Xanthine Oxidase: Enzymology and Pathophysiology

At the 661st Meeting of the Biochemical Society, held at Bath, the Host Colloquium, a joint meeting with the Society for Free Radical Research, took place on 10th and 11th April 1997. Its title was 'Xanthine oxidase: enzymology and pathophysiology'.

The two aspects of xanthine oxidase research specified in the Colloquium title have developed remarkably separately, and, for a number of reasons, it now seemed timely to bring together two groups of workers with quite different approaches to study of the same enzyme.

Xanthine oxidase is a complex enzyme, containing molybdenum, FAD and iron/sulphur redox centres, that has been known for at least 95 years and studied as the essentially pure enzyme for nearly 60 years. Its ready availability on a large scale from cows' milk has allowed the enzyme to become a model for structural and mechanistic analysis of molybdoenzymes in general, and a survey of the current position is now particularly appropriate.

Concerning pathophysiological involvement of xanthine oxidase, attention was focused on its role as a source of reactive oxygen species (ROS) in ischaemia–reperfusion injury by Granger and colleagues in 1981 [1]. Since that time, several hundreds of publications have addressed this issue. Meanwhile, ROS are increasingly being cited as agents of normal signal transduction, and, while their source is usually uncertain, xanthine oxidase is often a likely candidate. This candidature has been strengthened by recent analyses of promoter regions of human, mouse and rat enzymes, which suggest the presence of potential regulatory sites for cytokines known to stimulate generation of ROS. In view of all these recent developments, an assessment of the involvement of xanthine oxidase in both disease and normal physiology appeared to be timely.

Particularly because of the progress in the molecular biology of xanthine oxidase, enzymological and more clinically related studies on the enzyme, hitherto pursued essentially independently by relevant groups of investigators, have shown signs of converging, and this Colloquium represents an attempt to bring the two groups together, possibly for the first time. On the basis of many positive comments from representatives of both groups we are heartened by the feeling that this attempt has had a measure of success. The first day of the Colloquium was essentially devoted to enzymology and the second to pathophysiology. Of the seventeen invited speakers, over the two days, the representation was truly international, with nine contributions from the...
United States, four from the United Kingdom and one each from France, Germany, Italy and Japan.

The choice of topics and speakers seems to have been justified by capacity attendance at all sessions. Such a range would not have been possible without the generous sponsorship of Teijin Co. Ltd. and Rikaken Scientific Co. Ltd., both of Japan, and of the British Technology Group, to all of whom we are most grateful. We should also like to thank the Biochemical Society, all the student helpers who assisted in the organization and the session chairmen, Greg Bulkley, Robert Eisenthal, Bruce Freeman, Neil Granger and Vince Massey.

The Colloquium spawned a further meeting held at University of Sussex from 12th–15th April and organized by one of us (R.C.B.) and Russ Hille (Columbus, OH, U.S.A.), that dealt more generally with the chemistry, biochemistry and molecular genetics of molybdenum enzymes. It was attended by more than 90 research workers, with support from, amongst others, The Wellcome Trust, The Bioenergetics Group and the Society for Biological Inorganic Chemistry.


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Milk xanthine oxidoreductase: the first one hundred years
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In 1902, Schardinger demonstrated that samples of fresh milk decolorized Methylene Blue on addition of formaldehyde [1]. Some 20 years later, Morgan et al. [2] found an activity in bovine milk that converted hypoxanthine and xanthine into urate under both aerobic and anaerobic (with Methylene Blue) conditions. They found this activity was also present in the liver, kidney, spleen and lungs of the rat and ox. Boiling destroyed this xanthine oxidase (XO) activity. Dixon and Thurlow [3] partially purified XO and performed a kinetic analysis of the xanthine/Methylene Blue activity. In addition to hypoxanthine and xanthine, the partially purified XO was shown to utilize aldehydes as reducing substrates [4], supplying the first evidence that XO is identical with the Schardinger enzyme. An unusual type of enzyme inhibition was found when Dixon and Keilin [5] allowed XO to react with cyanide. Although cyanide added to assay mixtures did not inhibit the initial rate of Methylene Blue reduction or oxygen uptake, pre-inhibition of XO with cyanide abolished activity in a complete, irreversible and time-dependent manner. Addition of purines protected against inactivation.

In 1939, Ball [6] and Corran et al. [7] both obtained mostly pure XO, a golden brown material, the visible spectrum of which indicated the presence of a flavin (determined to be FAD) as well as another chromophore. Early purification procedures were quite harsh by current standards, involving solvent extraction, drying, acid treatment, heat treatment and exposure to proteases. Generally, only 5–40% of the visible spectrum was bleached on anaerobic addition of xanthine, causing much confusion over the role of the FAD and the unknown chromophore in catalysis. The purification of XO demonstrated that both purine- and aldehyde-oxidizing activities were found in the same enzyme, demonstrating the identity of XO with the Schardinger enzyme [8,9].

The first evidence of the existence of non-functional species of XO came in 1949 from Lowry and colleagues, who were able to titrate the number of active sites with the extremely tight-binding inhibitor 2-amino-4-hydroxy-6-formylpteridine [10]. These authors concluded that only 60% of the FAD in their sample was associated with active enzyme. Anaerobic mixing of XO with xanthine was observed to involve a fast phase (seconds) and a slow phase (minutes to hours) when the loss of visible absorbance of XO was monitored [6,7]. In 1952, Morell [11] discovered a linear relationship between the extent of XO bleaching in the first phase and the rate of xanthine/oxygen turnover. Treatment with cyanide resulted in XO that was not at all

Abbreviations used: XDH, xanthine dehydrogenase; XO, xanthine oxidase.