LXVI. THE XANTHINE OXIDASE-ALDEHYDE SYSTEM

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Booth [1938] concluded that xanthine oxidase and the Schardinger enzyme are identical and that the one enzyme activates all aldehydes. Although xanthine oxidase has been much studied as to its purine activity, less is known about its aldehyde activity. In this paper certain aspects of the dynamics of the enzyme-aldehyde system will be considered especially in regard to differences from the enzyme-purine system. The exceptional dual specificity of xanthine oxidase offers an experimental example of the fact that many properties of enzyme systems belong not to the enzymes but to the chemical reactions catalysed.

The enzyme preparation was made from whey by the method of Dixon & Kodama [1926] and dissolved in phosphate buffer *p*H 7·2 as required. Other experimental details are described by Booth [1938].

Destruction of enzyme by substrate

While there is no experimental indication that purines destroy the enzyme, formaldehyde destroys it anaerobically as the following experiment shows. A series of Thunberg tubes was set up with the same amount of methylene blue and buffered enzyme solution in each and with formaldehyde in optimum concentration as determined in Fig. 1. In the 1st tube the aldehyde was placed in the stopper with the methylene blue. In the 2nd tube the aldehyde was placed in the stopper but the methylene blue was put in the tube with the enzyme. In the other tubes the methylene blue was placed in the stoppers and the aldehyde with the enzyme. The tubes were incubated together, the first three being tipped after the usual 3 min. in the bath (+10 min. at room temp. necessary for greasing, evacuating etc.), and the others at graded intervals. The results in Table I show that the reduction times of the first two were the same but that

Table I. *Destruction of enzyme by aldehydes*

No.	In tube	In stopper	Tipped after min.	Reduction times: Formaldehyde min.	Reduction times: Acetaldehyde min.
1	Enzyme alone	Aldehyde + methylene blue	3	7·9	8
2	Enzyme + methylene blue	Aldehyde	3	7·9	8
3	Enzyme + aldehyde	Methylene blue	3	140	9
4	,,	,,	8	300	10
5	,,	,,	12	Incomplete	10
6	,,	,,	60	Partial	11

the times were increased by incubating the enzyme with the aldehyde. The last tubes were not completely reduced even overnight. Evidently formaldehyde destroys the enzyme sufficiently rapidly to make it an unsuitable substrate for quantitative studies, and presumably does so by reacting with amino groups on

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the enzyme molecule: these do not necessarily constitute the "active group" of the enzyme since no appreciable protection was afforded by uric acid. The experiment was repeated using acetaldehyde in concentration slightly above optimal. Destruction of enzyme by acetaldehyde was negligible in comparison with that by formaldehyde and may have been due to traces of peroxide. There was no indication of destruction by salicylaldehyde, a commonly used substrate. In presence of the enzyme preparation *o*-phthalaldehyde produced a black substance too rapidly to allow its use for methylene blue studies.

Michaelis constants

The optimal concentration of purine varies with the enzyme strength and the method of preparation (i.e. whether whole milk, "casein" or "whey" preparations are used). Hence the "Michaelis constant" is not strictly constant. Variation, however, is not great, and with the enzyme strength ordinarily used the value for hypoxanthine is 0·00015 *M*, i.e. the enzyme has a high affinity for hypoxanthine. The affinity for aldehydes is lower, for instance:

Acetaldehyde	0·062 *M*
Formaldehyde	0·0085
Salicylaldehyde	0·0014
Furfuraldehyde	0·004

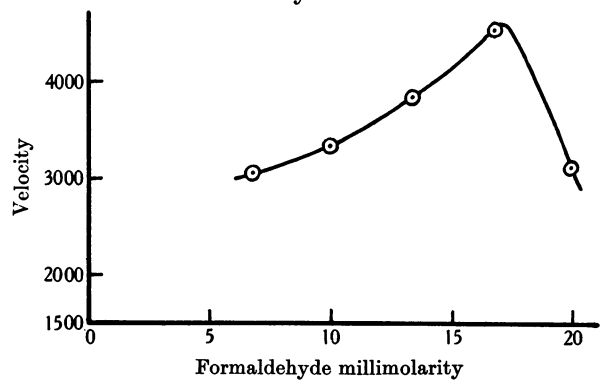

Fig. 1. Substrate concentration curve with formaldehyde. Anaerobic. Velocity is reciprocal of methylene blue reduction times in min. multiplied by 10,000.

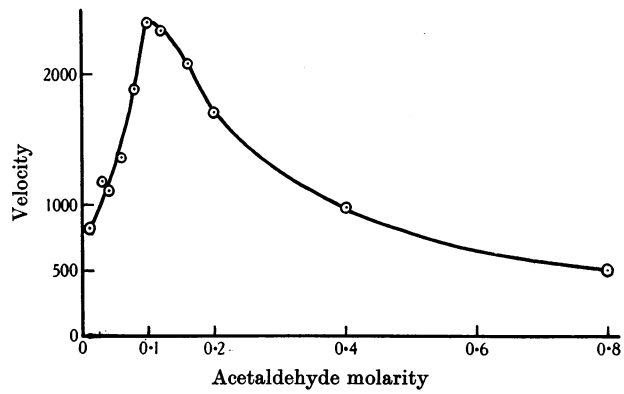

Fig. 2. Substrate concentration curve with acetaldehyde. Anaerobic.

The values for hypoxanthine and salicylaldehyde are taken from the data of Booth [1935], and the values for acetaldehyde and formaldehyde from Figs. 1 and 2. In the case of formaldehyde this estimate of the constant is low since the aldehyde destroys the enzyme and the true optimal concentration is unknown. Acetaldehyde (or an associated impurity) only destroys the enzyme slowly: the optimal concentration shown on the curve is therefore approximately correct. Many aldehydes are only slightly water-soluble. In many cases (e.g. glyceraldehyde) maximum velocity is probably not reached even with saturated solutions and the Michaelis constant cannot be determined.

Reversibility

Green [1934] made a study of xanthine oxidase equilibria with purines. He showed that the hypoxanthine-xanthine-uric acid system is reversible and that the potentials obtained agree with those predicted for a 2-stage oxidation. It seemed reasonable to expect that the aldehyde system might also be reversible, but thus far all attempts to reverse the system have failed. The experiments have been of two main types, with many variations.

The first was the colorimetric method of Green [1934]. Benzylviologen was reduced by aldehyde and enzyme in a Thunberg tube; the supposed oxidant was then tipped in from the hollow stopper, reoxidation of the dye indicating reversibility. The neutralized sodium salts of various organic acids have been used as oxidants. Benzylviologen is only partially reduced by the aldehyde system. This limitation was combated in two ways: (*a*) the *r*H of the dye was varied by alteration of *p*H; (*b*) in some experiments the indicator was reduced with a very small amount of hypoxanthine. Reoxidation was never observed.

In the second method hypoxanthine and a neutral solution of the salt of an organic acid were anaerobically incubated with enzyme in a series of tubes. At intervals a tube was opened and tests made for uric acid. If the system were reversible and the acid could be reduced to aldehyde some uric acid should be formed, but no trace was found in many experiments in which the *p*H and the concentrations of the reagents were varied.

The failure to reverse the aldehyde system suggests that the corresponding organic acid may not be the true oxidant. It is noteworthy that propionate does not inhibit the enzyme.

Oxidation products

The product of the enzymic oxidation of aldehyde is usually assumed to be the acid but this has never been isolated. Even the theoretical O_2 uptake [Keilin & Hartree, 1936] does not irrefutably establish that the acid is directly produced. Enzymically the aerobic oxidation almost certainly occurs in two stages, the first being the production of a powerful oxidizing agent. While this agent may be H_2O_2 the following facts support other possibilities.

(1) This agent destroys the enzyme much more rapidly than the oxidizing agent produced by the aerobic oxidation of hypoxanthine. The latter is probably H_2O_2 according to Dixon [1925] and Wieland & Rosenfeld [1930]. The difference is not due to protection by purine, for even when uric acid is added to the actively oxidizing aldehyde system the destruction still proceeds faster than in the case of the purine system.

(2) The protection afforded by catalase is variable and only obtained with powerful preparations.

(3) This oxidizing agent performs a coupled oxidation which H_2O_2 itself only performs when in much higher concentration. Enzyme, uric acid, and

salicylaldehyde were incubated anaerobically until the uric acid was mostly reduced to hypoxanthine by mixed dismutation [Booth, 1935]. The solution was then aerated at 38°. It was argued that aeration should reoxidize the hypoxanthine to uric acid. However, no uric acid was detectable and such traces as were present before aeration had also disappeared. Furthermore, in presence of aldehyde and the enzyme, uric acid disappeared more rapidly aerobically than anaerobically and no hypoxanthine could be isolated. The uric acid was oxidized, allantoin being apparently one product, since Ro's [1931–32] colour reaction for allantoin was positive and accounted for about one-third of the uric acid. Allantoin was not further oxidized when substituted for uric acid in the above experiment. The coupled oxidation only occurred in presence of the enzyme, aldehyde and O_2. That is to say xanthine oxidase and aldehyde together simulate the effect of a weak uricase. H_2O_2 also oxidizes uric acid but only when in high concentration. There is no reason to believe that uric acid is removed in this way during aerobic oxidation of hypoxanthine.

Keilin & Hartree [1936] suggested that the substance produced during aerobic oxidation of hypoxanthine may be called nascent H_2O_2 because it produced a coupled oxidation of alcohol unobtainable with added H_2O_2. The oxidizing agent produced during aerobic oxidation of aldehydes however appears to be more powerful than that produced during oxidation of purines. It may be a peracid or other highly oxidized unstable derivative of aldehyde.

Anaerobically the oxidative process must be different, but might occur also in two stages. In each case the problem of the oxidant remains unsolved.

Dismutation

Green [1934] showed that, under the influence of the enzyme, xanthine will dismute to form uric acid and hypoxanthine; and that in presence of hypoxanthine some uric acid disappears, presumably to form xanthine. I have confirmed these observations. On the other hand, aldehydes are not dismuted by xanthine oxidase as the following observations show.

A highly active xanthine oxidase solution was incubated anaerobically with 4% acetaldehyde for 9 hr. The solution was then acidified with H_3PO_4 and distilled into NaOH. The distillate was tested by the method of Krüger & Tschirch [1929; 1930] with lanthanum nitrate, iodine and ammonia, which produce a blue colour specific for acetate and propionate. None was found. The same experiment was performed with propaldehyde with the same negative result. Controls showed that the aldehydes had not destroyed the enzyme, and that a positive lanthanum test resulted from addition of either acid to the distillation mixture.

If aldehyde dismutes under influence of the enzyme, yielding alcohol and acid, aldehyde ought to react with hypoxanthine to yield alcohol and uric acid. Experimentally no uric acid was found although various aldehydes were tried. Were alcohols produced it is probable—though not necessarily certain—that they would also be activated by the enzyme, but none has been found to be so activated. Nor do alcohols inhibit the enzyme.

Since these experiments were done a paper has appeared by Dixon & Lutwak-Mann [1937] who have made a thorough investigation into the occurrence of mutase. They found too, using entirely different methods, that xanthine oxidase has no aldehyde mutase activity. They have critically examined the earlier literature in which the Schardinger enzyme is stated to catalyse aldehyde dismutation, and have clarified the position by the preparation of mutase completely free from aldehyde oxidase.

Dismutation or oxido-reduction is closely connected with reversibility, for to oxidize one molecule another must be reduced. The findings that neither reversal nor dismutation occur are therefore mutually confirmatory.

SUMMARY

Certain dynamics of the xanthine oxidase-aldehyde system which contrast with the xanthine oxidase-purine system are described.

(1) Formaldehyde destroys xanthine oxidase.

(2) Aldehydes have low affinity for the enzyme.

(3) The possible nature of the oxidation product is discussed. Aerobically some product is formed which performs an oxidation of uric acid to allantoin.

(4) Xanthine oxidase does not catalyse the reduction of acids or the oxidation of alcohols to aldehydes respectively.

(5) Xanthine oxidase has no aldehyde mutase activity.

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