Application of N-succinimidyl 4-[^{18}F](fluoromethyl) benzoate to protein labeling

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Abstract N-succinimidyl 4-[^{18}F](fluoromethyl) benzoate for protein labeling was prepared (57%, EOB) in about 30min. Reaction conditions of S^{18}FMB with IgG including pH of solutions, protein concentration, reaction temperature and time were studied. The optimal labeling conditions were: 0.2mg/mL IgG, pH = 7.8-8.5, 25℃, and reaction time 5min.Under these conditions the yield was about 80%. The ^{18}F-labeled protein was purified by size exclusion chromatography.

Keywords S^{18}FMB, IgG, Protein labeling, Size exclusion chromatography

CLC number Q503

1 Introduction

Positron emission tomography (PET) imaging coupled with monoclonal antibodies (Mabs) has generated interest in the development of methods for labeling proteins with positron-emitting nuclides. Among the positron emitters in routine use, ^{18}F is particularly attractive because its relatively long half life (110 min) is more compatible with Mab pharmacokinetics than ^{15}O, ^{13}N or ^{11}C[1-3].

One potential application of ^{18}F-labeled Mabs and PET would be to obtain quantitatively the uptake data of tumor and normal tissue for dosimetry estimation prior to radioimmunotherapy [4-6]. Several methods have been reported for the preparation of ^{18}F labeled proteins in general, and Mabs in particular[7,8].We recently reported a method for the one step synthesis of fluorine-18 labeled N-succinimidyl 4-[^{18}F](fluoromethyl) benzoate (S^{18}FMB)[9]. In this paper, the labeling of IgG was described, and several factors effecting the reaction were studied.

2 Materials and Methods

2.1 Materials

IgG was purchased from Sigma Chemical Company. Sephadex G-25 was obtained from Pharmacia. All other Chemicals were obtained from ACROS or commercially available and of analytical grade. NMR spectra were obtained on a Varian VXR-200 (200 MHz) instrument with tetramethylsilane as the internal standard. Thin-layer chromatography of the radioactive products was performed on GF254 silica gel glass-backed plates (5×20 cm^2, 250 μm).

2.2 Methods

2.2.1 Preparation of N-succinimidyl-4-[(4-nitrobenzenesulfonyl)oxymethyl]-benzoate(SNOB)

The preparation of N-succinimidyl-4-[(4-nitrobenzenesulfonyl)oxymethyl] benzoate was introduced elsewhere[9]. In short, 4-nitrobenzenesulfonyl chloride was mixed with silver oxide at mole ratio of about 1:2. The mixture was stirred at room temperature for 2 days in dark and unreacted silver oxide was filtered. The solvent was evaporated and the silver 4-nitrobenzene sulfonate was obtained in form of crystal without further purification. N-succinimidyl 4-(bromomethyl)benzoate was mixed with silver 4-nitrobenzene sulfonate(about 1:3 mol/mol) in acetonitrile protected from light and was stirred at room temperature for 5-7 days. The solid was filtered and the solvent was evaporated. EtOAc was added and the obtained white crystals were filtered. After evaporation of solvent, the SNOB was recrystalized in dichloromethane and hexane to give white crystal (75% yield).

1H NMR ($CDCl_3$)δ(ppm):2.91(s,4H,CH_2),5.25 (s,2H,CH_2),7.93-8.25(d,8H,arom) IR(KBr):1795,1769(C=O),1600(arom),1354,1532(NO_2).

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2.2.2 Preparation of N-succinimidyl 4- (fluoromethyl) benzoate (SFMB)

4 mg (67 μmol) potassium fluoride, 20 mg (52 μmol) kryptofix 222 , 3 mg (21 μmol) potassium carbonate and 1mL dry acetonitrile were mixed in a vial. The mixture was dried with nitrogen flow at 105℃. Azeotropic drying was repeated at least twice (depending on the amount of target water). After cooling, 22 mg (50μmol)SNOB in acetonitrile was added and the reaction mixture was heated at 80℃ for 10 min. Desired SFMB was purified by Sep-Pak silica and first washed with methylene chloride, then with CH_3OH. TLC analysis with $CHCl_3$ and CH_3OH (2/1 v/v) showed R_f=0.8-0.9; ^1H NMR($CDCl_3$) δ(ppm) 2.91 (s, 4H), 5.25 (s,2H), 7.25(m, 4H).

2.2.3 Radiochemical synthesis of N-succinimidyl 4-^{18}F-(fluoromethyl)-benzoate (S^{18}FMB)

[^{18}F]fluoride was produced via the $^{18}O(p, n)^{18}F$ nuclear reaction by bombardment of an isotopically enriched [^{18}O] water target with a 16.5 MeV proton beam at the Cyclone 3D cyclotron.

S^{18}FMB was synthesized using a procedure similar to the step above. For a typical run, 5-10 mCi ^{18}F (in 23 mg/mL potassium carbonate) was resolubilized with 1mg kryptofix 222 and 100 μL dry acetonitrile. The mixture was dried with nitrogen flow at 105-110℃. Azeotropic drying was repeated at least twice. After cooling, 1 mg SNOB in dry acetonitrile was added and the reaction mixture was heated at 80℃ for 5 min. The conditions of isolation were the same as the step above. The radiochemical yield was about 57% (decay corrected) by TLC (mobile phase: $CHCl_3/CH_3OH$=4/1 v/v). The radiochemical purity was greater than 97%, determined by TLC.

2.2.4 Labeling of IgG using S^{18}FMB

The protein was dissolved in 1 mL of potassium phosphate buffer to give protein concentrations of 0.01-1 g/L, and 0.1-0.5 mL of these solutions added to the solution of S^{18}FMB in about 50 μL acetonitrile. The mixtures were mixed at different temperatures for desired time. At the end of reaction, the mixtures were purified using sephadex G-25 column which was eluted with 0.05 mol/L saline solution.

3 Results and discussions

3.1 Synthesis of N-succinimidyl 4-[^{18}F](fluoromethyl) benzoate

The preparation of S^{18}FMB was shown in Fig.1. The radiochemical yield was strongly dependent on the mole ratio of kryptofix 222 to potassium carbonate, and it also varied with reaction time, temperature and dryness of the reaction solvent. The optimal labeling conditions were as follows: the mole ratio of K2.2.2 to K_2CO_3 was 1:1, reaction time 5 min at 80℃, and dry acetonitrile as solvent. The radiochemical yield was about 57% (EOS), a value more than 3 times that published in literature[10]. It was found that dryness of acetonitrile played a key role. 5-10% of S^{18}FMB would hydrolyze if the analytical grade acetonitrile was used, but less than 2% of that if dry solvent used. The product was separated from unreacted [^{18}F]fluoride and SNOB by simple filtration through a silica gel SEP-PAK. The product was suitable for use in the protein labeling step(after evaporation of solvent).

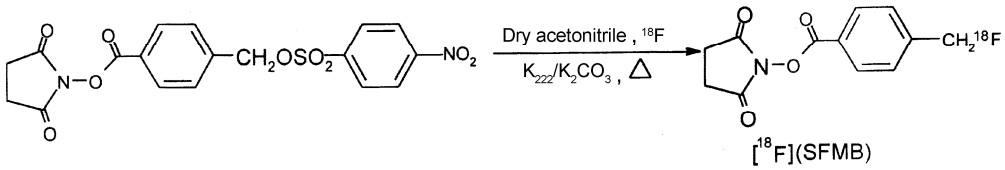

Fig.1 Synthesis scheme of N-succinimidyl 4-^{18}F-(fluoromethyl)- benzoate.

3.2 Labeling of IgG

The effects of protein concentration, reaction time, pH , reaction temperature and the amounts of S^{18}FMB on the reaction of S^{18}FMB with IgG were carefully studied. The results were shown in Fig.2-5.

The amidination reaction was strongly dependent on pH. There was a suitable range of pH to obtain higher yields. The labeling yields were higher when pH was

7.8-8.5. There was a competition between amidination and hydrolysis[11]. It was found that during the process of labeling IgG the stability of S^{18}FMB in aqueous solution at pH<8.5 was desirable. In higher pH solutions(pH>8.5), hydrolysis of S^{18}FMB became a main reaction. In addition, higher pH was not suitable for application to human bodies.

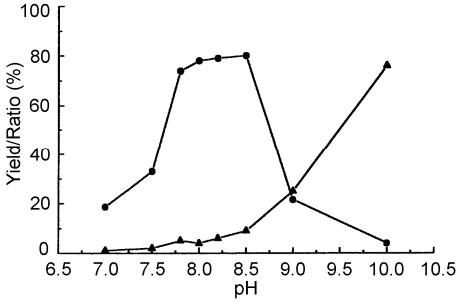

Fig.2 Yield of the reaction of S^{18}FMB with IgG vs. pH value of reaction solution (0.2 g/L IgG , 25℃, 15 min , n = 3) ●— reaction yield of S^{18}FMB with IgG; ▲—hydrolyzing ratio of S^{18}FMB.

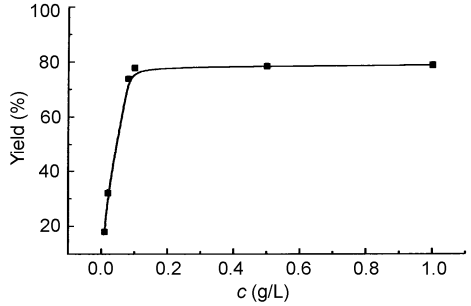

Fig.3 Dependence of yield of the reaction on concentration of IgG (pH 8.5, 25℃, 15 min, n = 3).

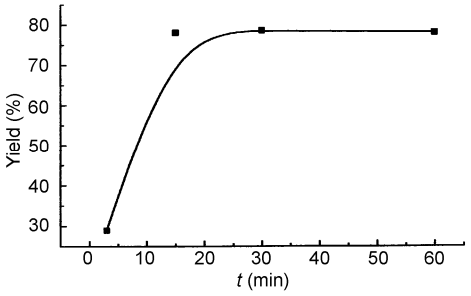

Fig.4 Dependence of yield of the reaction on reaction time (t) (pH 8.5, 25℃, 0.2 g/L IgG, n = 3).

Enough protein concentrations and reaction time were important in the labeling reaction. As antibodies were often precious commodities, selecting suitable amount of protein as little as possible would be necessary. Considering short half life of fluorine-18 and hydrolysis of S^{18}FMB, a long labeling time was undesirable.

The yield of labeling IgG was also temperature dependent. Lower temperature would give low yield. Higher temperature, however, would increase the probability of denaturalization of protein. The labeling reaction progressed smoothly at room temperature. There was no dependence of yield or labeling rate of IgG on the concentration of S^{18}FMB, as nearly identical results were obtained using larger reaction volumes.

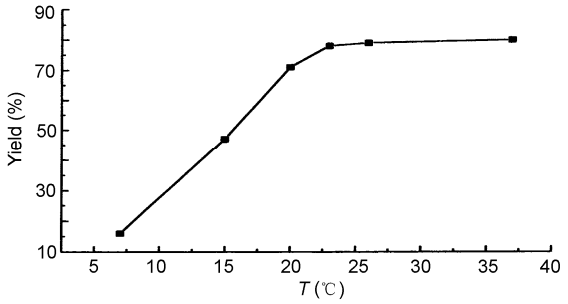

Fig.5 Yield of the reaction as a function of reaction temperature (T) (pH 8.5, 15 min, 0.2 g/L IgG, n = 3).

4 Conclusions

N-succinimidyl 4-[^{18}F](fluoromethyl) benzoate (S^{18}FMB) was obtained with high yield. IgG was labeled with S^{18}FMB. Under the optimal labeling conditions (0.2 g/L of IgG, pH = 7.8-8.5, 25℃, and reaction time 5 min) the yield would be about 80%.

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