A New Efficient Synthesis of Acetyltelluro- and Acetylselenomethionine and Their Use in the Biosynthesis of Heavy-Atom Protein Analogs

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It has recently been reported that both selenomethionine, Met(Se),[1] and telluromethionine, Met(Te),[2,3] can be incorporated into polypeptides *in vivo* using bacterial strains auxotrophic for Met, offering a new approach to solving the phase problem in protein crystallography. The electron density of tellurium is, in fact, sufficient to generate clear signals in the isomorphous and anomalous difference Patterson maps at the commonly used Cu Kα wavelengths,[2,3] and therefore, biosynthetic replacement of Met with Met(Te) represents a promising alternative to the soaking procedure for multiple isomorphous replacements.[4] 3D structures of Met(Se) proteins have also been resolved by the method of multiwavelength anomalous diffraction;[5,6] however, this requires intensity measurements with monochromatic synchroton radiation at a minimum of three different wavelengths.

Various efficient syntheses of Met(Se) have been reported,[7] but only two procedures have been described for Met(Te) so far. One is a multistep synthesis based on the reaction of 5-(β-bromoethyl)hydantoin with (methyltelluro)sodium (MeTeNa), followed by alkaline ring opening to generate the racemic Met(Te).[8] Since this method proved to be exceedingly difficult to reproduce, an alternative synthesis was proposed by Silks et al.[9] It relies on ring opening of (*S*)-2-amino-4-butyrolactone hydrochloride by (methyltelluro)lithium (MeTeLi) to afford directly L-Met(Te). Despite all the precautions adopted to operate in dry and oxygen-free media, we were unable to obtain the desired compound even by this procedure.

To allow for a more proper solvent choice, i.e., aprotic solvents like THF instead of methanol, and to exclude the presence of hydrochloride, we have examined the effect of N-protection of the 2-amino-4-butyrolactone. In contrast to what we observed with the unprotected lactone, reaction of *N*-acetyl-(*R*,*S*)-2-amino-4-butyrolactone[10] with MeTeLi[11] in THF and in the presence of tetramethylethylenediamine (TMEDA) produced the desired Ac-DL-Met(Te)-OH lithium salt in very good yields. As expected, reaction of *N*-acetyl-(*R*,*S*)-2-amino-4-butyrolactone with MeSeLi led to the corresponding Ac-DL-Met(Se)-OH lithium salt in a similarly efficient manner (Figure 1). Opening of the butyrolactone ring corresponds to an ester cleavage via an S_N2 reaction, where the soft nucleophiles methyl tellurolate and methyl selenolate are known to attack preferentially the soft sp^3 center.[9,12] Since small amounts of a byproduct were isolated and identified by FAB-MS and NMR as *N*-acetylhomoserine after workup of both reaction mixtures, a partial attack of the soft nucleophiles at the sp^2 center with formation of the related methyltelluro and methylseleno esters cannot be excluded.[12d] These esters are then apparently hydrolyzed to *N*-acetylhomoserine in the purification steps. The observation that, despite identical reaction conditions, Ac-DL-Met(Te)-OH was obtained in all syntheses in significantly higher yields (80−90%) than the corresponding Met(Se) derivative (60−70%) is attributed to the softer nucleophile character of the tellurolate compared to the selenolate.

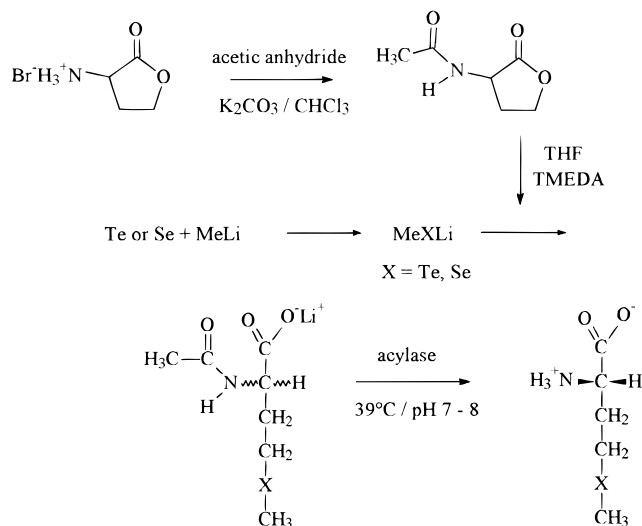

Figure 1. Synthetic scheme for L-Met(Te) and L-Met(Se).

Besides leading to a smooth nucleophilic ring opening of the 2-amino-4-butyrolactone, N-acetylation allowed the enantioselective enzymatic deacetylation of the Met analogs by aminoacylase-based procedures[13] to generate the desired L-Met(Te) and L-Met(Se). Monitoring of the enzymatic hydrolysis by capillary electrophoresis (CE) proved to be very advantageous (Figure 2). Both amino acids showed chromatographic and analytical properties identical with those of authentic probes (L-Met(Se), Sigma, München, Germany; L-Met(Te), L. A. Silks, Los Alamos National Laboratory, Los Alamos, NM).

Dialkyltellurium(II) compounds oxidize rapidly under various conditions;[14] therefore, L-Met(Te) and Ac-DL-Met(Te)-OH were analyzed for their stability in view of their application in the biosynthesis of Met(Te) proteins. In non-degassed aqueous solution at pH 7.0 and room temperature, Ac-DL-Met(Te)-OH is markedly more stable ($t_{1/2} \approx 20$ h) than L-Met(Te) ($t_{1/2} \approx 30$ min) as monitored by CE. In both cases, air oxidation leads to

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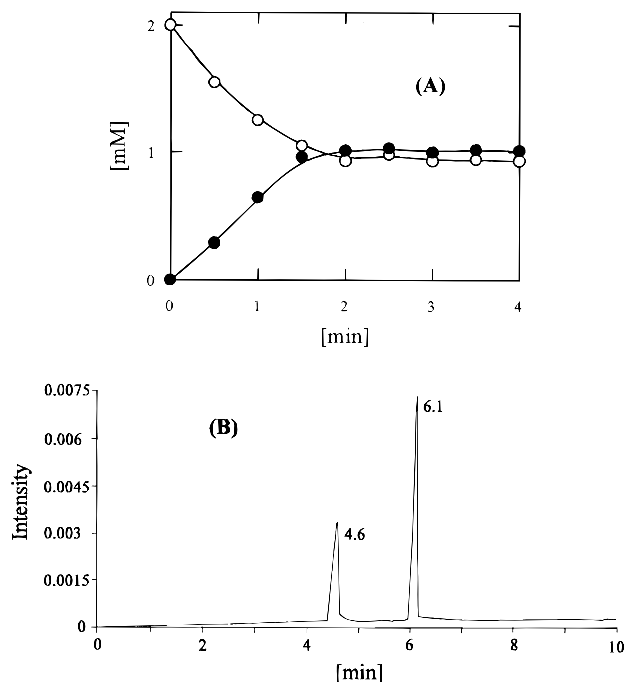

Figure 2. Enantioselective hydrolysis of Ac-DL-Met(Se)-OH with aminoacylase (EC 3.5.1.14) at pH 7−8 and at an enzyme/substrate ratio of 5:100 (w/w). (A) Rate of hydrolysis of Ac-DL-Met(Se)-OH (○) and formation of L-Met(Se) (●). (B) Monitoring of the hydrolysis (Met-(Se), $R_t = 4.6$; Ac-DL-Met-OH, $R_t = 6.1$) by CE (62 cm quartz capillary, 75 μm i.d., 50 mM sodium borate buffer, pH 8.5, 25 kV, UV at 215 nm).

main components of very similar UV spectra, together with minor byproducts. FAB-MS of the reaction mixture of L-Met-(Te) indicated the presence of the dihydroxytelluride, which might be reduced again to the telluride by glutathione in analogy to recent reports on diphenyltelluroxide.[15] Correspondingly, this L-Met(Te) oxidation product, if taken up by the bacteria, should be reconverted into L-Met(Te) by the reducing medium of the cell and thus become available for biosynthetic incorporation into proteins. Comparative experiments on expression of all-Met(Te)-annexin V (seven Met residues) were performed in methionine auxotrophic *Escherichia coli* using both L-Met(Te) and Ac-DL-Met(Te)-OH to analyze possible advantages of the higher stability of the acetyl derivative. Bacterial growth was performed as described previously[4] and, in the case of Ac-DL-Met(Te)-OH, in the presence and absence of aminoacylase in the growth media.

With *N*-acetyl-DL-Met(Te)-OH, all-Met(Te)-annexin V (Figure 3) was produced as efficiently as with L-Met(Te). Operating in the absence of external aminoacylase and thus allowing for deacetylation by endogenous aminoacylase proved to be even more advantageous, possibly because of the better bioavailability of the acetylated amino acid. All-Met(Se)-annexin V was obtained with similar efficiency using Ac-DL-Met(Se)-OH in the absence of aminoacylase in the growth medium. Quantitative replacement of the Met residues in annexin V by Met(Te) and Met(Se) was assessed by ES-MS (Figure 3) and amino acid analysis (absence of Met).

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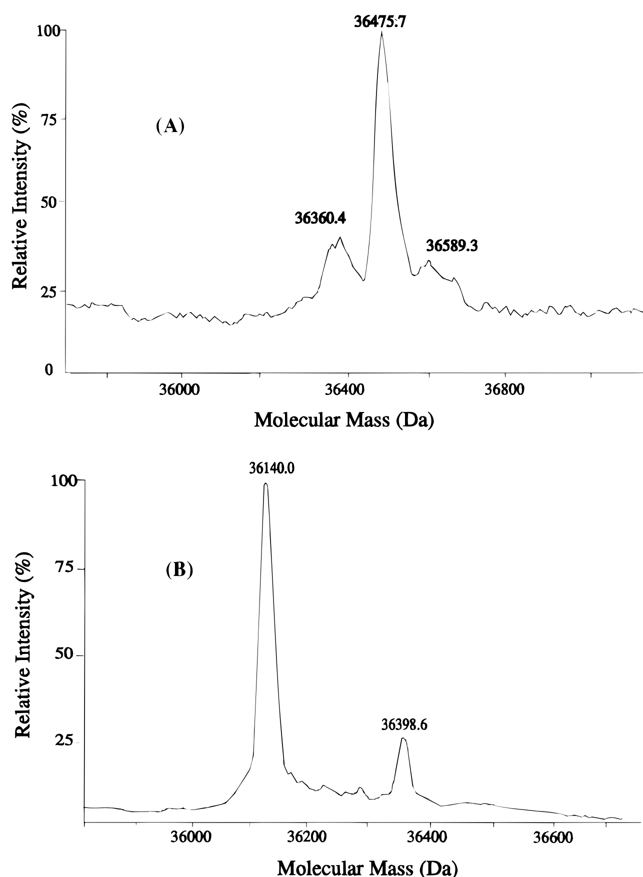

Figure 3. ES-MS spectra of (A) all-Met(Te)-annexin V (seven Met-(Te), $M_r = 36\,478$) and (B) all-Met(Se)-annexin V (seven Met(Se), $M_r = 36\,138$). The mass peak of 36 398 corresponds to Ca^{2+} adducts.

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Supporting Information Available: Experimental procedures and analytical data of Ac-DL-Met(Te)-OH, Ac-DL-Met(Se)-OH, L-Met(Te), and L-Met(Se) (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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