SELENIUM LABELED FATTY ACIDS AS POTENTIAL MYOCARDIAL IMAGING AGENTS

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SUMMARY

Six ^{75}Se labeled fatty acid analogues were synthesized : 3-selena-[^{75}Se]pentadecanoic acid, 4-selena[^{75}Se]hexadecanoic acid, 3-selena[^{75}Se]heptadecanoic acid, 4-selena[^{75}Se]octadecanoic acid, 3-selena[^{75}Se]nonadecanoic acid, and 4-selena[^{75}Se]-eicosanoic acid. Selenious acid was reduced with $NaBH_4$ in buffer pH 6.0. The nucleophile produced, NaSeH . was reacted with different alkyl halide to form the corresponding selenol. To this was added an aqueous solution of the sodium salt of either iodoacetic acid or 3-bromopropionic acid to form the sodium salt of the desired fatty acid . The free fatty acids were extracted in ether after acidification of the solution.

Keywords:

Se-75 Labeled fatty acids. 3-selena[^{75}Se]pentadecanoic acid, 4-selena[^{75}Se]hexadecanoic acid, 3-selena[^{75}Se]heptadecanoic acid, 4-selena[^{75}Se]octadecanoic acid, 3-selena[^{75}Se]nona-decanoic acid, 4-selena[^{75}Se]eicosanoic acid.

INTRODUCTION

Long-chain free fatty acids are a major energy source for the myocardium and are highly extracted from the blood by the heart (1). Several investigators have used fatty acids labeled with radioactive halogens (e.g. ^{123}I , ^{131}I, and ^{77}Br) for myocardial scintigraphy. The instability of the halogen derivatives, *in vivo*, results in rapid loss of radioactivity from the myocardium as free iodide (2-5). To minimize the loss of radioactivity due to dehalogenation radioiodine was attached to an aromatic ring positioned at

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the ω-carbon of a fatty acid (6).

In addition, β-oxidation plays a very important role in the rapid clearance of radioactivity from the myocardium. To block β-oxidation, branched chain fatty acids have been proposed (7). Also, selenium and tellurium derivatives have been suggested (8,9). Knapp et al (10) reported that 17-[^{131}I]-iodo-9-telluraheptadecanoic acid showed higher uptake by the myocardium than 16-[^{131}I]iodopalmitic acid but less than 9-[^{123m}Te]telluraheptadecanoic acid proving that the deiodination is not only due to β-oxidation but also due to the weakness of iodo-aliphatic carbon bond.

We felt that the synthesis and biodistribution studies of Se-75 labeled fatty acids with different chain lengths will be useful in developing a potential imaging agent for the myocardium. The selenium was positioned in the chain, replacing either the β-carbon or the γ- carbon and chain length was varied from 15 to 20 carbons.

RESULTS AND DISCUSSION

We synthesized six selenium-75 containing fatty acids: 3-selenapentadecanoic acid , 3-selenaheptadecanoic acid, 3-selenanonadecanoic acid whereby the selenium replaced the β-carbon and 4-selenahexadecanoic acid, 4-selenaoctadecanoic acid and 4-selenaeicosanoic acid whereby selenium replaced the γ-carbon.The synthesis of the selenafatty acids was accomplished according to Scheme I using NaHSe as a nucleophile. NaHSe can be produced by reducing selenium metal with one equivelant of sodium borohydride (11). Since Se-75 is available in the form of selenious acid (H_2SeO_3) in a very high specific activity and with a reasonable price, we developed a new method for generation of NaHSe from H_2SeO_3 (12). Reduction of H_2SeO_3 with three equivelants of $NaBH_4$ at pH 6.0 produced mainly NaHSe. The latter was used as a nucleophile to displace the halide of different alkyl halide forming

the corresponding selenol. Upon exposure to air the selenol oxidizes to the corresponding diselenide which has a yellow color. The reduction of the diselenide with $NaBH_4$ followed by the addition of the sodium salt of the suitable α- or β-haloacid afforded the sodium salt of the desired fatty acid.

Scheme I:

$$H_2SeO_3 + NaBH_4 \longrightarrow NaHSe$$

$$NaHSe + R\text{-}X \longrightarrow RSeH \xrightarrow{[O_2]} RSeSeR$$

$$RSeSeR + NaBH_4 + ICH_2COONa \text{ or } BrCH_2CH_2COONa \longrightarrow$$

$$CH_3(CH_2)_{n1}\text{-}Se\text{-}(CH_2)_{n2}\text{-}COOH$$

1: n1 = 11, n2 = 1 4: n1 = 13, n2 = 2

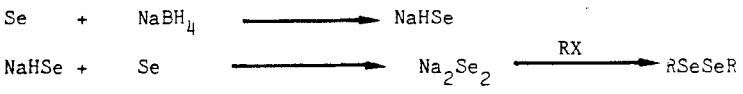
2: n1 = 11, n2 = 2 5: n1 = 15, n2 = 1

3: n1 = 13, n2 = 1 6: n1 = 15, n2 = 2

The purity and identity of the products were determined by TLC. A radioscan of the TLC plate using benzene:methanol (3:2) as an eluent showed all the activity coincident with the spot of the authentic selenafatty acids. The "cold" selenafatty acids were prepared from selenium metal according to Scheme II. Selenium metal was reduced with one equivelant of $NaBH_4$ to produce NaSeH which was converted to Na_2Se_2 by addition of one more equivelant of Se (13). Disodium diselenide reacts with two molecules of alkyl halide to form the corresponding dialkyl diselenide. The selenafatty acids were prepared from the corresponding diselenides in the same way as the radioactive compounds. The structure of the nonradioactive fatty acid

derivatives were confirmed using NMR and MS (Table 1).

Scheme II



EXPERIMENTAL

MATERIALS AND METHODS:

All reagents and solvents were analytical grade and were used without further purification. Se-75 was obtained as selenious acid in 1N HCl from Oak Ridge National lab.(Specific activity = 185 mCi/ mg). Melting points were determined on a Thomas Hoover melting point apparatus. Proton NMR spectral data were obtained using a Varian EM-360 NMR (60 MHz). Samples were prepared in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in parts per million (δ) relative to TMS and signals are described as s(singlet), d(doublet), q(quartet), and m(multiplet). Mass spectra data were obtained with a DuPont 21-492 B mass spectrometer.

SYNTHESIS OF NONRADIOACTICE DIALKYL DISELENIDE:

Selenium metal (0.39 g, 5 mmol) was suspended in 25 ml of water, under nitrogen. Sodium borohydride (0.38 g,10 mmol) in 10 ml of water was added to the selenium suspension dropwise (CAREFUL, EXOTHERMIC). A reddish brown solution initially formed and finally decolorized. To this solution, 0.39 g of Se was added to form a reddish brown solution. This was stirred for 15 min. and warmed for about 10 min for complete dissolution of selenium. The alkyl halide (10 mmol) in THF (25 ml) was added to this solution (THF:H_2O= 1:1) . In one hour the solution turned yellow indicating the reaction was

Table 1: Analytical Data of Selenafatty Acids I-VI.

Compound	mp (°C)	NMR (δ)	MS (m/e)
3-Selenapentadecanoic acid (1)	48-49	0.8-0.97(t,3H,CH_3), 1.2-1.4(m,20H,10 CH_2), 2.65-2.78(t,2H, CH_2Se), 3.6(s,2H, $SeCH_2COOH$).	308(M for ^{80}Se), 249(M - CH_2COOH)·
4-Selenahexadecanoic acid (2)	61-62	0.83-0.98(t,3H,CH_3), 1.23-1.4(m,20H,10 CH_2), 2.50-2.73(t,2H,CH_2Se), 2.78-2.89(m,4H,$SeCH_2$-CH_2COOH).	322(M for ^{80}Se), 249(M - C_2H_4COOH)·
3-Selenaheptadecanoic acid (3)	62-63	0.80-1.03(t,3H,CH_3), 1.26-1.43(m,24H,12 CH_2), 2.70-2.83(t,2H,CH_2Se), 3.16(s,2H,CH_2COOH).	336(M for ^{80}Se), 277(M - C_2H_4COOH)·
4-Selenaoctadecanoic acid (4)	70-71	0.79-1.02(t,3H,CH_3), 1.20-1.38(m,24H,12 CH_2), 2.50-2.73(t,2H,CH_2Se), 2.75-2.86(m,4H,CH_2-CH_2COOH).	350(M for ^{80}Se), 277(M - C_2H_4COOH)·
3-Selenanonadecanoic acid (5)	69-70	0.90-1.03(t,3H,CH_3), 1.20-1.50(m,28H,14 CH_2), 2.70-2.92(t,2H,2 CH_2Se), 3.17(s,2H,CH_2COOH).	364(M for ^{80}Se), 305(M - CH_2COOH)·
4-Selenaeicosanoic acid (6)	73-74	0.73-0.93(t,3H,CH_3), 1.23-1.36(m,28H,14 CH_2), 2.46-2.68(t,2H,CH_2Se), 2.73-2.80(m,4H,CH_2-CH_2COOH).	378(M for ^{80}Se), 305(M - C_2H_4COOH)·

complete. The reaction mixture was extracted with chloroform and the organic layer was dried over magnesium sulfate, filtered and evaporated to leave a yellow oil which solidified by cooling. Crystallization from ether/ methanol provided yellow needles of the desired diselenides:

Didodecyl diselenide : yield 82 %; mp 30°C, NMRδ 0.83-0.93 (t, 6H, 2 CH_3), 1.23-1.4 (m, 40 H, 20 CH_2), 2.8-3.03(t, 4 H, 2Se-CH_2).

Ditetradecyl diselenide : yield 84 %; mp 43°C; NMR δ 0.83-1.03 (t,6H, 2 CH_3), 1.3-1.45 (m, 48 H, 24 CH_2), 2.83-3.06 (t, 4H, 2 CH_2Se-).

Dihexadecyl diselenide: yield 85% ; mp 50-51°C; NMR δ 0.85-1.02 (t, 6H, 2 CH_3), 1.2-1.4 (m, 56 H, 28 CH_2), 2.8-3.03(t, 4H, 2 CH_2Se-).

SYNTHESIS OF NONRADIOACTIVE SELENAFATTY ACIDS:

To the diselenide (5 mmol) in THF , $NaBH_4$ (10 mmol) in ethanol/ water (1:1) was added dropwise under nitrogen. After the solution decolorized , the sodium salt of the suitable haloacid (20 mmol) was added and the solution was stirred for 30 min . Then, water was added and the solution extracted with ether. The organic layer was discarded. The aqueous layer was acidified with HCl and extracted with ether. The ether layer was dried over anhydrous magnesium sulfate , filtered and evaporated under vacuo leaving a white solid. Crystallization from water/ethanol provided white crystals of the desired compounds (see Table 1).

SYNTHESIS OF Se-75 DERIVATIVES OF FATTY ACIDS:

Method A:

To 10 µmol of H_2SeO_3 in a 25-ml three-necked round bottom flask, was added 4 mCi of Se-75 selenious acid. Sodium borohydride (1.193 mg, 30 µmol) in 0.1 ml water was added with a syringe, under nitrogen. A red precipitate was formed immediatly which was dissolved to form a red solution and finally a colorless solution. 0.5 ml of phosphate buffer (pH 6.0, 0.5 M) was added. To this solution, 10 µmol of the appropriate alkyl halide in 0.1 ml THF was added followed by the addition of one ml of THF. After stirring for two

hours, a few drops of $NaBH_4$ solution in water was added to reduce any diselenide to selenol. This was followed by the addition of sodium iodoacetate (3.54 mg,20 µmol) or sodium 3-bromopropionate (3.06 mg,20 µmol) in water. One ml of 10 % NaOH was added and the stirring was continued for two hours at room temperature. The reaction mixture was washed four times with ether to remove the undesirable Se-75 dialkyl selenide. The aqueous layer was acidified with 1 N HCl and extracted with ether . The radiochromatogram showed the desired compounds with a purity >97 %. The radiochemical yield was 65-80 %.

Method B:

The selenol, formed in the same way as in method A, was oxidized to the diselenide by exposure to air. The diselenide was extracted with ether. The organic layer was evaporated and the residue left was dissolved in 0.2 ml THF. This solution was transferred to a three- necked flask . $NaBH_4$ (0.38 mg, 10 µmol) in ethanol/water was added through a syringe under nitrogen followed by the addition of sodium iodoacetate (3.54 mg,20 µmol) or sodium 3-bromopropionate (3.06 mg, 20 µmol) in water. The solution was stirred for 30 min . The work up for separation of the free selenafatty acids was the same as in method A. The yield was 60-83 % with a radiochemical purity >99%.

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