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COMMENTARY

ALDEHYDE OXIDASE AND XANTHINE OXIDASE— FUNCTIONAL AND EVOLUTIONARY RELATIONSHIPS

THOMAS A. KRENITSKY
Wellcome Research Laboratories, Research Triangle Park, NC 27709, U.S.A.

Xanthine oxidase* and aldehyde oxidase† play important roles in the metabolism of many drugs. The evolutionary relationship between these closely related enzymes has not been previously considered, yet our knowledge has reached the point at which some plausible proposals on this subject can be made. Those presented here are relevant to the poorly understood functional relationship between these two enzymes.

The structures of xanthine oxidase and aldehyde oxidase are similar. Both enzymes have a particular weight of about 300,000 daltons. These large proteins can be dissociated into two inactive subunits of equal size. Each subunit contains one atom of molybdenum, one molecule of flavin adenine dinucleotide, and four nonheme iron–sulfur groups [1–3]. No other known enzymes share this combination of prosthetic groups. Both enzymes have a persulfide linkage at their active sites [4–6]. The absorption spectra of the enzymes are similar in both their native and deflavo forms [7].

These structural similarities have their catalytic counterpart. Both enzymes catalyze the transfer of an oxygen atom from water to a wide variety of compounds containing a group represented by the general formula, $R_1—CH=R_2$ [1, 8]. From this structural and catalytic evidence of relatedness, it seems quite probable that this pair of enzymes arose from a common progenitor by gene duplication and subsequent genetic modification. Genetic data support this view. In Drosophila, the structural genes for xanthine oxidase and aldehyde oxidase are vicinally situated on the same chromosome [9, 10], as would be expected if gene duplication was involved in their evolution. In humans, drug metabolism patterns are also consistent with the proximity of these genes. In a group of five patients with an inborn deficiency of xanthine oxidase, three of the patients were incapable of oxidizing allopurinol [11]. Since this oxidation is catalyzed by either xanthine oxidase or aldehyde oxidase [8, 12], the inability of these three patients to oxidize this drug indicates a deficiency in both enzymes. Occurrence of patients with this double deficiency suggests that in humans, as in Drosophila, the genes for these two enzymes are vicinally located.

From the above considerations, it can be inferred that xanthine oxidase and aldehyde oxidase were derived from a common progenitor. There is some data to further suggest that the progenitor enzyme resembled xanthine oxidase more than it resembled aldehyde oxidase. Although neither enzyme has been found in plants or fungi, xanthine oxidase, but not aldehyde oxidase, is present in some procaryotes [13, 14]. The most primitive species in which aldehyde oxidase has been found is the coelenterate, *Sagartia luciae* [15]. This pattern of distribution indicates that, of this enzyme pair, xanthine oxidase is primordial. The electron acceptor specificities of these enzymes are also consistent with this view. With procaryotic xanthine oxidase, oxygen and NAD^+ are poor electron acceptors [13, 14]. It has been proposed that in these organisms ferridoxin is the physiological electron acceptor [14]. This is consistent with the low levels of oxygen available to early life forms. Among the eucaryotic xanthine oxidases, NAD^+ is, in general, a more efficient electron acceptor than is oxygen [15]. On the other hand, oxygen is the preferred electron acceptor with aldehyde oxidase. This is consistent with the appearance of aldehyde oxidase in eucaryotes at a later time when atmospheric oxygen was more plentiful.

Why did aldehyde oxidase evolve from xanthine oxidase? What environmental pressure made this a desirable event? The answer lies in an understanding of the physiological function of both enzymes. Clues to the function of aldehyde oxidase have been sought in a comparison of its substrate specificity with that of xanthine oxidase [8]. Although both enzymes oxidize a wide variety of compounds, many have very low affinities for these enzymes and have slow rates of reaction. The most efficient substrates for both enzymes are substituted pyrimidines and 5,6-condensed pyrimidines. These heterocycles are probably the substrates of physiological importance. The major specificity difference between these two enzymes resides in the effect of ring substituents. This difference results in complementary specificities, i.e. in many instances, compounds not readily oxidized by one enzyme are good substrates for the other. Consequently, this pair of enzymes can oxidize, and thereby detoxify, a wide variety of pyrimidines, purines, pteridines and related compounds. The high concentration of

* The term "xanthine oxidase" is used here to denote any enzyme which catalyzes the oxidation of xanthine to uric acid, irrespective of the electron acceptor (EC 1.2.3.2).

† In literature, the term aldehyde oxidase is sometimes erroneously applied to the aldehyde dehydrogenases (EC 1.2.1.3, EC 1.2.1.4 and EC 1.2.1.5), enzymes very different from the molybdeno-flavoprotein referred to here (EC 1.2.3.1).

both enzymes in the small intestine and liver of mammals [15] appears to constitute a protective barrier against ingested nitrogen-containing heterocycles of the type most prevalent in nature. Viewed in more general terms, there seem to be two major mechanisms for the oxidation of aromatic compounds in animals: (1) the mixed function oxidase system (cytochrome P450-containing system) associated with the endoplasmic reticulum which most efficiently oxidizes the non-polar, lipophilic aromatics, and (2) the soluble system, consisting of xanthine oxidase and aldehyde oxidase, which most efficiently oxidizes the more polar aromatics, mainly nitrogen-containing heterocycles.

A comparison of the distribution of xanthine oxidase and aldehyde oxidase among mammals [15] provides a further clue to the physiological function of aldehyde oxidase. The levels of xanthine oxidase from species to species are within the same order of magnitude. In contrast, the levels of aldehyde oxidase vary by three orders of magnitude. Herbivores are at the higher end of the scale and carnivores are at the lower end. Thus, the function performed by aldehyde oxidase appears quantitatively more important to herbivores. It is well established that plants are much more versatile in their synthetic capabilities than are animals. Therefore, it seems probable that certain plants might have developed the ability to synthesize toxic heterocycles which xanthine oxidase was not capable of detoxifying. In order for these plants to become part of the food chain, it might have been necessary for early animals, especially herbivores, to acquire a second enzyme, aldehyde oxidase, with a specificity different from that of xanthine oxidase. Development of a second enzyme, rather than development of a single less specific enzyme, had the added advantage of allowing for the independent regulation of the oxidation of different types of heterocycles. The influence of herbivore–plant interactions on the development of the mixed function oxidase system has also been considered [16].

The proposed overall evolutionary view is that xanthine oxidase appeared in early life forms. In the beginning, its major function was to oxidize pyrimidines, purines and pteridines of the type produced endogenously. As the complexity of life increased, so did the variety of heterocycles synthesized, especially by plants. Primitive animals ingesting toxic heterocycles of a type not readily oxidized by the existing enzyme were endangered. The evolution of aldehyde oxidase from xanthine oxidase by gene duplication and subsequent genetic modification overcame this difficulty. Taken together, this pair of enzymes possesses the catalytic breadth necessary for animals to efficiently oxidize a wide variety of the nitrogen-containing heterocycles abundant in nature or manufactured by man.

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