FLUORINE-18 LABELLING OF ortho-FLUOROHIPPURIC ACID BY ISOTOPE EXCHANGE REACTION

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Received May 12, 1977
Revised June 28, 1977

ABSTRACT

The ^{18}F-labelling of o-fluorohippuric acid by isotope exchange reactions has been studied in several organic solvents under varying reaction conditions. The solvent mixture ethanol/water (1:1) was found suitable for the isotope exchange reaction: a 100 per cent isotope exchange was observed for this system after an exchange time of one and half hours. No isotope exchange was observed in the molten state of the exchanging species.

INTRODUCTION

Radionuclides and labelled molecules are applied to an increasing extent in science, technology (1-6) and medicine (7-9) in recent times. This has led to the development of adequate methods by radiochemists for producing the interesting radionuclides and introducing them into the appropriate chemical compounds for use in medical diagnosis and scientific investigations. There exists today a long list of such diagnostic radioisotopes, which - to name a few - include ^{32}P, ^{51}Cr, ^{59}Fe, ^{99m}Tc, ^{197}Hg, ^{64}Cu, ^{87m}Sr, ^{123}I, ^{125}I, and ^{131}I.

With regard to the half-life $t_{\frac{1}{2}}$ of the radioisotope, it is very important to employ radionuclides with relatively short half-life to maintain the lowest possible radiation load on the patients (e.g. ^{99m}Tc, $t_{\frac{1}{2}} = 6h$; ^{87m}Sr, $t_{\frac{1}{2}} = 2.8h$). The biological half-life of the labelled compounds in the organ and the type of

0362-4803/78/0514-0705$01.00

radiations emitted by the radionuclides are also important factors in deciding for a particular radionuclide.

Radioiodine, mostly in form of ^{131}I-labelled o-iodohippuric acid has been applied in clinical functions diagnostics, for example, radioiodine test of the thyroid glands and the functional investigation of the spleen, kidney and brain. Iodine-131 emits γ-quanta (E = 0.36 MeV and 0.64 MeV), which are used for the scintigraphic measurements and also β^--particles of maximum energy 0.8 MeV. These emitted β^--particles are not required for the measurements and can only increase the unrequired radiation load on the patients. The advantage of using the radionuclide fluorine-18 instead of ^{131}I is that ^{18}F emits only β^+-particles, which yield γ-quanta of energy 0.51 MeV (positron annihilation). Besides, the half-life for ^{18}F is shorter ($t_{\frac{1}{2}}$ = 2.56h) than that of ^{131}I ($t_{\frac{1}{2}}$ = 8.06d). It appears therefore interesting to label the hippuric acid with ^{18}F (o-fluorohippuric-^{18}F) instead of ^{131}I (o-iodohippuric acid-^{131}I).

EXPERIMENTAL

All the chemicals such as ethanol, glycine, o-fluorobenzoyl chloride and hydrochloric acid used in the experiments were Merck (Darmstadt) "analytical reagents" and were not further purified.

Preparation of o-fluorohippuric acid:

o-Fluorohippuric acid is not available commercially and so it was prepared by the action of glycine on o-fluorobenzoyl chloride in a weakly alkaline solution according to equations (1) and (2).

$$\text{o-F-C}_6\text{H}_4\text{-COCl} + \text{H}_2\text{N-CH}_2\text{-COOH} + 2\ \text{NaOH} \longrightarrow \text{o-F-C}_6\text{H}_4\text{-CONH-CH}_2\text{-COONa} + \text{NaCl} + \text{H}_2\text{O} \qquad (1)$$

$$\text{o-F-C}_6\text{H}_4\text{-CONH-CH}_2\text{-COONa} + \text{HCl} \longrightarrow \text{o-F-C}_6\text{H}_4\text{-CO-NH-CH}_2\text{-COOH} + \text{NaCl} \qquad (2)$$

A similar method was used by Schotten and Baumann for the preparation of o-iodohippuric acid. (10, 11)

0.1 Mole of glycine was dissolved in 150 cm^3 water in a four-necked round-bottomed flask, which was fitted with a stirrer and two dropping-funnels. To the stirred glycine solution were added slowly and simultaneously during 30 minutes through the funnels 0.1 Mole o-fluorobenzoyl chloride and a solution of 0.2 Mole sodium hydroxide in 100 cm^3 water. The reaction mixture was stirred for another two hours, while the temperature was maintained between 30°C and 40°C. Thereafter the mixture was treated with 30 cm^3 concentrated hydrochloric acid. The precipitated o-fluorohippuric acid was filtered off. The product was purified by heating it with about 50 cm^3 carbon tetrachloride for 10 minutes. After filtration the white, crystalline acid was recrystallized from a small quantity of absolute ethanol. The yield was 81% and m.p.: 145-146°C.

The determined melting point did not agree with the m.p. (121-121.5°C), given in a publication in the year 1883. (12) Because of this discrepancy the purity of the prepared o-fluorohippuric acid was further checked by elemental analysis. Found: C,54.52; H, 4.25; N, 7.02; F, 9.80. Calculated for $C_9H_8FNO_3$; C, 54.84; H, 4.06; N, 7.11; F,9.64%. Fluorine was quantitatively determined by fusing the acid with sodium peroxide and eventually precipitating it as PbClF. (13) Within experimental error the determined values agree with the theoretical values. It can only be assumed that the m.p. stated in the publication (12) is not correct.

Isotope Exchange reaction for the ^{18}F-labelling of o-fluorohippuric acid: For the ^{18}F-labelling of o-fluorohippuric acid the isotope exchange method was used according to the reaction equation (3):

$$\text{o-F-}C_6H_4\text{-CO-NH-}CH_2\text{-COOH} + {}^{18}F^- \rightleftharpoons \text{o-}{}^{18}F\text{-}C_6H_4\text{-CO-NH-}CH_2\text{-COOH} + F^- \quad (3)$$

The $^{18}F^-$-ions were used in form of $K^{18}F$. The fluorine isotope ^{18}F was produced by photonuclear reaction $^{19}F(\gamma,n)^{18}F$ in a linear accelerator. (14) The exchange reaction was studied at different temperatures in the solvents: ethanol, ethanol/water, dioxane and dioxane/water.

In addition the exchange reaction was also studied under the following conditions:

a) radiation - induced isotope exchange according to equation (3) in the solvent system ethanol/water (1:1); radiation source: ^{60}Co with dose rate of 0.45 Mrad/h.

b) Isotope exchange according to equation (3) in molten o-fluorohippuric acid. For all the cases the extent or degree of exchange was determined by the method of radio-thin-layer-chromatography. Ready-made thin-layer plates (silica gel F254, 5x20 cm, Merck Darmstadt) were used. A few drops of the reaction solution or dissolved molten reaction mixture were applied with a microlitre pipette to the thin-layer plates.

The development of the thin-layer plates was carried out with ethanol/chloroform/concentrated ammonia (8:4:1) solvent system. The R_f-value for o-fluorohippuric acid was 0.37 and zero for fluoride ions. The thin-layer plates were then exposed to UV-light and the developed spots were outlined with a pencil. The distribution of radioactivity on the spots was determined with a methane gas-flow thin-layer chromatogram scanner (Berthold LB 2720, Wildbad/W. Germany) fitted with a Geiger tube. The scan speed was about 120 mm per hour.

To evaluate the radio-thin-layer chromatogram the peak areas PA(F^-) (i.e. the area for $^{18}F^-$-activity) and PA(o-^{18}FHA) (i.e. the area for labelled o-fluorohippuric acid) were determined. If ^{18}F-activity is in form of $^{18}F^-$-ions before the exchange reaction according to equation (3), then the degree, D, of isotope exchange can be defined by equation (4). (15)

$$D = \frac{S(\underline{o}\text{-}^{18}\mathrm{FHA})}{S_\infty} \qquad (4)$$

where S(o-^{18}FHA) = specific radioactivity of o-fluorohippuric-acid-^{18}F after the exchange

S_∞ = specific radioactivity at equilibrium (i.e. after infinite exchange reaction time).

It can then be easily derived that the relationship (equation 5) is also valid for the degree of exchange, especially in this case that the isotope exchange is followed up by the method of radio-thin-layer chromatogram

$$D = \frac{PA(\underline{o}\text{-}^{18}FHA)}{PA(\underline{o}\text{-}^{18}FHA) + PA(^{18}F^-)} \cdot \left(1 + \frac{b}{a}\right) \qquad (5)$$

where $PA(\underline{o}\text{-}^{18}FHA)$ = peak area for the $\underline{o}$-^{18}fluorohippuric acid activity

$PA(^{18}F^-)$ = peak area for the $^{18}F^-$-ions activity

a = concentration of $\underline{o}$-fluorohippuric acid in the reaction solution $\left[\frac{\text{mole}}{\text{litre}}\right]$

b = concentration of the $^{18}F^-$-ions in the reaction solution $\left[\frac{\text{mole}}{\text{litre}}\right]$

To recover the ^{18}F-labelled o-fluorohippuric acid from the thin-layer plates, the ringed spots were scraped off the plates. The labelled acid was then eluted with very small quantities of absolute ethanol and could be concentrated to the desired specific radioactivity.

RESULTS AND DISCUSSION

The results of the ^{18}F-labelling of o-fluorohippuric acid in different solvents are summarised in Table I. Within experimental error, the accuracy of the determined D-values (degree of exchange) decreases with increasing reaction time because of the relatively short half-life of ^{18}F. For a reaction time of one hour the error is estimated at about ± 10% because of the low activity (counts per minute) observed in the evaluation of the thin-layer chromatogram. There was no isotope exchange between $^{18}F^-$ and o-fluorohippuric acid in pure dioxane at 100°C, but an exchange was observed when water was added.

Table I : Results of ^{18}F-labelling of o-fluorohippuric acid by isotope exchange reaction

Solvent	Concentration of $K^{18}F$ $\left[\frac{\text{mole}}{\text{litre}}\right]$	Concentration of o-FHA $\left[\frac{\text{mole}}{\text{litre}}\right]$	Temp. [°C]	Reaction Time [h]	Degree of Exchange D [%]
Water	0.0043	0.00254	100	0.25	28
				0,75	40
				1.50	55
Ethanol	0.00323	0.0019	78	0.25	10
				0,75	10
				1.00	10
Ethanol/ Water (1:1)	0.0043	0.00254	80	0.25	40
				1.00	68
				1.50	100
Dioxane	0.00323	0.0019	100	0.25	0
				1.00	0
Dioxane/ Water(2:1)	0.0043	0.00254	100	0.25	18
				1.00	32
a) –	b) 0.0577	b) 0.254	150	0.25	17

a) = molten state of o-fluorohippuric acid and $K^{18}F$

b) = concentrations are given in millimoles.

The irradiation of the exchanging species in the solvent system ethanol/water (1:1) with a ^{60}Co-source, with a dose rate of about 0.45 Mrad/h for one hour (radiation-induced exchange) yielded results similar to those in Table I obtained for ethanol/water without irradiation. The results did not indicate a faster rate of isotope exchange.

The exchange reaction experiments for the ^{18}F-labelling of o-fluorohippuric acid showed clearly that the isotope exchange depends strongly on the solvent. While the degree of exchange in the solvent system ethanol/water (1:1) at about 80°C amounted to 100% after an exchange time of one and half hours, little or no exchange was observed in pure ethanol under the same conditions. The exchange was clearly slower in pure water than in ethanol/water. Similar to pure ethanol, pure dioxane is not a suitable solvent for the isotope exchange. It can be explained that little or no exchange was observed in pure organic solvents because the labelled salt $K^{18}F$ is very slightly soluble or only slightly dissociated in these solvents.

The exchange time of about one and half hours in the suitable solvent mixture ethanol/water (1;1) is not all that favourable when the relatively short half-life of ^{18}F is taken into account and especially when a high specific radioactivity of the labelled compound is required. It may be possible to reduce the exchange time, if isotope exchange reactions are carried out in a suitable solvent in sealed ampoules at higher temperatures.

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