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ON THE MECHANISM OF IODINATION OF TYROSINE

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SUMMARY: Existing data contain proof that the iodinating species of tyrosine and its derivatives contained in mixtures of iodine and iodide is hypoiodous acid, HOI. It appears likely that the peroxidase-catalyzed iodination reaction with hydrogen peroxide, tyrosine or a tyrosine derivative and either iodide or iodine as substrates involves enzyme-activated HOI.

Mayberry, et al. recognized I_2, H_2OI^+ and HOI as the potential iodinating species in their superbly accurate kinetic study of the iodination of tyrosine derivatives (1). The purpose of the present communication is two-fold: first to point out that of these three possibilities only HOI is compatible with their experimental data; and second to discuss evidence which points to peroxidase-activated HOI as the iodinating species in the enzyme-catalyzed tyrosine iodination reaction.

MATERIALS AND METHODS

All materials and experimental procedures have been described in detail (2) and will be made available in more accessible form in a forthcoming publication.

RESULTS AND DISCUSSION

The uncatalyzed iodination reaction obeys accurately the second order rate equation based upon the stoichiometry

$$HTOH_{(total)} + I_{2(total)} \xrightarrow{k_{app}} H^+ + I^- + ITOH_{(total)} \qquad (1)$$

where the H in HTOH refers to a hydrogen in the position ortho to the phenolic group; $HTOH_{(total)}$ means the sum of all tyrosine species whether or not the phenolic OH group is ionized and $I_{2(total)}$ means $I_2 + I_3^-$ (1). The relevant equilibria involving iodine species are

$$I_3^- \underset{}{\overset{K_2}{\rightleftharpoons}} I_2 + I^- \qquad (2)$$

$$I_2 + H_2O \underset{}{\overset{K_3}{\rightleftharpoons}} H_2OI^+ + I^- \qquad (3)$$

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$$H_2OI^+ + H_2O \underset{}{\overset{K_4}{\rightleftharpoons}} HOI + H_3O^+ \quad (4)$$

where $K_2 = 1.3 \times 10^{-3}$ M (3), $K_3 = 10^{-11}$ M (4) and $K_4 = 4 \times 10^{-2}$ M (5,6). Iodide inhibits reaction 1 approximately to the second power and H^+ inhibits to the first power (1). The ionization of the phenolic group of the tyrosine derivative may be represented as

$$HTOH \underset{}{\overset{K_5}{\rightleftharpoons}} HTO^- + H^+ \quad (5)$$

With a slow reaction 1 it may safely be assumed that equilibria are maintained in Eqs. 2-5 (6). Thus in Eq. 2, in principle either I_2 or I_3^- might be the iodinating species but the total pool of $I_2 + I_3^-$ would be depleted with the equilibrium maintained. If I_2 is the iodinating reagent, then

$$HTO^- + I_2 \xrightarrow{k_6} H^+ + I^- + ITO^- \quad (6)$$

which at pH values where $(H^+) > K_5$ leads to

$$k_{app} = \frac{k_6 K_5}{(H^+)\left[1 + \frac{(I^-)}{K_2}\right]} \quad (7)$$

Eq. 7 explains proton inhibition to the first power but not iodide inhibition to the second power. Therefore I_2 (and I_3^-) are eliminated as the iodinating species. If either H_2OI^+ or HOI is active then the inhibition by H^+ requires that the mechanism be either of

$$HTO^- + H_2OI^+ \xrightarrow{k_8} ITO^- + H_3O^+ \quad (8)$$

$$HTOH + HOI \xrightarrow{k_9} ITOH + H_2O \quad (9)$$

which leads to either of

$$k_{app} = \frac{k_8 K_3 K_5}{(H^+)(I^-)\left[1 + \frac{(I^-)}{K_2}\right]} \quad (10)$$

$$k_{app} = \frac{k_9 K_3 K_4}{(H^+)(I^-)\left[1 + \frac{(I^-)}{K_2}\right]} \quad (11)$$

Both Eqs. 10 and 11 appear to explain the observed kinetics. However, a choice can be made based upon the kinetic exclusion principle: if a mechanism leads to a rate constant which exceeds the diffusion-controlled limit then that mechanism is eliminated (7). For N-acetyl tyrosine (1) at pH 7.20, ionic strength 0.64 and 25.00°C, $k_{app} = 31 \pm 1\ M^{-1}\ s^{-1}$ and $(I^-) = 0.015$ M.

Therefore $k_8 = 3.5 \times 10^{14}\ M^{-1}\ s^{-1}$ which exceeds the diffusion-controlled limit by a factor of 10^4. On the other hand, $k_9 = 9 \times 10^5\ M^{-1}\ s^{-1}$ which is physically possible and chemically reasonable. It would appear that the uncatalyzed iodination reaction occurs between a tyrosine derivative with an unionized phenolic group and HOI.

Three possibilities deserve consideration for the enzyme catalyzed reaction in which both peroxidase and hydrogen peroxide are present as initial reactants.

I. Molecular iodine is activated in the enzymatic reaction. If this were so one might expect evidence for an enzyme-iodine complex. Our results show that an optical spectrum attributed to such a complex (8) is merely the sum of the spectra of the enzyme and molecular iodine.

II. Tyrosine is first oxidized to a free radical which is then iodinated. We shall present evidence in a forthcoming publication which eliminates this possibility.

III. Enzyme-activated HOI is the iodinating species. In experiments in which the reaction mixture contains lactoperoxidase or horseradish peroxidase, H_2O_2, I^- and tyrosine the reaction sequence $I^- \rightarrow I_3^- \rightarrow I_2 \rightarrow$ monoiodotyrosine can be followed at low pH (2,9). It is known that iodide reacts with compound I by direct two-electron transfer (10), which probably indicates oxygen atom transfer. Therefore, the reactions

$$\text{Peroxidase} + H_2O_2 \longrightarrow \text{Compound I} + H_2O \qquad (12)$$

$$\text{Compound I} + I^- \longrightarrow \text{Peroxidase OI} \qquad (13)$$

$$\text{Peroxidase}\bullet\text{OI} + I^- \xrightarrow{\text{fast}} \text{Peroxidase} + I_2 + OH^- \qquad (14)$$

$$I_2 + I^- \underset{}{\overset{\text{fast}}{\rightleftharpoons}} I_3^- \qquad (15)$$

account for formation of the first product, I_3^-. In the presence of excess I^-, all molecular I_2 is complexed as I_3^-. However, reaction 13 continues to remove I^-, so that the equilibrium 15 is shifted to the left, producing the second observed product, molecular I_2. With little iodide left in the system then Eq. 14 becomes unimportant and the iodination of tyrosine is controlled by the hydrolysis

$$I_2 + H_2O \underset{}{\overset{K_{16}}{\rightleftharpoons}} HOI + H^+ + I^- \qquad (16)$$

Eq. 16 is a combination of Eqs. 3 and 4 so that $K_{16} = 4 \times 10^{-13}$ M^2 (5). The actual iodination step would follow reaction 13

$$\text{Peroxidase•OI + tyrosine} \longrightarrow \text{Peroxidase + monoiodotyrosine + } H_2O \qquad (17)$$

For any given pH and (I_2), if the equilibrium is maintained in Eq. 16, then (HOI) and (I^-) can be calculated. Figure 6 of reference (9) is relevant. At pH 3.6, $(I_2) = 1.5 \times 10^{-4}$ M so that (HOI) = $(I^-) = 5 \times 10^{-7}$ M. When the blank rate of disappearance of I_2 is subtracted, then the horseradish peroxidase catalyzed rate of disappearance of I_2 is 7×10^{-7} M s^{-1} when $(HRP)_o = 10^{-6}$ M. The above calculation can be checked using transient state kinetic results. With excess tyrosine present the rate-controlling step in the iodination reaction is Eq. 13 which has a rate constant of 10^6 M^{-1} s^{-1} for horseradish peroxidase at pH 3.6 (10). Therefore, the rate of iodide oxidation for the above set of conditions is equal to the rate of monoiodotyrosine formation and is given by

$$v = -\frac{d(I^-)}{dt} = k_{13}(HRP)_o(I^-)$$
$$= 10^6 \times 10^{-6} \times 5 \times 10^{-7}$$
$$= 5 \times 10^{-7} \text{ M s}^{-1}$$

in excellent agreement with the steady state calculation. Our steady state measurements are also in agreement.

The rate of iodide oxidation by compound I of lactoperoxidase is not known. However, from Figure 6, reference (9), one can calculate that for $(LP)_o = 10^{-8}$ M the rate of disappearance of I_2 is 4.3×10^{-6} M s^{-1}. Thus,

$$v = k_{13}(LP)_o(I^-)$$
$$k_{13} = \frac{v}{(LP)_o(I^-)} = \frac{4.3 \times 10^{-6}}{10^{-8} \times 5 \times 10^{-7}}$$
$$= 9 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$$

Our conclusions are confined to water-soluble peroxidases. The conclusion that iodine is an obligate intermediate (11) is correct in the presence of excess iodide. In the presence of only a small amount of iodide it appears that iodination can occur directly through Eqs. 13 and 17.

REFERENCES

1. Mayberry, W.E., Rall, J.E. and Bertoli, D. (1964), J. Am. Chem. Soc. 86, 5302-5307.
2. Ralston, I.M. (1980) Ph.D. Thesis, Chapter IV, University of Alberta, Edmonton, Canada.
3. Ramette, R.W. and Sandford, R.W. (1965) J. Am. Chem. Soc. 81, 5001-5005.
4. Bell, R.P. and Gelles, E. (1951) J. Chem. Soc. 2734-2740.
5. Allen, T.L. and Keefer, R.M. (1955) J. Am. Chem. Soc. 77, 2957-2960.
6. Eigen, M. and Kustin, K. (1962) J. Am. Chem. Soc. 84, 1355-1361.
7. Dunford, H.B. (1974) J. Theor. Biol. 46, 467-479.
8. Björkstén, F. (1970) Biochim. Biophys. Acta 212, 396-406.
9. Bayse, G.S. and Morrison, M. (1971) Arch. Biochem. Biophys. 145, 143-148.
10. Roman, R. and Dunford, H.B. (1972) Biochemistry 11, 2076-2082.
11. Thomas, J.A. and Hager, L.P. (1969) Biochem. Biophys. Res. Commun. 35, 444-450.