Gif-sur-Yvette, France).

Hydrogen-deuterium exchange reaction

Typically, 50 mg of glycolide was dissolved in 1 cm^3 of THF in a 16.5 cm^3 (3.2 cm diameter) round bottom flask prior to addition of 250 mg of the 5% $Pd/CaCO_3$ catalyst. The mixture was allowed to stir until it became homogeneous. The solvent was evaporated to dryness in order to provide a thin layer of solid deposit on the flask wall. The round bottom flask was then connected to a vacuum line. Then, gaseous deuterium was introduced at 550 mbar pressure. The flask was sealed by a valve and immersed into a temperature-controlled silicone oil bath. At the selected reaction time, the flask was opened and 5 cm^3 of THF was added to the reaction mixture. The catalyst was removed by centrifugation and filtration using a 0.45 μm filter. The white powder recovered after solvent evaporation, was purified by sublimation at 50°C under vacuum (10^{-3} mbar).

Polymerization

Typically, lactide and glycolide were introduced into a round bottom flask. The selected initiator, namely Zn lactate or stannous octoate, was then introduced. The reaction mixture was melted at 120°C and carefully degassed by the vacuum/argon cycle method. At the end, the flask was sealed under vacuum by glass melting.

The sealed flasks were allowed to stand in a temperature-controlled oven at 140°C for the selected reaction time. At the end, the polymers were recovered by dissolution in chloroform

for PLA100, PLA96 or PLAGA. The unreacted lactide and glycolide, and the low molecular weight oligomers, were removed by precipitation in ethanol. The resulting polymeric materials were cooled with liquid nitrogen and the residual solvent was evaporated.

Molecular weights were determined by size exclusion chromatography (SEC) using a Waters equipment fitted with a 30 cm long 10^4 Å Ultrastyragel column, the mobile phase being THF delivered at a flow rate of 1 cm^3/min. Detection was achieved using a Waters 410 differential refractometer with a flow scintillation analyser on line, Flo-one™ Beta Packard. The data was expressed according to polystyrene (PS) standards.

Mass spectrometry

Mass spectra were obtained using an HP 5989 spectrometer equipped with a particle beam interface which separates the eluted molecules from the solvent. The solvent vector was methanol. The ionization potential was 70eV and the source temperature was set at 220°C. Spectra were obtained by chemical ionization, the reaction gas being CH_4. The samples were dissolved in chloroform. The exchange yield was calculated using the following expression :

$$\text{Yield} = \frac{\sum_{j=0}^{4}\left(W_{\%j} \times j\right)}{4} \times 100 \qquad (1)$$

$W_{\%j}$ stands for the area of one j signal as referred to the sum of j areas, j being the number of substituted hydrogens on the glycolide molecules ; 4 was the maximum number of protons available for substitution.

RESULTS AND DISCUSSION

Belonging to the same 1,4-dioxane-2,5-dione family of cyclic dilactones as lactide, glycolide was submitted to the same successful exchange method. Identical experimental conditions were used namely $Pd/CaCO_3$ as catalyst, catalyst /substrate ratio equal to 5, pressure fixed at 550 mbar and volume of the flask fixed at 16.5 cm^3. Only the reaction time and the reaction

temperature were varied. In the case of lactide, the isotopic exchange was found to be primarily dependent on the reaction temperature as in the case of peptide labeling by the HSCIE method (3-8). However, we have also seen that high temperatures can cause degradation of the substrate. Therefore, the reaction temperature used in the case of glycolide was kept below 120°C. After recovery, samples were purified by sublimation. The minimal temperature tested was 70°C because the lactonic glycolide cycle was more fragile than the lactide one. In the case of lactide, NMR and mass spectrometry analyses revealed that only the methine protons were substituted and thus methyl proton resonances could be used as an internal reference. For glycolide, it was impossible to use the same method because there was no protonated group to be used as reference. The only possibility was to introduce a foreign standard. The investigations were simplified by measuring the substitution yield after each test. The data are reported in Table 1.

Table 1 : Influence of temperature and reaction time on the $^{1}_{1}H \rightarrow ^{2}_{1}H$ exchange in glycolide using the HSCIE method.

N°	Temperature (°C)	Time (min)	Recovery yield[a)]
1	70	45	55%
2	80	"[b)]	55%
3	90	"	53%
4	100	"	34%
5	110	"	23%
6	120	"	/[c)]
7-1[d)]	80	45	55%
7-2	"	"	29%
8-1	90	60	52%
8-2	"	"	25%

a) Percentage of recovered glycolide compared to the initial quantity (at the beginning of the first stage of the two reactions both run).
b) " : for idem.
c) / : the glycolide was totally degraded.
d) The first number corresponds to the number test, and the second to the stage number.

These showed a large decrease in the recovery yield (table 1 runs 4 to 6) when temperature was raised above 100°C. At 120°C, the glycolide was completely degraded (run 6). Between 70°C and 90°C, the recovery yield was virtually constant (runs 1 to 3). Finally, 90°C corresponded to a temperature allowing the recovery of 50% of the glycolide after 45 to 60 minutes of reaction (runs 3 and 8-1). In the case of lactide, repeating the process twice in a row led to higher substitution yields. Therefore, the same procedure was used for glycolide (runs 7-1, 7-2 and 8-1, 8-2). Between the two successive reactions, glycolide was recovered and purified by sublimation. The final recovery yield was 25%. The product recovered from run 8-2 was analysed by mass spectrometry. The molecular peak of genuine glycolide was at M+1 i.e. 117. On the spectrum corresponding to deuteriated glycolide molecules, the previous peak was split and appeared as four peaks i.e. 117, 118, 119 and 120 (Figure 1).

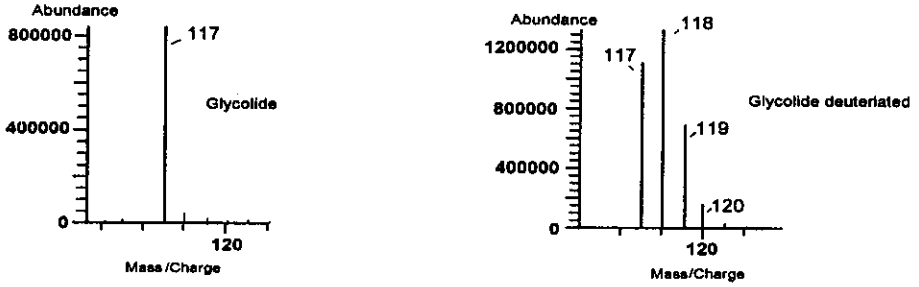


Figure 1 : Mass spectra of genuine glycolide (left) and deuteriated glycolide (right).

Up to 3 substitutions were possible on the same molecule as shown by the presence of a peak at 120. In the case of lactide, the maximal number of substitution was 2 and these substitutions affected the 2 H in the α position of the carbonyl according to ^1H NMR and mass spectra data. The glycolide having 4 H in the α position, one could expected up to 4 substitutions (Figure 2).

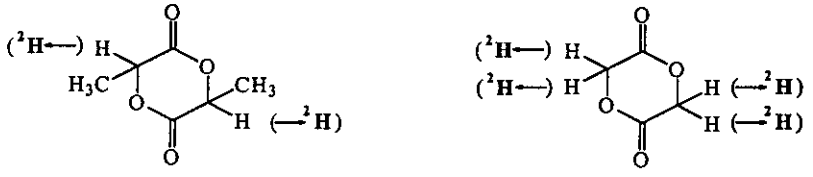
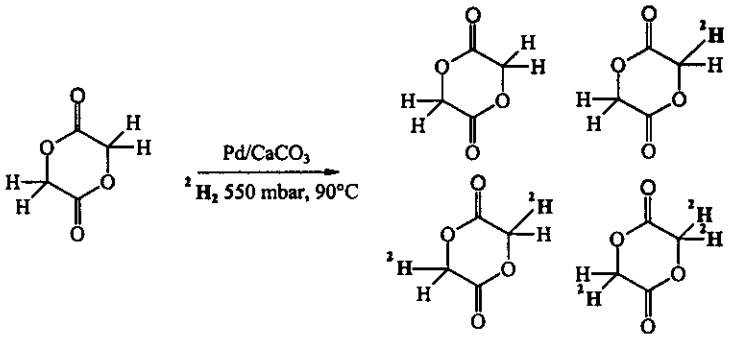


Figure 2 : Chemical formula of DL-lactide (left) and glycolide (right).

According to the mass spectra, the peak at 121 could not be differentiated from the background, thus showing that substitution was limited to 3, the resulting glycolide being a mixture of zero, mono, di and tri-deuteriated glycolides. The relative proportion of each of these isomers was 34%, 40%, 21% and 5%. The average substitution yield estimated from equation 1 was 24%. As the number of protons was smaller on glycolide than on lactide, a 24% labeling on glycolide turned to be higher in specific radioactivity (RAS) than the 43% yield found in the case of lactide (1). The exchange reaction in the solid state can thus be represented by the following reaction :



Reaction 1 : Exchange reaction on solid state applied to glycolide.

Comparison of lactide and glycolide labeling conditions showed that in both cases the optimal reaction temperature was close to the melting temperature. For DL-lactide, the optimal temperature was 120°C for a melting temperature of c.a. 123-125°C, whereas for glycolide the optimal temperature was 90°C for a melting temperature of 83-85°C. This observation suggested that the melting played a significant role in the exchange reaction. On the other hand, sublimation was observed from the heated bottom of the flask to the cold neck. This feature showed that glycolide molecules in the vapor state were also present during the exchange reactions. Therefore, whether the prevailing substitution occurred on solid, liquid and/or gaseous glycolide remains to be seen. Deuteriated lactide and glycolide being available, various poly(α-hydroxy acid)s were synthesized that were labeled either at lactyl or at glycolyl-type units (Table 2).

Table 2 : Examples of polymers and copolymers obtained from deuteriated monomers.

N°	Monomers	Copolymers PLAXGAY[a)]	$\overline{Mw}$ [b)]	Ip[c)]
1	Deuteriated DL-lactide	PLA50*[d)]	91 000	2.2
2	Deuteriated DL- + deuteriated L-lactides	PLA96*	11 300	1.7
3	Deuteriated L-lactide	PLA100*	26 000	1.6
4	DL-lactide + deuteriated glycolide	PLA37.5GA25*	17 900	2
5	DL-lactide + deuteriated glycolide	PLA25GA50*	32 100	1.6

a) X=100n/(n+p), n and p corresponding respectively to the ratio of repetition units of L-lactyl and D-lactyl. Y=100q/(n+p+q), n, p and q corresponding respectively to the ratio of repetition units of L-lactyl, D-lactyl and glycolyl.
b) $\overline{Mw}$: mass molar weight.
c) Ip : index of polymolecularity.
d) * : deuteriated.

Polymers with molecular weight in the range of 10^4-10^5 g.mole^{-1} were obtained suggesting that the synthesis of similar tritium-labeled polymers should be possible after convenient tritiation of glycolide.

CONCLUSION

The labeling of glycolide by deuterium was successfully achieved by a similar route to that investigated previously for lactide. Only the temperature was different. The optimal reaction temperature was found once more to be similar to the melting temperature. Analysis by mass spectrometry showed that the recovered glycolide was a mixture of zero, mono, di and tri substituted glycolides. From labeled lactide and glycolide, it was possible to synthesize PLAGA copolymers labeled either at lactyl or glycolyl sites or at both. These results suggest that any PLAGA copolymers could be labeled differently e.g. having lactyl and glycolyl units labeled respectively by ^{14}C and deuterium and/or tritium.

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