Determination of selenomethionine in biological sources by chromatography and neutron activation analysis

Some investigators believe that selenium is an essential trace element only in the role that it assumes incorporated into amino acids and not in the inorganic form. The majority of research that has been conducted on selenium in biological sources has involved the analysis of total selenium levels in serum or tissues, and only a few workers have been interested in the seleno-organic compounds such as selenomethionine. A greater interest in the seleno-amino acids would perhaps develop if methodology could be perfected for the practical separation of these amino acids from their sulfur analogs.

MARTINS AND CUMMINS[1] have recently effected a separation of methionine and selenomethionine by an ion exchange technique and MILLAR[2] has perfected a solvent mixture for separation of these analogs using thin-layer chromatography. The separations by these authors were accomplished by using standard mixtures and further work will decide whether or not these techniques can be used for the identification of selenomethionine from natural sources.

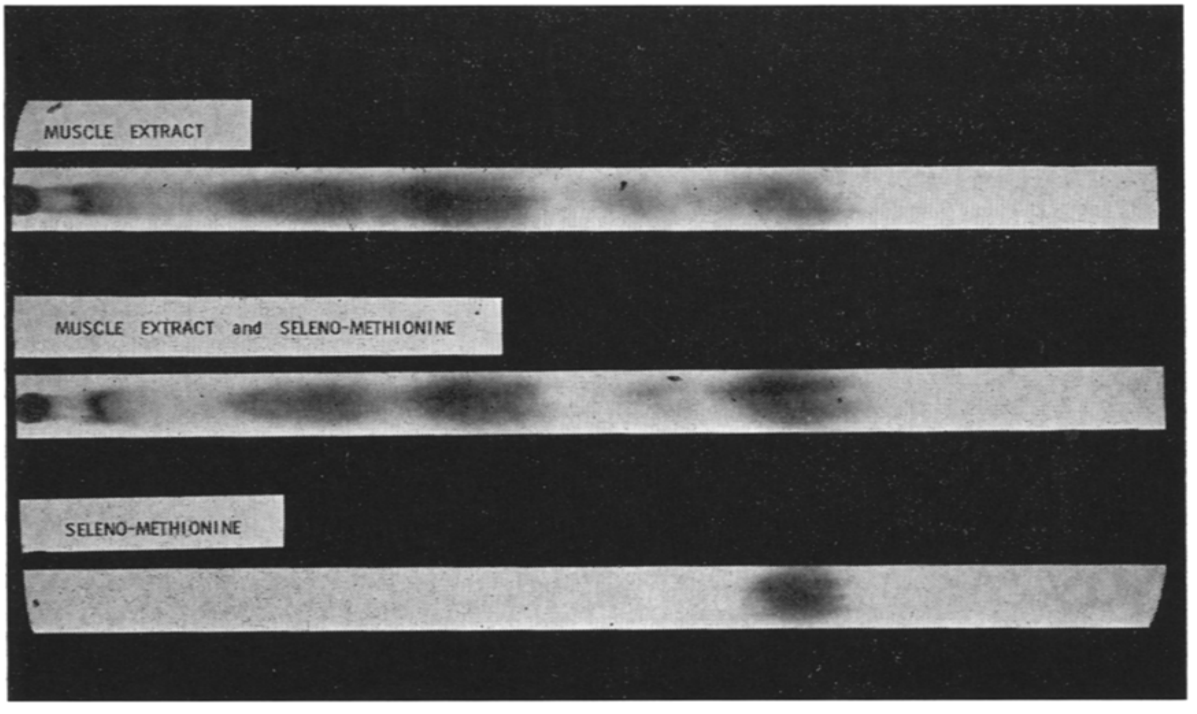

Fig. 1. Typical chromatographic separation of methionine–selenomethionine from a biological source.

By coupling the techniques of paper chromatography and neutron activation analysis, a method has been perfected for quantitating selenomethionine in the presence of methionine and in sources such as serum, liver or muscle.

One gram of liver or muscle was homogenized in the presence of 9 volumes of 50% ethyl alcohol or if serum was analyzed, 1 ml was added to 9 volumes of ethyl alcohol to obtain a protein-free filtrate. Following centrifugation or filtration, 5 ml of the filtrate was lyophilized to dryness. The residue from lyophilization was taken

into solution in 1 ml of 50% alcohol and 20 μl were placed quantitatively on to Whatman No. 1 filter paper strips. The strips were placed in a descending chromatography bath containing solvent 1 of PETERSON AND BUTLER[3] (*n*-butanol–pyridine–water, 1:1:1 v/v). The strips were irrigated for a 12-h period, after which they were sprayed with ninhydrin and developed in an oven at 80° for 10 min. Fig. 1 shows how well the lead spot, the methionine area (which includes the selenomethionine) was separated from the rest of the ninhydrin-positive areas of muscle. The same separation was effected with liver and serum. Elution and rechromatographing of the solute in this area in other solvent mixtures has shown it to be a single entity, methionine.

Next in the procedure the methionine area was cut from the paper, placed in a tygon tube and irradiated for 20 sec in a Triga Mark I atomic reactor, using a rapid-transit pneumatic rabbit system and a neutron flux of $1.1 \cdot 10^{11}$ n/cm^2/sec. Following

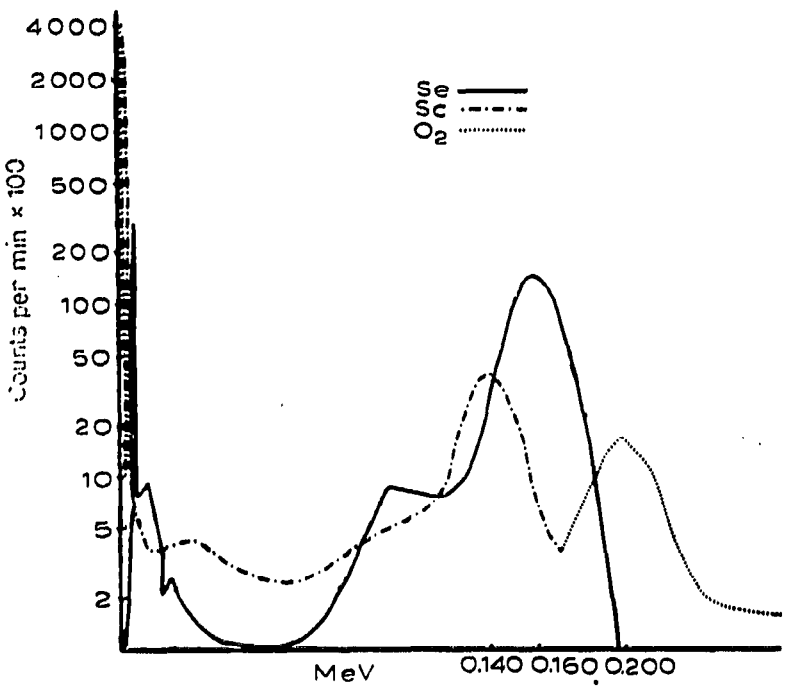

Fig. 2. Gamma ray spectra for ^{77m}Se and closest possible interfering isotopes produced from scandium and oxygen.

a delay of 20 sec, the sample was analyzed in an RIDL 400 channel gamma ray spectrometer using a 1-min live time count. This provided a spectrum from 0 to 1 MeV, which included the peak due to ^{77m}Se, a 17.5 sec isotope of selenium peaking at 0.160 MeV (Fig. 2). The selenium in the sample was estimated by a direct comparison method using selenium standards. The micromoles of selenium within the spot represent the micromoles of selenomethionine because of the one to one stoichiometric relationship between selenium and selenomethionine. Fig. 2 shows the two isotopes most likely to interfere with neutron activation analysis of ^{77m}Se. These are ^{46m}Sc which could be derived from the scandium in natural sources and ^{19}O which we have found arises mainly from irradiated samples containing water. By using the dried, ninhydrin-sprayed spots in the neutron activation analysis for selenium the ^{19}O interference is eliminated. Scandium was found to remain at the origin of the

chromatogram with the butanol–pyridine–water solvent and creates no problem in this technique. The paper background of Whatman No. 1 offers very little interference in this analysis. This is of immediate interest in view of the findings of WEST[4], who has reported high concentrations of selenium in various types of paper, particularly cigarette paper.

Recovery studies have shown that 100% of selenomethionine were recovered from liver and muscle homogenates but only 60 % of the selenium added as selenomethionine to serum was recovered from the serum methionine area. The remainder of the selenium associates itself with the third ninhydrin-positive spot on the chromatogram and suggests a metabolic pathway for selenomethionine at the level of the serum.

The lower limit of detectability of this method is 0.1 μmoles of selenomethionine per g of tissue or per ml of serum. Levels of natural, free selenomethionine in rat liver, muscle and serum and in human serum apparently exist at a level lower than this because none could be detected in these sources. Consequently, this procedure is useful only for studies involving the metabolism of selenomethionine where this amino acid is added to the organism or biochemical system. A reactor with a neutron flux ten-fold or one hundred-fold greater than that used in this laboratory may increase the sensitivity of the procedure.

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Rapid automatic methods for the determination of lanthionine and lysinoalanine using an amino-acid analyser

When wool is treated by a variety of setting processes, as, for example, in hot water, steam or alkalis, particular interest attaches to the fate of the cystine residues. Some of these are transformed into either lanthionine or lysinoalanine residues, thereby creating new, stable cross-linkages in the proteins of wool. The rapid and accurate assay of these two amino acids is thus of considerable benefit in the study of mechanisms of set in wool[1–4]. Of wider interest are the amounts of lysinoalanine formed during the alkali-treatment of a number of well-characterised proteins[5,6].

The work described here concerns the rapid, automatic determinations of lanthionine and lysinoalanine in protein hydrolysates, using the analytical train of the Technicon Automatic Amino-Acid Analyser[7].