PTERINE OXIDASE

By OLIVER H. LOWRY, OTTO A. BESSEY, AND ELIZABETH J. CRAWFORD

(From the Department of Pharmacology, Washington University School of Medicine, St. Louis, the Department of Biological Chemistry, University of Illinois College of Medicine, Chicago, and the Division of Nutrition and Physiology, The Public Health Research Institute of The City of New York, Inc., New York)

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When pteroylglutamic acid (PGA) is irradiated with ultraviolet light, oxidative cleavage occurs and three pteridines are formed in succession (1). It was observed that the last of the series, 2-amino-4-hydroxypteridine (AHP), could be oxidized to isoxanthopterin by a concentrate of xanthine oxidase from cream. It was also observed that the first photolytic product, 2-amino-4-hydroxy-6-formylpteridine, was a remarkably effective inhibitor of this enzymatic oxidation. As little as 2×10^{-4} γ of the aldehyde per ml. produced demonstrable inhibition (1). This enzyme and this inhibition have been further explored and it appears that a single enzyme in cream is responsible for the oxidation of xanthine, xanthopterin, and AHP. The dissociation constant for the aldehyde-enzyme complex is so small that it has been possible to estimate an upper limit of the number of equivalents of enzyme present.

Materials and Methods

The 2-amino-4-hydroxypteridine (AHP), xanthopterin, and isoxanthopterin were obtained through the courtesy of Dr. G. H. Hitchings of Burroughs Wellcome and Company. The 2-amino-4-hydroxy-6-formylpteridine was kindly furnished by Dr. T. H. Jukes of the Lederle Laboratories Division, American Cyanamid Company. The oxidase preparations will be described below.

The enzymatic oxidation of xanthine to uric acid was measured by the increase in optical density at 295 mμ (Kalckar (2)). For substrate concentrations of the order of 10^{-5} M, the enzymatic oxidations of xanthopterin to leucopterin, and AHP to isoxanthopterin were similarly followed by measuring the increases in the absorption spectra at 340 mμ (Kalckar and Klenow (3)) and 335 mμ respectively. For work with higher dilutions of these two substrates the oxidations were followed fluorometrically. The fluorescence of xanthopterin disappears in the neutral pH range on conversion to leucopterin, and may be conveniently followed in the fluorometer (Kalckar and Klenow (3)).

In 0.1 M phosphate or acetate buffer in the pH range from 4 to 7 there is

a 5- to 10-fold increase in fluorescence upon conversion of AHP to isoxanthopterin (1). This increase can be used to measure the oxidation. At pH values more alkaline than 7 the enzymatic change in fluorescence becomes less and is reversed at pH 9. With the Farrand micro fluorometer (4) the oxidation of either xanthopterin or AHP can be followed with substrate concentrations from 10^{-5} down to as low as 3×10^{-8} mole per liter (0.005 γ per ml.). It is thus possible by using both spectrometric and fluorometric means to measure the enzymatic oxidation of xanthopterin or AHP through a 1000-fold range in substrate concentration.

Flavin-adenine dinucleotide (FAD) present in the enzyme was measured by a method detailed elsewhere (5). This method is based on the fact that FAD has only about 10 per cent of the fluorescence of flavin mononucleotide or riboflavin. The flavin was split from the proteins by heating 4 minutes at 100°. The fluorescence of an aliquot was measured, following which an FAD-splitting enzyme from potato (6)[1] was added. The increase in fluorescence, with appropriate internal standards, was used as a measure of the FAD.

Pterine Oxidase—The procedure given by Ball (7) for the preparation of xanthine oxidase was followed closely. The resultant material seemed very comparable to his preparation. The activity toward xanthine appeared to be at least as great, and the ratio of optical density at 280 mμ to that at 450 mμ was 16 as compared to 12, as reported by Ball. This material was used for most of this study. A small sample was further fractionated at 0° with neutralized ammonium sulfate (pH 6.8), as suggested by Kalckar (8). The flavin-adenine dinucleotide was measured in each of five successive fractions. The FAD accounted for more than 98 per cent of the total riboflavin of all the fractions. The protein was estimated from the light absorption at 280 mμ by means of the coefficient determined by Ball (7). The enzyme activity and FAD content maintained a constant ratio in all fractions through a 9-fold change in activity relative to protein (Table I). The most active fraction had properties quite similar to the best preparation of Corran, Dewan, Gordon, and Green (9). They found a ratio of 6.2 between the optical densities at 275 and 450 mμ, which is close to the $D_{280}:D_{450} = 7.8$ for the most active fraction listed in Table I. In this fraction 200,000 gm. of protein were associated with each mole of FAD (calculation from data of Corran *et al.* indicated 170,000 gm. of protein per mole of FAD). The FAD as measured fluorometrically agreed exactly with the optical absorption of the heated extract, but only accounted for about a third of the optical density at 450 mμ of the unheated enzyme solution (Table I, last column). This agrees exactly with Corran *et al.* who

[1] Lowry, O. H., Bessey, O. A., and Love, R. H., in preparation.

measured FAD through its function as coenzyme for D-amino acid oxidase. Part of this apparent absorption may be due to light scattering by the protein; it is also possible that the absorption spectrum of FAD is altered by its combination with protein. The failure to find extra absorption in the heated extract argues against the presence of a second chromogenic prosthetic group.

If the FAD is a direct measure of the number of moles of enzyme present, the turnover number at 30° is about 125 for xanthine at pH 6.8 in 0.1 M phosphate buffer (see below for the means used to measure xanthine oxidation). Evidence to be presented in another section indicates that the

TABLE I

Fractionation of Pterine Oxidase

$(NH_4)_2SO_4$* fraction	$\frac{D_{280}}{D_{450}}$†	Total‡ protein	Total FAD	Protein / FAD	Enzyme activity			$\frac{D_{450}}{D_{450}}$ calculated from FAD
					Vs. AHP		*Vs.* Xanthine	
					$V_{max.}$§	$V_{max.}$‖	$V_{max.}$‖	
M		*mg.*	10^{-6} *mole*	10^5 *gm. per mole*				
1.35		11.3	6.0	18.2	2.0	37		
1.48	40.0	8.4	6.9	12.1	3.3	40	128	3.5
1.69	21.6	9.8	15.3	6.4	5.8	37		3.4
1.96	12.4	12.5	38.2	3.3	12.4	41		3.0
2.42	7.8	11.9	58.5	2.0	18.5	37	123	3.0

* The fraction analyzed is that which precipitated between the next lower and the given concentration of ammonium salt (0°, pH 6.8).

† Optical density.

‡ Calculated from D_{280}.

§ Moles of substrate split per minute per 100,000 gm. of protein at 30° at pH 6.8 in 0.1 M phosphate.

‖ Moles of substrate split per minute per mole of flavin-adenine dinucleotide.

number of active molecules or active centers cannot exceed about 60 per cent of the number of FAD molecules in this preparation; hence the turnover number is 210 or more. Corran *et al.* calculated a turnover number of 306 for hypoxanthine at 38°. In what follows the active enzyme will be considered to be numerically equal to 60 per cent of the molecules of FAD.

Activity toward AHP—With sufficient AHP to saturate the enzyme there is only minor dependence of the initial velocity of reaction, $V_{max.}$, on pH over a wide pH range (Fig. 1). The pH optimum is in the neighborhood of 5.5. The Michaelis-Menten constant, K_s, is quite low, varying from 0.5×10^{-6} to 3×10^{-6} M over the pH range measured (Fig. 1). Under a given set of conditions the velocity data fit the equation $K_s = (S)(V_{max.} -$

$V)/V$ over the entire range of substrate concentration (S). (Both $V_{max.}$ and K_S vary somewhat with the kind and amount of salt present. The data (Fig. 1) thus represent the mean values of measurements made under a variety of conditions.) The greater dependence of rate on pH at very low substrate concentrations is also indicated in Fig. 1 ($V_{dil.}$). The temperature coefficient is 1.04 per degree ($Q_{10} = 1.5$) in the range 15–45°.

Inhibitors—Several related pterines are competitive inhibitors for the enzymatic oxidation of AHP. Isoxanthopterin, the product of enzymatic oxidation of AHP, has a dissociation constant with the enzyme which is

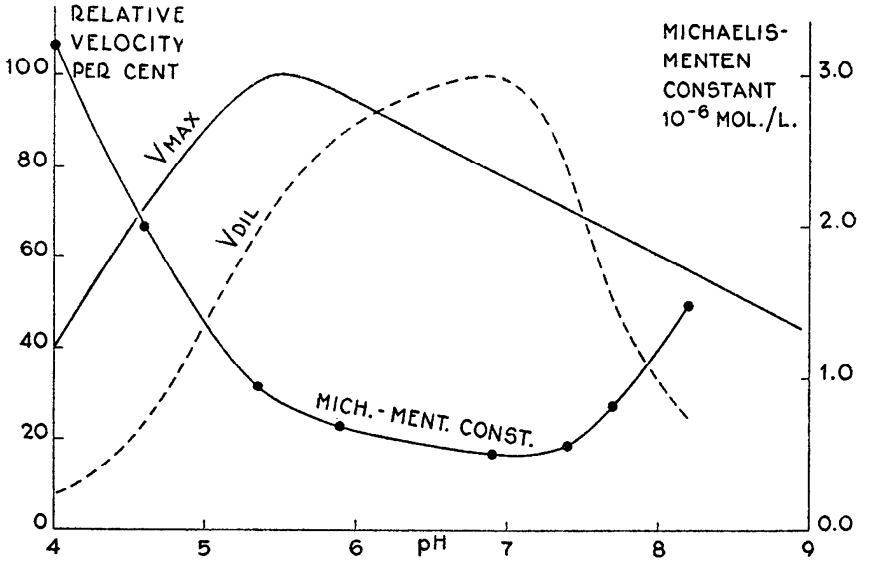

FIG. 1. The effect of pH on the velocity of enzymatic oxidation of 2-amino-4 hydroxypteridine at saturating concentrations of substrate ($V_{max.}$) and at high dilution of substrate ($V_{dil.}$), and on the Michaelis-Menten constant.

not very different from the K_S of AHP. An interesting consequence of this is that during the oxidation of AHP there is an approximation to a first order reaction not only at high dilution but also at substrate concentrations up to several times the K_S.

The most potent inhibitor found is 2-amino-4-hydroxy-6-formylpteridine (Tables II, III, IV). The inhibitory constant, K_i, is of the order of 10^{-9} M, or 1000 times smaller than the K_S for AHP. Other pterines inhibit, but to a much lesser degree, and it is necessary in each case to rule out the possibility that a trace of the aldehyde might be present. Kalckar *et al.* (10) have given evidence that the inhibition by folic acid of the enzymatic oxidation of xanthine and xanthopterin results from contamination

with a little of the aldehyde. In the present investigation it was similarly found that folic acid inhibits AHP oxidation and that this inhibition is sharply diminished if some of the fluorescent impurities are removed by extraction. Xanthine (1 γ per ml.) causes temporary inhibition of AHP oxidation, until it is itself oxidized by the enzyme. Hofstee has studied a more extensive series of inhibitors for xanthine and xanthopterin oxidation with similar findings (11). No other inhibitor has been found, however, with activity approaching that of the 6-aldehyde.

TABLE II

Inhibition of Cream Enzyme by 2-Amino-4-hydroxy-6-formylpteridine

Substrate, 2-amino-4-hydroxypteridine, 78 $\times$ 10^{-9} mole per liter. All values recorded as 10^{-9} mole per liter, except V which is moles per minute per mole of E_T. $V_{max.}$ = 62 moles per minute per mole of E_T. $K_S = 0.71 \times 10^{-6}$ mole per liter. The symbols are defined in the text.

FAD of enzyme	6-CHO (iT)	(E_T)*	V	(ES)	(E)	(Ei)	(i)	K_i
9.3	0	5.3	6.0	0.52	4.8			
9.3	1.18	5.3	5.1	0.44	4.0	0.86	0.32	(1.5)†
9.3	2.26	5.3	3.9	0.34	3.14	1.82	0.44	0.8
9.3	5.76	5.3	1.57	0.14	1.26	3.90	1.86	0.6
2.56	0	1.53	6.1	0.153	1.38			
2.56	0.62	1.53	4.3	0.107	0.97	0.45	0.17	(0.4)†
2.56	1.18	1.53	3.5	0.089	0.80	0.64	0.54	0.7
2.56	2.26	1.53	2.19	0.057	0.50	0.97	1.29	0.7
2.56	5.76	1.53	0.85	0.021	0.19	1.32	4.44	0.6

* Assuming moles of enzyme = 0.6 $\times$ moles of FAD present.

† These values are less reliable than the rest due to the very low concentration of aldehyde.

The apparent dissociation constant of the 6-aldehyde-enzyme complex is so small that partial inhibition is observed with amounts of the aldehyde which are lower, on a molar basis, than the enzyme itself. Indeed, with low substrate concentrations, each mole of the 6-aldehyde appeared to inhibit more than 1 mole of enzyme, as measured by its FAD content. For example, in one case (Table II) the concentration of enzyme FAD was 9.3 $\times$ 10^{-9} M, and the substrate (AHP) concentration was 78 $\times$ 10^{-9} M, or only sufficient to keep about 10 per cent of the enzyme combined with substrate; *i.e.*, V was equal to 10 per cent of $V_{max.}$. When the solution was made 2.26 $\times$ 10^{-9} M with respect to the 6-aldehyde inhibitor, the reaction velocity was reduced to 65 per cent of the control. That is, the 2.26 $\times$ 10^{-9} mole per liter of inhibitor had apparently inactivated 35 per cent of the enzyme or 3.3 $\times$ 10^{-9} mole per liter as measured by the FAD. Since

the inhibition is clearly competitive and reversible, the most plausible explanation would seem to be that part of the FAD was not present in active enzymatic combination. The data to follow suggest that only 60 per cent of the FAD is associated with active enzyme. (It would, however, also be reasonably consonant with the data if 2 moles of FAD were associated with each active enzyme molecule or center. Philpot (12), as a result of ultracentrifugal data on the enzyme preparation of Corran *et al.* (9), concluded that 1.4 to 3.1 moles of FAD were associated with each mole of enzyme.) The extremely low dissociation constant of the enzyme-inhibitor complex permits a virtual titration of the enzyme.

In most instances of competitive inhibition, the amount of competitor, i, combined with enzyme, E, is negligible in comparison with the total amount of inhibitor present, and hence in the mass law equation, $((E)(i))/(Ei) = K_i$, it is possible and usual to consider $(i) = (i_T)$, the concentration of total inhibitor (13, 14). In the present instance, however, a substantial proportion of inhibitor is combined with enzyme and cannot be ignored. The following equations serve to evaluate K_i, the enzyme-inhibitor dissociation constant, and the number of moles, n, of FAD (whether active or inactive) which are associated with each mole or equivalent of active enzyme.

Let (E_T), (ES), (Ei), and (E) represent respectively molar or equivalent concentration of total enzyme, enzyme combined with substrate, enzyme combined with inhibitor, and free enzyme. By definition (FAD) $= n(E_T)$, $V_{max.}$ is the velocity of enzyme activity with excess substrate and without inhibitor, and V is the observed velocity with given amounts of substrate and inhibitor. (S) is the substrate concentration. Then

$$(E_T) = (ES) + (Ei) + (E),\ i_T = i + Ei,\ \frac{(E_T)}{(ES)} = \frac{n(E_T)}{n(ES)} = \frac{V_{max.}}{V},$$

$$K_S = \frac{(E)(S)}{(ES)} = \frac{n(E)(S)}{n(ES)},\ K_i = \frac{(E)(i)}{(Ei)} = \frac{n(E)((i_T) - (Ei))}{n(Ei)}$$

$$= \frac{n(E)(i_T)}{n(Ei)} - \frac{n(E)(Ei)}{n(Ei)} = \frac{n(E)(i_T)}{n(Ei)} - (E)$$

As (E) approaches zero $(n(E)(i_T))/(n(Ei))$ will approach K_i. Therefore $n(E)$ and $(n(E)(i_T))/(n(Ei))$ were plotted against each other, with use of the data of the experiment presented in Table III. A value of 0.6×10^{-9} M was obtained for K_i, from which n was calculated to be approximately 1.7. Thus the data suggested that only about 1/1.7 or 60 per cent of the FAD in this preparation was associated with active enzyme centers. The consistency of the calculated values for K_i, except with the lowest inhibitor concentrations, suggests the validity of the above presentation. This

approach gives an upper limit for the amount of enzyme present. There might be inactive enzyme present, still capable of combining with aldehyde; in addition, the aldehyde itself is slowly oxidized by the enzyme (see below), which means the amount of aldehyde present is overestimated.

TABLE III

Activity of Cream Enzyme toward Xanthopterin and AHP; Inhibition of This Activity by 6-Aldehyde

All values recorded as 10^{-9} mole per liter, except V which is moles per minute per mole of E_T. The symbols are defined in the text.

(S)	6-CHO (i_T)	(E_T)*	V	(ES)	(E)	(Ei)	(i)	K_i
S = 2-amino-4-hydroxypteridine†								
2590	0	9.8	51	8.0	1.8			
500	0	3.7	26.5	1.57	2.13			
128	0	3.7	10.2	0.60	3.10			
51	0	3.7	4.3	0.255	3.45			
2590	20.7	9.8	9.0	1.41	0.39	8.0	12.7	0.6
2590	5.5	9.8	24.8	3.89	1.08	4.8	0.7	0.2
51	5.5	3.7	0.90	0.053	0.73	2.92	2.6	0.6
51	2.4	3.7	2.08	0.123	1.74	1.84	0.56	0.5
51	1.1	3.7	2.98	0.177	2.50	0.92	0.18	0.5
S = xanthopterin‡								
2650	0	9.8	17.6	9.2	0.6			
510	0	9.8	16.0	8.3	1.5			
132	0	3.7	7.6	1.50	2.20			
53	0	3.7	4.1	0.81	2.89			
2650	54.6	9.8	3.4	1.77	0.128	7.9	46.7	0.8
2650	20.7	9.8	6.0	3.12	0.225	6.5	14.2	0.5
53	5.5	3.7	0.77	0.15	0.55	3.0	2.5	0.5
53	2.4	3.7	2.03	0.40	1.45	1.85	0.55	0.4
53	1.1	3.7	2.90	0.57	2.07	1.06	0.04	(0.1)§

* Moles of enzyme = 0.6 × moles of FAD present.

† $V_{max.}$ = 62 moles per minute per mole of E_T. $K_S = 0.72 \times 10^{-6}$ mole per liter.

‡ $V_{max.}$ = 18.8 moles per minute per mole of E_T. $K_S = 0.19 \times 10^{-6}$.

§ This value is less reliable than the rest due to the very low concentration of the aldehyde.

Other Substrates—It is of interest to compare the oxidation of AHP with that of other substrates attacked by the cream enzyme. Therefore, with the same enzyme preparation, the oxidation of xanthine, xanthopterin, and AHP was measured in 0.1 M phosphate buffer at pH 6.8. Since xanthine is not measurably fluorescent with present instruments, it was diffi-

cult to work with sufficiently low substrate concentrations to evaluate the Michaelis-Menten constant. However, by using absorption cells with a 10 cm. light path, it was possible to measure the oxidation with 1.2×10^{-6} M substrate, which gave a submaximal rate and therefore permitted calculation of the constant.

The different dissociation constants and maximal velocities are of interest (Tables III and IV). The observed values agree with the findings of Hofstee who estimated that the dissociation constants for xanthine and xanthopterin were of the order of 10^{-6} M, and that the xanthine constant was about 10 times larger than that of xanthopterin (11). If these oxidations are all effected by the same enzyme, then a single value should be obtained for the dissociation constant of the enzyme-inhibitor complex,

TABLE IV

Activity of Cream Enzyme toward Various Substrates; Inhibition by 6-Aldehyde

S is 10^{-6} mole per liter, V is moles per minute per mole of E_T (turnover number), and all the other values are 10^{-9} mole per liter.

Substrate	(S)	6-CHO (i_T)	(E_T)*	V	(ES)	(E)	(Ei)	(i)	K_i
AHP ($K_S = 340 \times 10^{-9}$)	37.0	0	86	48	85.2	0.79			
	37.0	250	86	11.5	20.6	0.19	65	185	0.54
Xanthopterin ($K_S = 100 \times 10^{-9}$)	43.0	0	86	12.8	85.8	0.20			
	43.0	250	86	6.6	44.2	0.10	42	208	0.51
Xanthine ($K_S = 900 \times 10^{-9}$)	43.0	0	86	234	84.2	1.8			
	43.0	250	86	37.6	13.5	0.27	72	178	0.67
	1.22	0	25						
6-Aldehyde ($K_S = 0.6 \times 10^{-9}$ (from K_i above))	35		1350	0.165					

* Assuming moles of enzyme = 0.6 × moles of FAD of enzyme.

K_i. Tables III and IV indicate that within experimental limits the same dissociation constant is obtained for all three substrates. This strongly suggests that one enzyme is responsible for all three oxidations. Hofstee has concluded that xanthine and xanthopterin are oxidized by the same enzyme (11).

Both Corran *et al.* (9) and Ball and Ramsdell (15) reported that milk or cream oxidase will oxidize reduced diphosphopyridine nucleotide ($DPNH_2$). This substrate[2] was oxidized by the present enzyme preparation with a molar velocity only 3 or 4 per cent as great as that with xanthine. The oxidation of $DPNH_2$ was unaffected by concentrations of 6-aldehydepteridine which completely blocked xanthine or AHP oxidation. It therefore

[2] The $DPNH_2$ was kindly supplied by Dr. F. Edmund Hunter.

seems likely that either two separate enzymes are involved or, as suggested by Corran *et al.* (9), that two separate active centers are present in the same enzyme.

The 6-aldehyde is itself slowly oxidized. If a small amount of the aldehyde is allowed to remain with the enzyme, its inhibitory capacity is slowly destroyed, in agreement with Kalckar *et al.* (10). A total of 70 μM of the

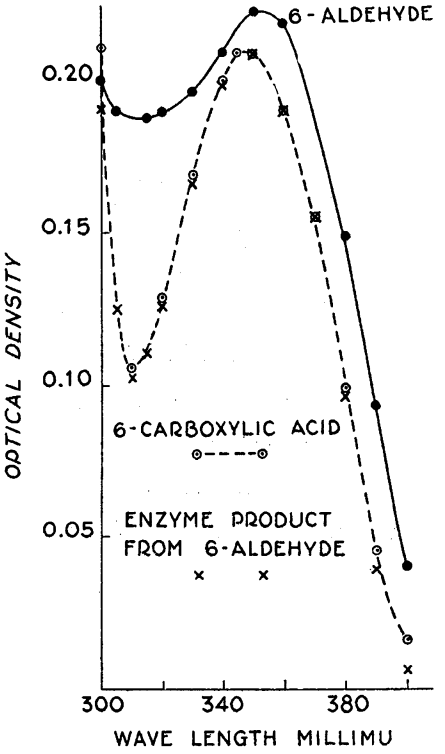

FIG. 2. The absorption spectra of 2-amino-4-hydroxy-6-formylpteridine, 2-amino-4-hydroxy-6-carboxypteridine, and the product obtained by treatment of the aldehyde with cream enzyme.

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6-aldehyde in 2 ml. of phosphate buffer of pH 6.8 was treated with an amount of the enzyme which contained 4.5 μM of FAD. The reaction was followed by measuring the decrease in optical density at 310 mμ. The reaction was complete in about 2 hours. The final optical absorption, after correction for the contribution of the enzyme, was identical with that of 2-amino-4-hydroxy-6-carboxylic acid (Fig. 2), proving that oxidation of the aldehyde group had occurred. The rate of oxidation of the aldehyde was a thousand times slower than that of xanthine (Table

IV). The turnover number was calculated to be 0.16; *i.e.*, an average of 6 minutes would be required for 1 molecule of enzyme to oxidize 1 molecule of this substrate. It was found that, when a molar excess of the 6-aldehyde was mixed with the enzyme for a few minutes and AHP (or other substrate) was then added, the initial velocity of AHP oxidation was almost zero but increased during a 2 or 3 minute period to a steady rate determined by the amount of inhibitor and substrate. On the other hand, when AHP and the 6-aldehyde were mixed together, and the enzyme was added last, the initial rate was not discernibly inhibited, but the velocity fell during the first 2 or 3 minutes to the same steady rate observed when the AHP was the last addition. The delay in the development of inhibition is presumably due to the slowness of the reaction enzyme + inhibitor → enzyme-inhibitor when they are both present in such high dilution. The delay in "deinhibition" resulting from the addition of the competing substrate AHP indicates that *both* the following reactions are slow: enzyme-inhibitor → enzyme + inhibitor, and enzyme-inhibitor → enzyme + oxidized inhibitor. Thus the turnover time of the last reaction must be several minutes at least in support of the directly observed rate of conversion of the 6-aldehyde to the 6-carboxyl compound. The opportunity presented for direct study of these reactions would seem to merit more thorough investigation.

DISCUSSION

The existence of an inhibitor of purine oxidation, which is active in such low concentration as 10^{-9} M, may not be without biological consequences. If the 6-aldehyde were to be released slowly from PGA in the tissues, it might tend to preserve hypoxanthine or adenine from oxidation. PGA is known to reduce or eliminate the adenine requirement of certain microorganisms. Keith *et al.* have indeed shown that the xanthine oxidase activity of chick liver is increased 3-fold when the chicks are made deficient in PGA (16).

It is rather remarkable that the dissociation constants for xanthine, AHP, xanthopterin, and the 6-aldehyde (900, 340, 100, and 0.6×10^{-9} M) are roughly proportional to the calculated turnover numbers (238, 48, 13, and 0.16, respectively). This may be fortuitous; however, the apparent dissociation constants actually describe the situation,

$$E + S \underset{(2)}{\overset{(1)}{\rightleftharpoons}} ES \xrightarrow{(3)} E + \text{product}$$

in which monomolecular reactions (2) and (3) are inseparable unless the reactions (1) are known (17). If reactions (2) are slow compared to reactions (3) (Case VI of Lineweaver and Burk (14)), then the apparent

dissociation constants would indeed parallel the velocity of oxidation. One consequence of the above parallelism is that in spite of a 1000-fold range of oxidation velocity between these four substrates, all four substances would be oxidized at about the same rate when present at concentrations of 10^{-9} M or less.

SUMMARY

1. 2-Amino-4-hydroxy-6-formylpteridine is a powerful inhibitor of the enzyme in cream which oxidizes xanthine, xanthopterin, and 2-amino-4-hydroxypteridine. The inhibitory constant appears to be the same relative to all three oxidations. It is approximately 0.6×10^{-9} M. Thus with low substrate and low enzyme concentrations, 10^{-4} γ per ml. will produce appreciable inhibition.

2. Because the amount of aldehyde inhibitor in actual combination with the enzyme is an appreciable fraction of the total added, it is possible to measure the combining proportions of enzyme and inhibitor and hence estimate the number of moles or equivalents of enzyme present. The amount of enzyme combining with 1 mole of aldehyde inhibitor contained not quite 2 moles of flavin-adenine dinucleotide. This might mean that about half of the flavin was inactive, or that 2 moles of flavin coenzyme were associated with each active center.

3. The inhibitor itself was slowly oxidized to 2-amino-4-hydroxy-6-carboxylic acid. The turnover number for this reaction was about 0.16, compared to turnover numbers of 234, 48, and 13 observed for xanthine, 2-amino-4-hydroxypteridine, and xanthopterin, respectively, as substrates. The slow turnover number for the aldehyde was confirmed by the lag period of several minutes for "deinhibition" when substrate was added to the enzyme after the inhibitor.

4. The oxidation of reduced diphosphopyridine nucleotide by the enzyme from cream was not inhibited by the 6-aldehyde, and hence another active group or another enzyme is probably responsible for its oxidation.

BIBLIOGRAPHY

1. Lowry, O. H., Bessey, O. A., and Crawford, E. J., *J. Biol. Chem.*, **180**, 389 (1949).
2. Kalckar, H. M., *J. Biol. Chem.*, **167**, 429 (1947).
3. Kalckar, H. M., and Klenow, H., *J. Biol. Chem.*, **172**, 349 (1948).
4. Lowry, O. H., *J. Biol. Chem.*, **173**, 677 (1948).
5. Bessey, O. A., Lowry, O. H., and Love, R. H., *J. Biol. Chem.*, in press.
6. Kornberg, A., *J. Biol. Chem.*, **174**, 1051 (1948).
7. Ball, E. G., *J. Biol. Chem.*, **128**, 51 (1939).
8. Kalckar, H. M., *J. Biol. Chem.*, **167**, 461 (1947).
9. Corran, H. S., Dewan, J. G., Gordon, A. H., and Green, D. E., *Biochem. J.*, **33**, 1694 (1939).

10. Kalckar, H. M., Kjeldgaard, N. O., and Klenow, H., *J. Biol. Chem.*, **174**, 771 (1948).
11. Hofstee, B. H. J., *J. Biol. Chem.*, **179**, 633 (1949).
12. Philpot, J. S. L., *Biochem. J.*, **33**, 1707 (1939).
13. Michaelis, L., and Menten, M. L., *Biochem. Z.*, **49**, 333 (1913).
14. Lineweaver, H., and Burk, D., *J. Am. Chem. Soc.*, **56**, 658 (1934).
15. Ball, E. G., and Ramsdell, P. A., *J. Biol. Chem.*, **131**, 767 (1939).
16. Keith, C. K., Broach, W. J., Warren, D., Day, P. L., and Totter, J. R., *J. Biol. Chem.*, **176**, 1095 (1948).
17. Briggs, G. E., and Haldane, J. B. S., *Biochem. J.*, **19**, 338 (1925).