6-HYDROXYLATION OF THE PTERIDINE RING BY XANTHINE OXIDASE

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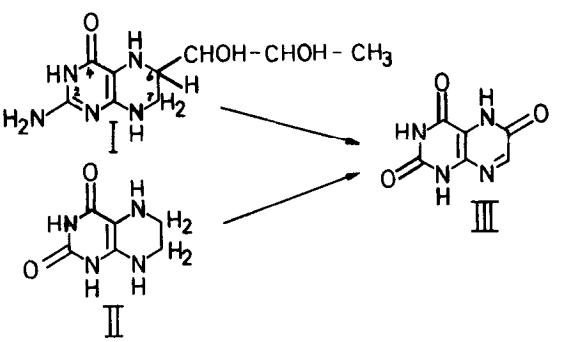
H. Rembold and H. Metzger (1965a, b) investigating the metabolism of tetrahydrobiopterin (I) found a degradation to 6-hydroxylumazine (III) by rat liver homogenates.



The above structures indicate, that this degradation must occur in several steps. The sidechain is cleaved, the amino group in position 2 is replaced by an oxodihydro group, and finally a new oxygen function is introduced into position 6.

This last step can be examined apart from the preceding ones, when tetrahydrolumazine (II) is used as a substrate, which yields 6-hydroxylumazine too, when incubated in rat liver homogenates. The enzyme responsible for this reaction appeared to be rat liver xanthine oxidase: figure 1.

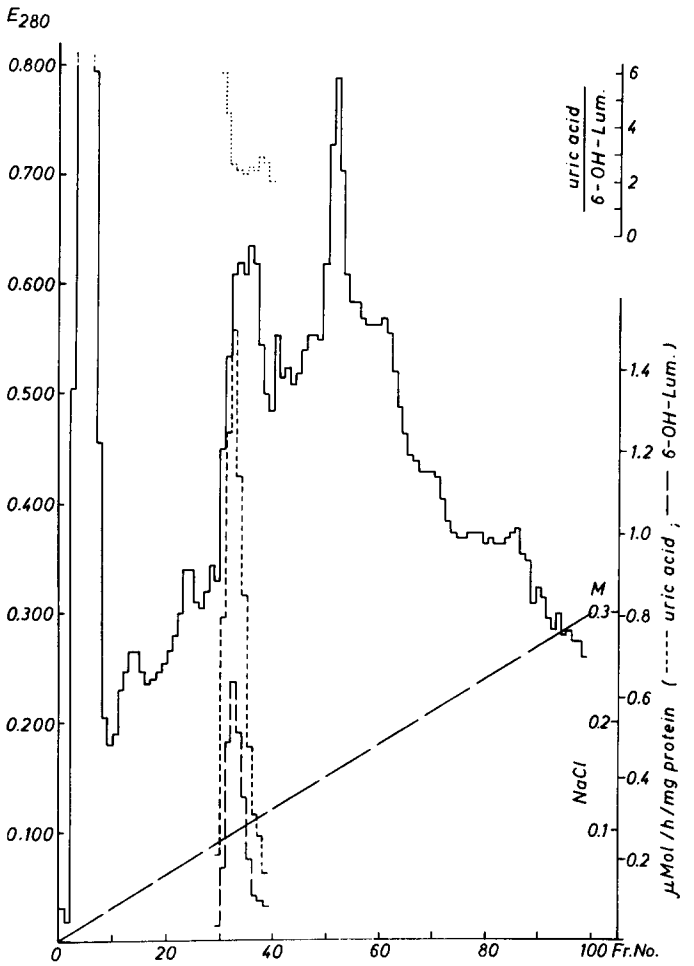
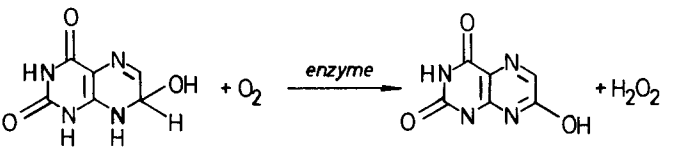


Fig. 1. DEAE Chromatography of 450 mg of an ammonium sulfate precipitate (30-50 % saturation) from rat liver homogenate. Column volume 37 ml, fraction volume 5 ml.

The specific activities shown in this figure were determined by two tests described under "Experimental". The two activity peaks are exactly symmetrical and the ratio of the specific activities remains constant throughout the peak. Pure xanthine oxidase from milk also catalyzes the formation of 6-hydroxylumazine.

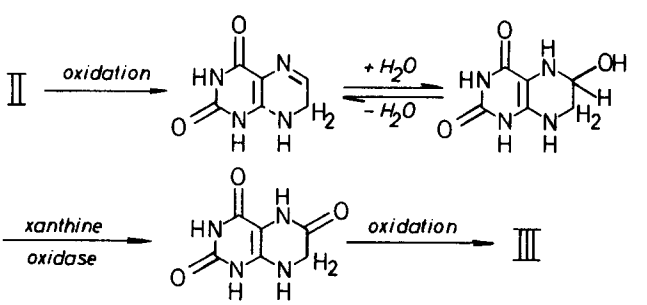
From experiments with nonhydrogenated pteridines as substrates for xanthine oxidase, F. Bergmann and H. Kwietny (1959) concluded, that this enzyme is able to attack the

ring system in positions 2, 4 and 7 only. These results and similar ones from the purine series indicate clearly, that xanthine oxidase does not show a high specificity as far as the attacked position of the ring systems is concerned. The exact mechanism of the xanthine oxidase reaction on CN-heterocyclic compounds, such as the formation of 7-hydroxylumazine from lumazine, is shown below.



As can be seen from the structure, the proper substrate for the dehydrogenation reaction of xanthine oxidase is the hydrated form of a C=N double bond. With all nonhydrogenated pteridines, hitherto investigated, the 7,8-double bond always seems to be hydrated much faster than the neighbouring 5,6-double bond. In presence of xanthine oxidase the 7-hydroxy derivatives are formed rapidly according to the above scheme. With these compounds a hydration of the 5,6-double bond seems unlikely.

On the other hand a hydration of the 5,6-double bond must have taken place in the case of a 6-hydroxylumazine formation described above. Assuming this, the proper substrate would not be tetrahydrolumazine, but rather its oxidation product, 7,8-dihydrolumazine, in its hydrated form. We consider the following reaction sequence to be the most probable.



That the 5,6-double bonds of 7,8-dihydrolumazine and 7,8-dihydropterin are readily susceptible to nucleophilic

attack including an addition of water, has been shown by H.S. Forrest and coworkers (1960). The product of the enzymatic reaction, 7,8-dihydro-6-hydroxylumazine, cannot be isolated, because it is oxidized rapidly to 6-hydroxylumazine. The test described below is based on this fact; after complete oxidation by standing overnight 6-hydroxylumazine is determined spectrophotometrically.

The UV spectra of the enzyme product at four different pH values are shown in figure 2.

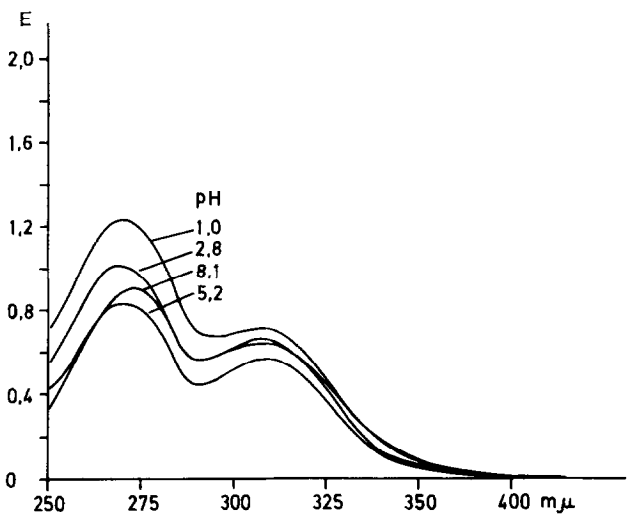


Fig. 2: UV spectra of the enzyme product formed from tetrahydrolumazine.

This UV spectrum and its high stability to changes in pH favour the tautomeric form of the enzyme product shown in the above scheme. Moreover this spectrum is completely identical with that of the known 7,8-dihydroxanthopterin, the analogous compound from the pterin series.

The structure of the enzyme product so confirmed shows that also in this case, the mechanism of xanthine oxidase is the normal one. It seems therefore, that with nonhydrogenated pteridines the enzyme is unable to attack position 6 only because the 5,6-double bond is not available in a hydrated form.

Further experiments indicated, that the analogous reaction steps, described here for the lumazine series, proceed in the pterin series too. Starting from tetrahydrofolic acid, tetrahydrobiopterin or tetrahydropterin, 7,8-

dihydroxanthopterin is formed by the action of xanthine oxidase via 7,8-dihydropterin as a common intermediate. 7,8-dihydroxanthopterin is finally oxidized to xanthopterin (H. Rembold et al., 1968). This compound has been isolated from many natural sources, but its biogenetic origin has not been satisfactorily explained as yet. From our results it can be regarded as a degradation product of hydrogenated cofactors like tetrahydrofolic acid or tetrahydrobiopterin.

EXPERIMENTAL

Tetrahydrolumazine was obtained from lumazine by catalytic hydrogenation in 1 N HCl with palladium oxide in an apparatus described by H. Rembold and H. Metzger (1967).

The formation of 6-hydroxylumazine is tested as follows. 250 µg (1,5 µMol) tetrahydrolumazine are incubated with the protein fraction in 2 ml of 0.1 m TRIS-HCl buffer, pH 7.5, for 90 min. at 37°C under aerobic conditions by shaking. The reaction is stopped by the addition of 1 ml of 30 % TCA. The protein suspension is boiled shortly and allowed to stand in air overnight. The solution is separated from the precipitated protein and brought to a volume of 4 ml. The concentration of 6-hydroxylumazine is measured spectrophotometrically by its absorption at 365 mµ (ε = 5500). The absorption of lumazine (from reoxidized substrate) at 365 mµ is negligible at this low pH value.

Xanthine oxidase activity was tested in the protein fractions with 50 µg (0.33 µMol) xanthine in 3 ml of 0.1 m TRIS-HCl, pH 7.5, by measuring the increase of absorption at 293 mµ (ε uric acid at 293 mµ = 12 300).

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