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REVIEW

Arsenic compounds in marine and terrestrial organisms: analytical, chemical and biochemical aspects

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Arsenic has a reputation as a poison, because arsenic trioxide was used during medieval times as an agent for murder. Lingering memories of these events make any arsenic-containing material suspect. Toxicity is a property of a specific compound and varies with the composition and structure of compounds. Developments in analytical methodology made it possible not only to determine total arsenic in a variety of matrices but also arsenic compounds. Knowledge about the arsenic cycle in marine systems has expanded considerably during the past decade. The marine arsenic cycle appears to be more complex than the cycle in the terrestrial environment. More attention must be given to the minor arsenic-containing compounds detected in organisms and experiments should be undertaken that provide information about the biochemical pathways used for the transformation of arsenic compounds.

Keywords: Arsenic, arsenic compounds, transformation, determination, biological significance

HISTORICAL ASPECTS

Arsenic is a fascinating element. Its sulfides, orpiment (As_2S_3) and realgar (As_4S_4), were known at least 2000 years ago.[1] The oxide of trivalent arsenic, As_2O_3, could not have escaped detection by workers attending roasting or smelting furnaces, in which arsenic-containing ores were processed. Most sulfide minerals, such as pyrite (FeS_2), chalcopyrite ($CuFeS_2$), sphalerite (ZnS), and galena (PbS), contain arsenic in concentrations ranging from trace to several per cent.[2,3] Arsenopyrite (FeAsS), orpiment, realgar, and arsenides of iron, copper, and nickel have arsenic as a major component. The arsenic in these ores is converted to arsenic trioxide at elevated temperatures attained during the roasting processes, in which air is used as the oxidizing agent. Arsenic trioxide sublimes under these conditions and condenses on cool surfaces within the roasting facilities and their environs as a white to yellowish material. This arsenic compound was, therefore, easily available to animals and man. The toxic and lethal effects of arsenic trioxide were discovered by trial and fatal error, probably during the mining and processing of ores in regions controlled by the ancient Greeks. Strabo mentions an arsenic sulfide mine near Pompeiopolis, in which — because of the poisonous character of the ore — only slaves were used. Knowledge about arsenic compounds came to Europe with the invading Arabic armies. The numerous mines in the mountain ranges of Europe that were the source of arsenic-containing ores supplied as one of the poducts of ore-processing activities 'white arsenic', as arsenic trioxide was commonly called. Alchemists investigated arsenic trioxide and one of them, Paracelsus, used arsenic-containing formulations, for instance a preparation obtained from arsenic trioxide and potassium nitrate, to combat illnesses.[4] Arsenic trioxide — taken repeatedly in doses of approximately 20 mg — was known or at least believed to increase appetite, confer blushing cheeks, improve general appearance, and give the impression of well-being. Arsenic trioxide-containing pills, traded under the name of asiatic pills, were — not suprisingly — in great demand by females, young and old.[5]

White arsenic gained considerable reputation during medieval ages as a lethal poison. The oral lethal dose of arsenic trioxide is approximately 100 mg for an adult. Symptoms are delayed from one-half to several hours. Violent abdominal pain, vomiting, and watery diarrhea resembling the stools of cholera set in accompanied by cold, clammy skin, feeble pulse and weak, sighing respiration. Death occurs within 24 h to four days.[6] Poisoning by arsenic trioxide, a powdery white substance without smell and with no taste when mixed into food or dissolved in drinks, was the method of choice to settle political differences and feuds, speed up transfer of wealth through inheritances, and end domestic disagreements and troubles.[7] How many

men and women from the highest to the lowest social classes were lethally poisoned by arsenic trioxide will never be known. The use of arsenic trioxide as a deadly poison began in the thirteenth century and peaked in the fifteenth and sixteenth centuries. Murder by arsenic trioxide was so common at this time that mysterious illnesses and deaths were initially always suspected to have been caused by white arsenic. The state of medical and chemical knowledge made the detection of arsenic in the tissues of victims and samples of food difficult to impossible. Although medieval justice did not require 'proof beyond reasonable doubt', the chances for the poisoners to remain undiscovered were excellent until 1836, when Marsh reported his test for arsenic.[8] Criminal poisonings by arsenic trioxide declined rapidly. Analytical chemistry has come to our rescue and murders with white arsenic have been very infrequent during the past 150 years.

Although murders by arsenic trioxide have mostly disappeared, the memories of the toxic and lethal effects of arsenic trioxide are still much alive in the general population and in the scientific community. However, over time the term 'arsenic', a short form of 'white arsenic' meaning arsenic trioxide, lost its specific meaning and became to be applied as a general term for all arsenic-containing materials. The toxic characteristics of arsenic trioxide remained attached to the general term 'arsenic'. The result of this change in terminology is the common — and often incorrect — perception, that any arsenic-containing material is a threat to life, even when arsenic is present at very low concentrations. This attitude is at least partially responsible for the many determinations of arsenic in environmental samples that were carried out as soon as analytical methods of sufficient detection power had become available. The interest in arsenic is well expressed by the number of papers published during the past 20 years. An average of 100 publications have appeared annually since 1976 in many different journals describing results of investigations in the area of the environmental chemistry of arsenic.

ANALYTICAL ASPECTS

The methods for the quantitative determination of arsenic have been steadily improved since Marsh's discovery, in 1836, that the thermal decomposition of arsine (AsH_3) leads to the deposition of an arsenic mirror that is easily detected and identifiable. The wet chemical methods for the determination of arsenic, described in older textbooks of analytical chemistry, were based on the formation of insoluble precipitates containing arsenite (AsO_3^{3-}) or arsenate (AsO_4^{3-})[9,10] and were compound-specific, allowing for differentiation between trivalent and pentavalent inorganic arsenic compounds. The instrumental methods of analysis that have been developed during the past 30 years have made it possible to detect and quantify arsenic at ever-lower concentrations. Today, 1 ng and even smaller quantities of arsenic can be determined without great difficulties. The analyst has many methods[11,12] from which to choose. Flame atomic absorption and emission spectrometry, graphite furnace atomic absorption spectrometry, colorimetry, polarography and other electrochemical techniques, hydride generation with its many modifications, X-ray fluorescence and atomic fluorescence spectrometry, plasma emission spectrometry, gas chromatography, neutron activation analysis, and proton-induced X-ray emission are examples. Most of these methods destroy the sample and with it the molecules that contain arsenic. If the analysis should reveal only 'total arsenic' concentrations, the destruction of arsenic-containing molecules and the concomitant loss of chemical information do not cause much harm. The destructive analytical techniques fostered, however, a peculiar attitude that denied that arsenic may occur in environmental samples in forms of molecular entities with distinct physical, chemical and biological properties. Dedicated efforts during the past 15 years uncovered ample proof that 'total arsenic' concentrations are largely inadequate for the assessment of an arsenic-related threat to the health of organisms.[13] We know now that arsenite, arsenate, methylarsonic acid, dimethylarsinic acid, trimethylarsine oxide, tetramethylarsonium salts, 2-hydroxyethyl(trimethyl)-arsonium salts (arsenocholine), 2-carboxymethyl-(trimethyl)arsonium zwitterion (arsenobetaine), dimethyl(ribosyl)arsine oxides, and perhaps arsenic-containing lipids occur in organisms. Because the biological properties of these arsenic compounds differ vastly from each other, total arsenic determinations must be followed by the identification and quantification of each of the arsenic compounds present.

Methods for the identification and quantification of arsenic compounds are now available. Volatile arsenic compounds, e.g. methylarsines $(CH_3)_nAsH_{3-n}$ $(n=1,2,3)$ which have been infrequently detected in the environment, and methylated arsenic compounds, e.g. $(CH_3)_nAs(O)(OH)_{3-n}$ $(n=1,2,3)$ which are frequently found in the environment and can be reduced to methylarsines, can be identified and quantified by the methods applicable to organic compounds. Gas chromatography, mass spectrometry, and GCMS may serve as examples of such methods. The most widely used method for these two groups of arsenic compounds is the hydride generation technique.[4] After a

pH-controlled reduction of the arsenic compounds with sodium borohydride (selective reduction of arsenite at pH > 4; reduction of all arsenic compounds at pH 1) the arsines can be separated according to their boiling point or by gas chromatography.[15]

The group of non-volatile and non-reducible arsenic compounds, to which arsenocholine, arsenobetaine, dimethy(ribosyl)arsine oxides and arsenic-containing lipids belong, requires a different analytical approach. These arsenic compounds can be separated by liquid chromatography. To facilitate the detection, identification and quantification of arsenic compounds, arsenic-specific detectors for ion and high-pressure liquid chromatography were developed. Graphite furnace atomic absorption spectrometers and plasma atomic emission spectrometers were coupled to the liquid chromatographs.[15] Whereas the arsenic-specific detector systems perform adequately, the chromatography of crude extracts is still troublesome and needs to be considerably improved to allow quick screening of large numbers of samples. Organic compounds present in the extracts from organisms function as ion-pairing reagents, compete with the ion-pairing reagent used in the paired-ion chromatography, and produce several arsenic-containing bands. This interference can be overcome by column-chromatographic purification of the extract or — in some cases — by increasing the concentration of the ion-pairing reagent.[17] Dimethyl-(ribosyl)arsine oxides, the most complex arsenic compounds unambiguously identified in marine organisms, have been extracted with methanol, the extracts defatted with diethyl ether and then purified by column chromatography with several solvents and by thin-layer chromatography.[18–20] The arsenic compounds were identified by ^{1}H and ^{13}C NMR spectroscopy after the structure of one of the compounds had been established by X-ray crystallography.[18] The arsenic-containing riboses were shown to be separable by a high-pressure liquid chromatography–plasma emission spectrometry system.[20]

The non-reducible arsenobetaine and dimethyl-(ribosyl)arsine oxides can now also be determined by the hydride generation technique[15] after their conversion to reducible methylarsenic compounds by hot 2 mol dm^{-3} aqueous sodium hydroxide. Arsenobetaine is converted to trimethylarsine oxide and the dimethy(ribosyl)arsine oxides to dimethylarsinic acid. Reduction of the sample with sodium borohydride before and after treatment with sodium hydroxide makes it possible to determine and distinguish between reducible di- and tri-methylated arsenic compounds orignally present in the extract and generated by digestion.

Investigators identifying arsenic compounds in organisms were often satisfied with learning the nature of the major arsenic species and neglected minor components that produced distinct peaks in the chromatograms. These minor components must now be identified in an attempt to clarify the biochemical pathways traveled by arsenic on its journey through organisms. The analytical methods are now available to make this task feasible but not necessarily easy.

Knowledge accumulated about arsenic compounds in nature and the availability of methods for their identification and quantification provide the opportunity for investigators to join the growing community of 'speciators'. Although complex and expensive instrumentation is of great help in this work, much can be done with a liquid chromatograph and a graphite furnace atomic absorption spectrometer, even when these instruments are not coupled. Manual transfer of the fractions into the graphite furnace will ultimately also produce an HPLC—GFAA chromatogram and lead to the identification of arsenic compounds. With scientists switching from 'total arsenic' to 'arsenic compound' methods, much will be learned at a quicker pace about the distribution of arsenic compounds in nature.

CHEMICAL ASPECTS

Unless the structure of an unknown arsenic compound isolated from an organism has been determined by X-ray methods, the ultimate identification rests on the availability of synthetic materials. Analytical methods require standards. For instance, chromatographic peaks and their retention times are useful for the identification of compounds only when chromatograms of standards are available for comparison. Many of the arsenic compounds found in nature can be easily prepared in the laboratory. A dimethyl(ribosyl)arsine oxide has been successfully synthesized.[21] Attempts to prepare arsenolecithins, in which arsenic replaces nitrogen in the choline moiety, have thus far been unsuccessful. The hope that the methods developed decades ago for the preparation of lecithins would yield the arsenolecithins was not fulfilled. The reasons for these failures are not yet clear, but might be associated with the capability of arsenic to expand its valence shell. However, a lipid, in which a 1-(trimethylarsonio)-2-ethylphosphonic acid and a 1,2-dipalmitoylglycerol are joined was successfully prepared. Attempts to prepare the arsenic-containing phospholipid continue.[22]

Although the methylation of arsenite by microorganisms to methylarsenic compounds has been known for almost a century,[23] the detailed mechanism of this reaction and the methyl donors have not yet been

identified with complete certainty. The powerful techniques of nuclear magnetic resonance spectroscopy promise to provide much information about these methylation reactions. By felicitous choice of organisms and conditions, the transformations of arsenic compounds should be detectable in intact organisms. Concerted efforts in this area should lead to a much better understanding of the pathways of arsenic.

It is generally accepted that only trivalent arsenic can be methylated. The arsenic compound that has just acquired an additional methyl group contains now pentavalent arsenic and must be reduced to a trivalent-arsenic compound before further methylation can occur. The intermediate trivalent arsenic compounds have not yet been detected. Whether these intermediates are methyl(hydroxy)arsines, $(CH_3)_nAs(OH)_{3-n}$, is not known. Methylhydroxyarsines, which undoubtedly exist in solutions of aqueous or organic solvents, have not been studied to any extent even in purely synthetic systems. Much work awaits the organometallic chemists, who are expected to provide samples of organic arsenic compounds much needed as standards by analytical and environmental scientists and as test materials by toxicologists.

BIOCHEMICAL ASPECTS

Much has been added in recent years to knowledge of the arsenic cycle in nature.[24] Most of the work on natural arsenic compounds has been carried out with marine organisms, in which arsenobetaine, arsenocholine, arsenic-containing riboses and perhaps arsenolipids have been detected. Arsenobetaine is certainly the most ubiquitous arsenic compound in marine animals. Very little is known about trophic levels and how these arsenic compounds are formed from arsenite or arsenate present in seawater at 2 $\mu g\ dm^{-3}$. Phillips and Depledge[25] suggested that arsenocholine is formed biochemically in analogy to choline and is then oxidized to arsenobetaine. Whereas the biochemical oxidation of arsenocholine to arsenobetaine has been experimentally proven to occur in mice, rats and rabbits,[26] the choline pathway for the formation of arsenocholine awaits experimental verification. Much evidence has recently accumulated that dimethyl-(ribosyl)arsine oxides might be the precursors of arsenobetaine.[27] Our expanded knowledge of the arsenic cycle in the marine environment should allow the design of well-focused biochemical experiments that should clarify the important pathways that are used for the transformation of arsenic compounds.

A striking difference appears to exist between the way in which marine organisms and terrestrial (including freshwater) organisms deal with arsenic. In contrast to marine organisms, terrestrial animals and plants seem to convert inorganic arsenic only to simple methylarsenic compounds with trimethylarsine oxide as the most complex arsenic derivative. Whether this difference is real, or only the result of insufficient attention given to terrestrial organisms, cannot be decided at this time.

After the progression from total-arsenic determination to the more complex task of determining arsenic compounds, an even more complex assignment awaits the analyst. Although arsenite, arsenate and the organic arsenic compounds might be present as dissolved 'independent' molecules in cellular and extracellular fluids, these arsenic compounds will certainly interact with biologically important molecules. The reaction of trivalent arsenic compounds with thiol groups in enzymes is the accepted molecular cause for the observed toxicity. Arsenic compounds might be associated with other molecules in living organisms. Nothing, for instance, is known about the transport of arsenic. Many ions such as copper(II) (Cu^{2+} and iron(III) (Fe^{3+}) are transported in the blood in form of complexes with specific ligands. Does a specific transport protein exist for any of the arsenic compounds? Our analytical methods are probably not gentle enough for an 'association' between an arsenic compound and another molecule — unless held together by a covalent or strong ionic bond — to survive.[28] Better, gentler, preferably *in-situ*, methods must be used to explore the presence and identity of these associates. Nuclear magnetic resonance might be useful for these purposes.

Concern about arsenic and its compounds is heightened by fear of the ill effects including painful death that might come from exposure to arsenic compounds. It is now abundantly clear that a blanket condemnation of 'arsenic' as evil, extremely toxic and undesirable is not defensible. Whereas some arsenic compounds (arsine, arsenite, arsenic trioxide) are certainly toxic and exposure to them must be minimized, other arsenic compounds such as arsenobetaine and arsenocholine[29] are not toxic. In this context one should not forget that the dose makes the poison.[30] Fortunately the dose of arsenobetaine, ubiquitous in seafood at the milligram-per-kilogram level, does no harm to seafood lovers. On the contrary, a daily small dose of 'arsenic' might be life-supporting. Evidence based on animal experiments is accumulating that arsenic has an essential function.[31] The physiological function of arsenic is unknown. The arsenic requirement of man cannot be estimated with any certainty, but requirements for animals were extrapolated to man

and suggested to be 30 μg per day. This amount is not furnished by the typical diet in the USA, of which the Market Basket Survey is representative.[32]

The work on arsenic and arsenic compounds carried out by scientists from many disciplines has greatly increased our understanding of the interaction of arsenic compounds with biological systems. Our increased knowledge has made it possible to ask deeper-probing questions and has made it likely that we will find the answers. Progress in this endeavor depends on the close co-operation of scientists of various backgrounds: the instrument builder provides the instruments with improved power of detection and discrimination, the analytical chemist develops the methods for the detection and quantification of arsenic compounds in matrices far removed from distilled water, the crystallographer determines the structures of unknown compounds that were laboriously isolated from animals and plants, the toxicologist explores the poisonous characteristics of the compounds, and the biochemist and the molecular biologist contemplate the molecular significance of these compounds to life. Together we make progress quicker and keep each other from making mistakes. An interdisciplinary approach to the various aspects of arsenic in the environment promises to be most fruitful.

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