

The Metabolism of Oxalic Acid*

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Abstract: Oxalic acid is a widely occurring natural product of animals, plants and other organisms. It sometimes occurs as a free acid, but more commonly as a calcium salt. Organisms exhibit various levels of oxalate content which even show fluctuations among the organs of the same individual. Although oxalate seems to engage in the metabolism and adaptation of animals, plants and fungi, its mechanism is far from being completely understood. There is now an appreciable body of information regarding the occurrence and significance of oxalic acid in living tissues but the data are widely scattered in various scientific areas due to the broad distribution of oxalate among organisms. It is my goal in the present review to gather this information together to make it available for forthcoming research in the area of oxalate.

Key Words: Oxalate metabolism, Oxalate pathogenic activities, oxalate oxidase.

Oksalik Asidin Metabolizması

Özet: Oksalit asit hayvan, bitki ve fungus metabolizmalarının bir ürünü olarak yaygın bir şekilde bulunur. Oksalit asit bazen serbest asit durumunda görülse de genellikle kalsiyum tuzu durumunda bulunur. Oksalit asit miktarı organizmalar arasında farklılık gösterdiği gibi aynı organizmanın organları arasında da farklılık gösterir. Oksalitt asidin hayvan, bitki ve fungus metabolizmalarında ve adaptasyonlarında rol aldığı bilinmekle beraber bu işlevlerin mekanizmaları tam olarak anlaşılmaktan uzaktır. Günümüzde oksalik asidin oluşumu ve önemi hakkında önemli bir bilgi birikimi olmuştur. Bu biriken datalar oksalik asidin çok değişik organizmalarda çalışılmasından dolayı geniş bir bilimsel alana yayılmıştır. Benim bu derlemedeki amacım oksalit asit ile ilgili bilgileri bir araya getirmek ve böylece ileride oksalit asit alanında yapılacak araştırmalar için bir zemin hazırlamaktır.

Anahtar Sözcükler: Oksalit asit metabolizması, oksalik asidin patojenik aktiviteleri, oksalit asit toksikolojisi.

Introduction

Oxalic acid is one of the strongest organic acids with pKa values of 1.3 and 4.3 (1). Oxalic acid is used as an analytical reagent, it also has a wide variety of industrial and household applications. It is a constituent of cleaning solutions for removing paint, varnish, rust and ink stains as well as being used extensively in laundries as a scouring agent to remove excess alkalinity remaining in washed fabrics. It is also used for cleaning of bleaching wood and straw, as a chrome stripper and as a bleach in leather manufacture.

Oxalic acid has been detected in various organisms, including animals, plants and fungi. The occurrence and the distribution of oxalate vary enormously among organisms. For instance, in plants the highest oxalate concentrations commonly occur in the leaves and the lowest in roots. Meanwhile, the oxalate content of plants can vary according to their age, the season, the climate and the type of soil. In some plants, such as rhubarb,

oxalate content tends to increase as the plants mature, whereas, in other plants, e. g. spinach, sugar beet leaves, and bananas, there is a large increase in oxalate content during the early stages of development, followed by a decrease as the plants mature.

Synthesis and storage of oxalate

Early studies on plant organic acids, including oxalic acid, yielded evidence that the production of these acids was related to photosynthesis and carbohydrate metabolism. Myers (2) noted that oxalate concentration in rhubarb leaves increased in parallel with the growing seasons, being correlated with the seasons of most active photosynthesis. Later, experiments on rhubarb and *Begonia* indicated that oxalic acid was not a direct product of photosynthesis but it was synthesised from precursors synthesised in the photosynthetic pathway (3, 4).

It is well known that oxalic acid is synthesised via several major pathways. Glyoxylate and L-ascorbic acid appear to be the major precursors of oxalic acid in plants

* Oxalic acid and oxalate are not used to define any particular forms of oxalic acid. Therefore they are interchangeable in this text.

(5, 6). Glucose, acetate and some acids of the tricarboxylic acid cycle were determined to be involved in oxalate biosynthesis in red beetroots and young spinach leaves (7). Moreover, glycolic and isocitric acids (8), and oxaloacetic acid (7) are known to donate carbon to oxalic acid in plants. The relative significance of these metabolites as precursors of oxalic acid has not been established (9).

The enzymes involved in the synthesis of oxalic acid are of interest. In lettuce, two enzymes identified to have a role in the oxidation of glycolate and glyoxylate to oxalic acid were lactate dehydrogenase and glycolate oxidase (5). Since oxalic acid could be formed from several precursors, there is no clear evidence of its synthesis and deposition site. However, it was observed that the primary site of deposition of oxalic acid formed from L-ascorbic acid was the vacuole in barley which is a low level oxalic acid accumulator (9).

The involvement of oxalate in animal metabolism

Many plants and animals produce oxalic acid, and it is of interest that they share some common pathways of oxalic acid synthesis. Oxalate may be present as the free oxalic acid, as soluble sodium and potassium salts or as insoluble calcium oxalate crystals. Unlike plants, calcium oxalate crystal formation in animals is generally considered to be pathological. In a related context, most analytical studies that have dealt with the occurrence and distribution of oxalates in plants have focused on their possible antinutritive calcium-sequestering influence in the human diet (10). High oxalate contents of rhubarb and spinach are of particular interest, and in extreme cases, oxalate-rich rhubarb leaves are acutely toxic for humans just as high-oxalate pasture grasses are acutely toxic for grazing cattle (11). The pathological role of oxalic acid in the formation of urinary stones in animals and humans has been known since the early 18th century. For example, excess consumption of oxalate-rich foods leads to hyperoxaluria which is recognised as a key risk factor for calcium oxalate stone formation (12). Furthermore, there is evidence that high ascorbic acid intake increases urinary oxalate levels which could lead to the formation of calcium oxalate stones in the kidneys and other regions of the urinary system (13, 14). Oxalic acid is regarded as an undesirable component of our food not only because it raises the risk of urinary stones but also because it sequesters calcium, which is one of the essential ions, as insoluble calcium oxalate. The toxicology of oxalic acid in humans has been reviewed by several researchers (15, 16). In medicine, the knowledge of the oxalate concentration in blood and other body fluids can

be very important in certain clinical situations such as primary hyperoxaluria (17). Various methods have been formulated for the assay of oxalate from different sources, for instance, gas-liquid chromatography, ion chromatography, high performance liquid chromatography, mass spectrometry and enzymatic determination methods have all been developed. Of these, the oxalate oxidase activity-based determination of oxalate has become very popular and is used widely because of its simplicity, specificity and sensitivity (18).

Oxalic acid has usually been seen as an inert end product of the metabolism and only plants have been reported to be able to metabolise oxalic acid and oxalates. However, recent studies indicate that the levels of oxalate are too high for the substance to only be an end-product of the metabolism in animals (19). Therefore, it has been suggested that there could be an oxalate oxidase pathway in animals which uses oxalate to produce H_2O_2 , which could then be used to promote a "burst" of phagocytes: cells that engulf and break down foreign particles, cell debris and disease-producing micro-organisms.

The involvement of oxalate in plant metabolism

Unlike animals, plants are highly tolerant of oxalic acid and oxalates. Oxalic acid and oxalates have been detected in varying quantities in all parts of most plants' leaves, leaf stalks, flowers, tubers and roots (20). It is well established that plants are capable of metabolising oxalate by observing fluctuations in oxalate concentrations under certain conditions (21), and enzymes degrading oxalic acid have been detected in numerous plants (22, 23). Several functions have been proposed for the presence of oxalic acid in plants. It has been implied that oxalic acid might be related to ionic balance, since it can combine with various plant ions to form soluble or insoluble compounds. It was suggested that oxalate synthesis occurred to balance the excess of inorganic cations (represented by K^+ , Na^+ , NH_4^+ , Ca^{++} and Mg^{++}) over anions (represented by NO_3^- , Cl^- , $H_2PO_4^-$, SO_4^{-2}) normally present in the plant—the ability of nitrate ions and chloride ions to inhibit oxalic acid oxidase activity in *Beta vulgaris* results in an accumulation of oxalate.

Calcium oxalate crystals were amongst the first objects observed in plants in the early days of light microscopy in the late 17th century. Insoluble calcium oxalate formation enables plants to control the concentration both of ionically active oxalic acid and calcium. Both of these molecules might have a toxic effect when accumulating in excess quantities. Thus plants could induce calcium oxalate crystal formation to remove excess oxalic acid or calcium. Although calcium is essential to

biological growth and development, free calcium at high concentrations is toxic to cells. So it was suggested that calcium oxalate precipitation serves to sequester excess calcium and remove it from the active metabolism (24). The rapid induction of calcium oxalate crystal formation by calcium in *Lemna* plants suggests that the crystals may serve as a storage form for calcium for future needs (25). Further support for this came from the observation that in some plants the crystals appear to be dissolved during calcium deficient conditions, presumably to supply calcium for growth and cell maintenance (26). Calcium is required for the activation and/or stabilisation of certain enzymes; for example plant cells need calcium to release peroxidases which are related to the control of cell elongation since they can rigidify walls by their cross-linking activity and their ability to participate in the formation of lignin. Thus, for this role they are under the control of cellular calcium levels (27).

One of the major roles of calcium in plant cells is its action in the formation of the middle lamella where Ca^{2+} ions from stabilising ionic bridges between pectin chains. Although it has been suggested that calcium oxalate crystals are a means of detoxifying excess oxalic acid, the fact that many plants are able to retain high concentrations of soluble and free oxalic acid within their vacuoles indicates that oxalic acid may not be particularly toxic to plant tissues. However, accumulation of oxalic acid may have some toxic consequences (for example in causing osmotic problems and destabilisation of cells), unless it is readily metabolised (28).

The involvement of oxalate in fungus metabolism

It is well established that certain plant pathogenic fungi secrete oxalic acid as part of the process for invasion of plant tissues. For example, *Sclerotium rolfsii* Sacc., a fungus, causes diseases of plants in nearly 100

plant families. Considerable quantities of oxalate were detected in infected but not in healthy tissues. Oxalic acid produced by this pathogenic fungus played an essential role in its pathogenic capabilities (29). Another pathogenic fungus infecting a wide range of plant species is *Sclerotinia sclerotiorum*. During infection, the fungus produces high levels of a necrosis phytotoxin identified as oxalic acid (30). The role of oxalic acid in the pathogenicity process is still unclear. However, oxalic acid may have a number of functions in the infection process including chelating calcium from the cell was thus making the pectic fraction more available to fungal hydrolases, and providing an acid pH needed for maximum activity of the wall degrading enzymes released by the pathogenic fungus (31). Furthermore, it was suggested that oxalic acid produced by fungi played a key role in lignin biodegradation through its stimulation of lignin-degrading enzymatic activities (e.g. Mn-peroxidase activity) (32). One part of the plant's defensive response to this may be the production of oxalate oxidase which is an oxalic acid degrading enzyme (22). A second response would be the induction of deposition of oxalic acid in the form of soluble or insoluble salt.

Oxalic acid, which is a widely occurring natural product of animals, plants and fungi, has been seen as an inert end product of the metabolism. Moreover, oxalate accumulation was thought to be toxic to living tissues because not only does oxalic acid induce some pathological circumstances in animals and plants but also oxalic acid's involvement in pathological activities of some fungus. The growing body of information has changed this notion and it is suggested that oxalic acid and its salts, oxalates, may function in the metabolic regulation of plant, animal and fungus development. A better understanding of oxalate biology might enable us to manipulate various aspects of these organisms.

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