Selenium research in mammals using nuclear analytical methods and related techniques in conjunction with biochemical procedures

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Due to the essential functions of selenium-containing enzymes and the relationships between changes in the selenium status and diseases, the determination of the element and its compounds is of great interest. Radiotracer studies with $^{75}$Se have been valuable tools in selenium research. NAA and ICP-MS allow both total element and stable isotope measurements. ICP-MS in conjunction with chromatographic separation techniques and gel electrophoretic procedures coupled with scanning methods such as XRF, PIXE and laser ablation ICP-MS have been used in the determination of the selenium compounds. In this survey the application of these methods in selenium research is discussed with the help of examples on the regulation of the selenium metabolism and the detection and investigation of novel selenium-containing proteins.

Introduction

Selenium is a ubiquitous element, although in most environmental and biological materials it is present only in very low concentrations. It is now well known that it has essential effects and that Se deficiency can lead to various disorders. The pathological changes observed in Se deficiency or a combined Se and vitamin E deficiency include growth retardation, skin lesions and hair loss, visual defects, reproductive disorders, pancreas atrophy, liver necrosis and dystrophies of the skeletal muscle and the heart muscle. In humans, muscular dystrophy, cardiomyopathy and disturbances in the thyroid hormone metabolism have been observed. Special interest was aroused by the recent findings that the element has protective effects in carcinogenesis and in several viral infections such as hepatitis and HIV.

Selenium is also highly toxic and the range between deficiency and the first signs of toxicity is relatively small. Therefore, very reliable analytical methods are required in Se research and in the determination of the Se status in health and disease.

However, the determination of the total Se is not always sufficient as the bioavailability and the effects of the element depend to a large extent on its biological forms. We know now that Se is present in a variety of important enzymes such as the glutathione peroxidases, the iodothyronine deiodinases and the thioredoxin reductases, but there are also Se-binding proteins and non-specifically incorporated forms. After ingestion of selenite, selenate or selenocysteine, nearly all of the metabolized element is incorporated into specific biologically active selenoproteins in which it is present in the form of selenocysteine. Their levels are homeostatically controlled and cannot be further increased by additional Se supplementation. In the case of dietary selenomethionine, a part of the element is metabolized in the same way. A certain percentage, however, is deposited directly and non-specifically into proteins in place of methionine. This part depends on the ratio of selenomethionine and methionine in the diet and can be considerably increased by raising the dietary selenomethionine intake.

In the evaluation of the Se status it is, therefore, necessary to distinguish between specifically and non-specifically bound Se and to determine the Se compounds responsible for the biological effects. The analytical methods to be applied in Se research on mammals should thus allow not only the determination of the total Se and, in case of stable tracer studies, that of the Se isotopes, but also the identification and measurement of the Se species present in the different biological materials to be investigated. In this short survey some of the most important features of the analytical methods used in our studies in Se research and their combination with biochemical separation procedures are discussed.

Methods

Several nuclear analytical methods and related techniques listed in Table 1 have been shown to be suited to the determination of Se and the Se species.

Radiotracer methods

Tracer techniques using $^{75}$Se have been developed for various applications in Se research. $^{75}$Se with the high specific activity of 37 MBq/µg Se has been produced by long-term irradiation of $^{74}$Se, enriched from the natural abundance of 0.9% to more than 99%. The tracer has been administered, mostly in the form of sodium selenite, either to rats or to cell cultures.

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The activity of $^{75}\text{Se}$ in bulk samples was analyzed via its gamma-rays whereas with histological sections and the flat surfaces of gels and blot membranes used in protein separation the distribution of the tracer is determined by autoradiography of its 11.2 keV X-rays. The studies include the detection and characterization of Se-containing proteins, the investigation of the distribution of Se and the Se-containing compounds in tissues and subcellular compartments, the identification of novel selenoproteins and the quantitative Se analysis in small protein fractions where very low limits of detection are required.\(^6\)

**Analysis of total Se and Se isotopes**

Of the nuclear analytical methods and related techniques, neutron activation analysis (NAA) and inductively coupled plasma – mass spectrometry (ICP-MS) are suited to the determination of trace amounts of Se and of stable Se isotopes.

With the exception of $^{77}\text{Se}$ the other five stable Se isotopes, $^{74}\text{Se}$, $^{76}\text{Se}$, $^{78}\text{Se}$, $^{80}\text{Se}$ and $^{82}\text{Se}$, produce radioisotopes by thermal neutron capture and can thus be determined by NAA. With the latter three, however, radiochemical NAA has to be applied, as the half-life of their radioisotopes is in the range of several minutes to about one hour where interfering gamma-radiation, mainly from $^{24}\text{Na}$ and $^{35}\text{Cl}$, will prevent their direct detection in biological materials. For the determination of total Se we mainly use instrumental NAA for the measurement of $^{74}\text{Se}$ via $^{75}\text{Se}$ with a very long half-life (120.4 d) or $^{76}\text{Se}$ via $^{77}\text{Se}$ with a very short half-life (17.5 s). Here, either a long decay time or a short irradiation period minimizes interferences from other radionuclides.\(^7\)

ICP-MS likewise allows the determination of the stable Se isotopes. However, the limits of detection are considerably affected by mass interferences from polyatomic ions which stem primarily from the argon used to produce the plasma and some of the elements enriched in biological materials. These polyatomic interferences (e.g., $^{34}\text{S}^{40}\text{Ar}$ for $^{74}\text{Se}$, $^{36}\text{Ar}^{40}\text{Ar}$ and $^{40}\text{Ca}^{36}\text{Ar}$ for $^{75}\text{Se}$, $^{37}\text{Cl}^{40}\text{Ar}$, $^{39}\text{K}^{38}\text{Ar}$ and $^{1}\text{H}^{40}\text{Ar}^{36}\text{Ar}$ for $^{77}\text{Se}$, $^{38}\text{Ar}^{40}\text{Ar}$ and $^{40}\text{Ca}^{38}\text{Ar}$ for $^{78}\text{Se}$, $^{40}\text{Ar}^{40}\text{Ar}$ and $^{40}\text{Ca}^{40}\text{Ar}$ for $^{80}\text{Se}$ and $^{44}\text{Ca}^{38}\text{Ar}$ for $^{82}\text{Se}$) can be avoided by reactions with gases such as helium or hydrogen in a collision cell. Its mechanism is based on the fact that the polyatomic interference has a larger ionic radius than the monoatomic analyte of the same mass. Therefore, it collides more often with the collision gas, and, due to the greater energy loss and the resulting stronger deflection in the ion guide, can be prevented from entering the mass analyzer. The development of the collision cell now allows the determination of all six Se isotopes and has greatly improved the detection limits.\(^8\)

**Determination of small Se-containing molecules**

The main essential functions of Se are due to selenoproteins, but the determination of small Se-containing molecules is likewise of importance in various fields of Se research, e.g., in studies on the chemical forms of dietary Se, on toxic compounds and on the Se metabolites. Here separation of the liquid samples with chromatographic methods or capillary zone electrophoresis and on-line determination of the isolated Se compounds by ICP-MS has proved to be a very useful method.

A special challenge in the determination of small Se compounds is the identification of novel selenocysteine-containing selenoproteins by analysis of the selenoamino acid in the purified Se-containing protein, as in most cases only a very small amount of the protein is available for amino acid analysis. For this purpose a tracer method has been developed in which the

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**Table 1. Nuclear analytical methods and related techniques in selenium research**

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