REACTION OF TRITIUM ATOMS WITH UNSATURATED COMPOUNDS*

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SUMMARY

The reaction of tritium atoms with unsaturated compounds was shown to proceed both by addition to the double bond yielding the reduced product as well as by tritium/hydrogen exchange resulting in labeled parent. Reduction occurred to varying degrees in all cases investigated but appears to be far less significant than in Wilzbach labeling. The addition/substitution ratio producing labeled products for oleic acid was 2.6(compared to 24.4 by Wilzbach) for the unsaturated amino acids 3,4-dehydro-D,L-proline and L-2-amino-4-(2'-aminoethoxy) trans-3-butenoic acid was 12.8 and 11.6 respectively and for morphine (resulting in 6,7-dihydromorphine) only 0.02.

Key Words: Tritium Atoms, Reduction, Addition, Tritium/Hydrogen Substitution, Microwave Discharge.

INTRODUCTION

Microwave discharge activation (MDA) of tritium gas has been shown to effectively initiate tritium-hydrogen exchange in a variety of compounds (1-3). Purification of compounds labeled in this manner has uncovered the presence of carrier-free tritium labeled impurities (3). A search of the literature has shown that addition

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of hydrogen to isolated double bonds to be an important reaction pathway in Wilzbach labeling (4-6), β-selfradiolysis (7), γ-radiolysis (8-10) and may also play an important role in radiation-induced hydrogen atom reaction with unsaturated compounds in vivo.

Similar reductive pathways of hydrogen atom reactions must be considered in microwave discharge activation (MDA) of tritium gas labeling of unsaturated compounds. We have therefore investigated the reaction of tritium atoms with oleic acid and 3,4-dehydro-DL-proline and determined the amounts of addition (to stearic acid and proline respectively) and substitution (leading to labeled parent) in each case. Oleic acid was also Wilzbach labeled for comparison with both literature values (4,5) and tritium atom values for the extent of addition to the double bond.

EXPERIMENTAL

(a) Materials

Oleic acid and stearic acid were purchased from Analabs and were used without further purification. [^{14}C]-oleic acid and [^{14}C]-stearic acid were purchased from New England Nuclear and were purified by chromatography of their methyl esters on silica gel/silver nitrate columns prior to their use. The 3,4-dehydro-DL-proline was purchased from Calbiochem, L-proline from Schwarz-Mann. Both were used without further purification.

(b) MDA Labeling Apparatus

The samples were labeled in a reaction system previously described (3a) with light trap modifications between discharge and sample regions (3b).

(c) Oleic Acid-MDA Labeling

Oleic acid (5.89 mg) was labeled on a glass reaction tray for 5 minutes with liquid nitrogen cooling at a pressure of 4 mm Hg of tritium gas (1 Ci) with a microwave power of 20 watts (2450 MHz). After the exposure carrier stearic acid (10.4 mg) was added to the oleic acid (containing 802 μCi of tritium). The stearic-oleic mixture was then estrified with diazomethane (11).

Prior to purification [^{14}C]-methyl stearate (32.2 μCi) and [^{14}C]-methyl oleate (20.2 μCi) were added along with carrier methyl esters giving a final mixture of 71.5 mg methyl oleate and 44.5 mg methyl stearate. Purification was carried out on three successive silica gel-silver nitrate columns using petroleum ether-benzene

mixtures, to constant $^{3}H/^{14}C$ ratios (as well as constant specific activities).

(d) Oleic Acid-Wilzbach Labeling

Oleic acid (5.68 mg) was exposed to 687 mCi of tritium gas at 69 mm Hg in a 3.2-cc glass reaction vessel. The sample was kept dark at room temperature for two months.

Carrier stearic acid (16.2 mg) and oleic acid (21.1 mg) were added to the product which contained 40.31 μCi tritium prior to esterfication with diazomethane (11). To 8% of the sample (2 of 25 ml) containing 3.22 μCi was added [^{14}C]-methyl oleate (20.2 μCi, 20.6 mg) and [^{14}C]-methyl stearate (53.7 μCi, 56.2 mg) prior to purification. Purification was the same as above.

(e) Labeling of 3,4- dehydro-DL-proline

Microwave discharge labeling of 3,4- dehydro-DL-proline (5.65 mg) was the same as for oleic acid. Carrier proline (4.45 mg) was added to the crude tritium labeled dehydroproline prior to purification by Dowex 50W-X8 chromatography using 0.1 M pyridine/acetate buffer at pH 3.1. The sample initially contained 1.36 μCi.

RESULTS

The results of oleic acid labeling by tritium atoms (MDA) and Wilzbach methods are shown in Table I.

Table I. Addition vs substitution in the labeling of oleic acid by MDA and Wilzbach.

Labeling Method	μCi [^{3}H]-Oleic Acid (substitution)	μCi [^{3}H] Stearic Acid (addition)	Addition/Substitution Ratio[c]
Microwave discharge[a]	17.4	46.4	2.6
Wilzbach[a]	38.4	945	24.4
Wilzbach[b]			14.2
Wilzbach (Hg-sens)[b]			5.4

(a) Performed in our laboratories.

(b) See Ref. 4. Hg-sensitized.

(c) μCi [^{3}H]-steric/μCi [^{3}H]-oleic

The formation of stearic acid by addition of tritium to the 9,10-double bond of oleic acid predominated over the substitution reaction in both microwave discharge (tritium atom) and Wilzbach labeling. However, the reductive pathway is considerably less significant in MDA labeling than for Wilzbach.

Similarly the reaction of tritium atoms with unsaturated amino acids, 3,4-dehydro-DL-proline and L-2-amino-4-(2'-amino ethoxy)-trans-3-butenoic acid resulted mainly in formation of the reduction products (proline and L-2-amino-4-(2'-amino ethoxy) butanoic acid respectively).

Table II. Reaction of tritium atoms with unsaturated amino acids.

Compound Labeled	μCi parent (substitution)	μCi Reduction Product (addition)	Addition/Substitution Ratio
3,4-dehydroproline	21.3[a]	273	12.8
L-2-amino-4-(2'-amino ethoxy)-trans-3-butenoic acid[b]	170	1980	11.6

(a) 1360 μCi initial total tritium activity.

(b) This compound was labeled in our laboratory and purified by Dr. M. Lieberman, U.S.D.A., Beltsbille, Md.

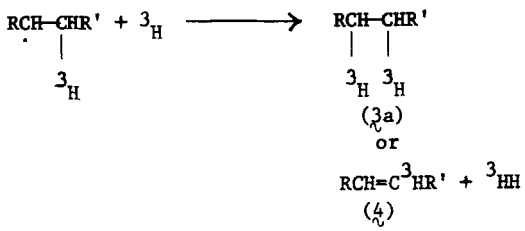
DISCUSSION

The large amounts of reduction products formed during the tritium atom (MDA) labeling of oleic acid and the unsaturated amino acids are not surprising, and can be attributed mainly to the very low activation energies (E_a 1-2 kcal/mole) of H (or ^3H) addition to isolated unsaturated bands (8) compared to the much higher E_a for abstraction (6-12 kcal/mole), see Ref. 14). These observed differences in activation energies can lead to rates of addition 10-100 times greater than for abstraction (15). A proposed reaction scheme is shown below (Scheme I)

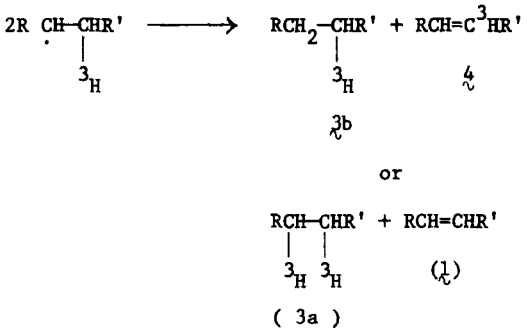
Upon formation of radical 2 by addition of tritium to the double bond several alternate paths are possible leading to the observed products.

Scheme I

$$\underset{(1)}{RCH{=}CHR'} + {}^3H \longrightarrow \underset{(2)}{RCH(\cdot)-CH(^3H)R'} \qquad (1)$$



(2)



(3)

Radical II may react at the gas solid interface with a second tritium by further addition to form the saturated compound ($\underset{\sim}{3}a$) or by abstraction leading to labeled olefin ($\underset{\sim}{4}$). Disproportionation in the solid phase, on the other hand, would lead again to the reduced product ($\underset{\sim}{3}a$, $\underset{\sim}{3}b$) and labeled olefin ($\underset{\sim}{4}$) as well as unlabeled parent ($\underset{\sim}{1}$). Abstraction elsewhere along the carbon chain and subsequent recombination with tritium would also lead labeled parent.

It is interesting to note that although there are large differences in the addition/substitution ratios of Wilzbach vs MDA labeling (attributable to large contributions of ionic reactions in Wilzbach labeling) that in the Hg sensitized "Wilzbach" involving exclusively tritium atoms (4) the addition/substitution ratio is only twice that of MDA (tritium atom) labeling. This agreement is quite good considering differences in reaction temperature, time, and phase conditions in the two systems.

Morphine has also been labeled in these laboratories by MDA method. Analysis performed by Fishman et al. (16) has shown the reduction products, 6,7-dihydromorphine, to be a very minor product with a morphine/6,7-dihydromorphine ratio of only 0.02.

Extension of this work to tritium atom reactions with aromatic amino acids both free as well as in peptides is currently underway. Although such addition reactions are known to occur (17-19) preliminary results indicate that in tritium

atom reactions with phenylalanine and tryptophan reduction of the aromatic ring may not be a major reaction pathway. Analysis of labeled phenylalanine (both in free amino acid as well as in peptides) has shown that no β-cyclohexylalanine is produced. This is in contrast to Wilzbach labeling where the β-cyclohexylalanine is a major product (20).

Similarly in the labeling of tryptophan formation of addition products 4,7-dihydro, 4,5,6,7-tetrahydro, and octahydrotryptophan have been looked for but have not been observed. In both cases, however, large amounts of carrier free, tritium labeled products have been observed. Identification of these products is currently in progress.

CONCLUSION

Addition to isolated double bonds does occur in tritium atom reactions. The extent of the reduction is quite variable from one system to another but appears to be far less than by Wilzbach labeling.

Tritium labeling of unsaturated compounds by microwave discharge activation of tritium gas can be a useful method if one is aware of the presence of reduction products and if caution is exercised in purification.

Dute to the facile nature of hydrogen (or tritium) atom addition to unsaturated bonds, reduction may play an important role in radiation-induced H-atom reactions in vivo.

Tritium atom addition products (i.e., reduced Phe, Trp) appear not to represent a major reaction pathway in reactions with aromatic amino acids. Other, as yet unidentified products are formed. Identification of these products is currently in progress.

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