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Stereo and Regioselectivity in "Activated" Tritium Reactions

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SUMMARY

To investigate the stereo and positional selectivity of the microwave discharge activation (MDA) method, the tritium labeling of several amino acids was undertaken. The labeling of L-valine and the diastereomeric pair L-isoleucine and L-alloisoleucine showed less than statistical labeling at the α-amino C-H position mostly with retention of configuration. Labeling predominated at the single β C-H tertiary (methyne) position. The labeling of L-valine and L-proline with and without positive charge on the α-amino group resulted in large increases in specific activity (greater than 10 fold) when positive charge was removed by labeling them as their sodium carboxylate salts. Tritium NMR of L-proline labeled both as its zwitterion and sodium salt showed also large differences in the tritium distribution within the molecule. The distribution preferences in each of the charge states is suggestive of labeling by an electrophilic like tritium specie(s).

INTRODUCTION

Methods of labeling involving "activated" or "reactive" tritium species such as Wilzbach, electrical and microwave discharge activation (MDA) etc., have been well described in the literature. These general methods may have initially been expected to result in nonspecifically (i.e. randomly) labeled products, however, even the earliest studies, clearly indicated reaction patterns of selectivity in these "activated" tritium reactions. Specifically, such is the case for the MDA method of labeling. Our original studies of this method (ref. 1), in the evaluation of the specific activity differences between classes of compounds, we observed variations of more than one million fold (see Table 1). Clearly, these vast observed differences were indications of some kind of selectivity being expressed in this "general" method of labeling. With this as the starting point we have over, the years, investigated many parameters of MDA labeling. The goal of these studies was to document observable labeling patterns and their sensitivity to a variety of physical and chemical effects thus ultimately resulting in optimization and control of specific activities and labeling patterns.

TABLE 1
Specific activity range in MDA labeling.

Compound Labeled	Specific Activity (mCi/mmol)
Naphthalene	0.01
Androstenedione	0.03
L-Valine	0.50
Oleic Acid	0.80
L-Leucine	1.40
L-Tryptophan	4.30
L-Leu-L-Trp-L-Leu (Peptide)	700.0
L-Val-L-Ala-L-Ala-L-Phe (Peptide)	2000.
ACTH[a]	14,500.
LH[b]	45,000.

[a] Adrenocorticotropic Hormone (39 Amino Acid Peptide)
[b] Luteinizing Hormone (Protein Hormone)

For the study specifically of stereo and regioselectivity described below, amino acids were selected for investigation due to their chiral (enantiomeric and/or diasteriomeric) nature and since they are the constituants of peptides and proteins frequently labeled by this method.

METHODS

The MDA method used in these studies is a modification of that originally described by Ghanem and Westermark (ref. 2) and later by Gosztonyi and Walde (ref. 3). Our modifications and the apparatus used are described in detail in ref. 1 and 4. The labelings were conducted using solid powdered amino acids (~5 mg) except for the proline, which was carried out on an inert microporous membrane surface (ref. 4,5) so as to produce sufficient amounts of labeled material needed for the tritium NMR studies. The conditions used were a 5-15 minute exposure to activated tritium specie(s) produced via microwave discharge (2450 MHz) in 1-2 Ci of tritium gas (4 Torr) at a foward power of 20-30 watts. The samples were cooled with liquid nitrogen, although the actual temperature of the samples is unknown.

RESULTS

L-Valine [$(CH_3)_2CH$-$C(NH_2)H$-CO_2H]

Both the positional distribution and the stereochemistry of the labeling at the chiral α-carbon were determined (ref. 6). These results are shown in Table 2. The most striking feature is the non-randomness of the tritium distribution in the labeled valine with the single tertiary β C-H containing nearly one-third of the tritium, far greater than the one-eigth statistically predicted. In contrast both the α-amino C-H and the primary positions are well under statistical predictions. In addition retention of configuration (L vs D) predominated by a factor of nearly 3.5/1.

TABLE 2
Distribution of tritium in MDA labeled L-valine.

Position	% H-3/Position	% H-3/C-H Bond
Alpha(Total)	7.1	7.1
(L-Alpha)	(5.5)	- - -
(D-Alpha)	(1.6)	- - -
Beta	32.7	32.7
Gamma	60.2	10.0

L-Isoleucine and L-alloisoleucine [$C_2H_5{}^*C(CH_3)H{-}{}^*C(NH_2)H{-}CO_2H$].

L-Isoleucine and L-alloisoleucine are two amino acids in a unique group of amino acids with two chiral centers (*C), one at the α-carbon and one in the β-position, along the aliphatic side chain These amino acids are stereochemically diasteromers of one another. Thus inversion of either of the chiral centers results in the one of the other diastereomeric amino acid pairs. For example, inversion at the α-position of L-leucine (2S, 3S absolute configuration) results in D-alloisileucine (2R, 3S), and inversion of the β–position results in L-alloisoleucine (2S, 3R). A similar relationship can be shown for L-alloisileucine (2S, 3R) where α or β inversion results in either D-isoleucine (2R, 3R) or L-isoleucine (2S, 3S) respectively. Results of these labelings are shown in Table 3 and demonstrate several points: 1) In the labeling of either diastereomer the labeled parent predominates (~70/30); 2) formation of the diastereomeric products in each case is almost exclusively by reaction at the tertiary β C-H position; and 3) total labeling at the α–position (determined by base exchange) is low in the case of L-isoleucine (7.9%) or essentially nonexistent when labeling L-alloisoleucine (<1%).

TABLE 3
Diastereomeric distribution of tritium from MDA labeling of L-isoleucine and L-alloisoleucine.

Tritiated Product	% From L-Ile Labeling[a]	% From L-Alloile Labeling[b]
L-Isoleucine	72.2 (Parent)	29.1 (β)
D-Isoleucine	(1.1)[c]	0.3 (α)
L-Alloisoleucine	23.3 (β)	67.6 (Parent)
D-Alloisoleucine	3.4 (α)	(3.0)[c]

a Total alpha labeling by base exchange = 7.9% (see ref. 7)
b Total alpha labeling by base exchange = <1% (see ref. 7)
c Presence of these isomers may be the result of an unlikely double α/β inversion, inversion of excited reaction intermediates or GLC analysis artifact.

These data are also consistent with the L-valine data described above. The perdominance of β C-H exchange again shows great selectivity for reaction at the tertiary (methyne) C-H position. The energetics may be the determining factor in this observed selectivity. It is known that bond dissociation energies (ref. 8,9) and energy of activation of hydrogen atom abstraction (in the gas phase) decrease (ref. 10,) going from primary>secondary>tertiary, with a corresponding increase in hydrogen atom rate constants (ref. 11). This has also been observed in solution (ref. 12,13). Other explainations may also be relevant if other than atomic tritium species play an important role in MDA labeling reactions (ref. 14).

Effect of Charge

During our studies with amino acids we have observed that charge state of the alpha amino has a great effect on their resulting specific activity obtained by MDA labeling (ref. 15). In both cases a large increase in labeling specific activity was observed when removing the positive charge from the α–amino group (Table 4).

TABLE 4
Tritium labeling as a function of amino acid charge.

Compound	Charge on α–Amino	Rel. Specific Activity
L-Valine (Zwitterion)	+1	1.0
L-Valine·HCl	+1	2.4
L-Valine⁻Na⁺	-1	45.0
L-Proline (Zwitterion)	+1	1.0
L-Proline⁻Na⁺	-1	12.1

Secondly, we determined the tritium distribution within each of the L-prolines by tritium NMR (see Table 5). The L-proline zwitterion showed a decidedly nonstatistical distribution particularly with selectivity for the 3(α) position containing 21.7% and with only 14.5% total in the two 5(α,β) C-H positions adjacent to the protinated amino group. The 3(β) and 4(α,β) positions are not resolved enough in the spectrum to quantitate each individually. In contrast, labeling of the unprotonated L-proline as the carboxylate anion, leads to a redistribution of the tritium pattern. In this case, the 5(α,β) positions (next to the now neutral amino group) contain, relative to the other positions, three times the tritium (44.0%) as in the zwitterion (14.5%), at the expense of the 3(α,β) and 4(α,β)positions. These patterns would again be consistent with labeling by electrophilic like tritium specie(s).

Table 5
Tritium distribution in the labeling of L-proline as a function of amino acid charge.

[Position] [Compound]	2	3(α)	Σ 3(β)+4(α,β)	Σ 5(α,β)
L-Proline (±)	17.4	21.7	46.4	14.5
L-Proline⁻Na⁺	14.8	9.7	31.4	44.0

CONCLUSIONS

From these amino acid labeling studies using the MDA Method, several generalizations can be made. 1) Minimal (i.e. statistical or less than statistical) labeling occurs at the chiral α C-H position. 2) Retention of configuration predominates. 3) Tertiary (methyne) positions are more reactive. This has also been reported by others (ref. 16) and may be a bond energy effect. 4) The charge on the α-amino group has a dramatic effect both on level of labeling (specific activity) as well as on the distribution of the tritium within the molecule. These effects are consistent with an electrophilic like labeling specie(s).

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