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Os(VIII)-catalyzed and uncatalyzed oxidation of biotin by chloramine-T in alkaline medium: Comparative mechanistic aspects and kinetic modeling

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Abstract

Sodium *N*-chloro-*p*-toluenesulfonamide (chloramine-T or CAT) is the prominent member of aromatic sulfonyl haloamines and has received considerable attention as a mild oxidant for several organic compounds. D-Biotin, a member of the B-vitamin family, is an essential nutrient in human nutrition as is commonly referred to as Vitamin H. Controlled oxidation of biotin to biotin sulfoxide forms a very important synthetic route in biochemical reactions. Optimum conditions have been developed for the oxidation of biotin to biotin sulfoxide. Literature survey has revealed that no attention has been paid towards the controlled oxidation of biotin to biotin sulfoxide by *N*-haloamines from the kinetic and mechanistic view points. This prompted us to undertake the title investigation. The kinetics of Os(VIII) catalyzed and uncatalyzed oxidation of biotin by CAT have been studied in NaOH medium at 303 K under identical experimental conditions. The stoichiometry (1:1) and the oxidation product (biotin sulfoxide) are the same for Os(VIII) catalyzed and uncatalyzed reactions. Biotin sulfoxide was confirmed by GC–MS analysis. In Os(VIII) catalyzed oxidation, the rate law is $-\mathrm{d[CAT]/dt} = k\mathrm{[CAT][Os(VIII)]/[NaOH]}$ but it takes the form $-\mathrm{d[CAT]/dt} = k\mathrm{[CAT][Biotin]}^{x}\mathrm{/[NaOH]}^{-y}$ for uncatalyzed reaction, where x and y are less than unity. The reaction was subjected to changes in: (a) concentration of added reduction product of CAT, *p*-toluenesulfonamide, (b) ionic strength, (c) dielectric permittivity and (d) halide ions effect in both the cases. Proton inventory studies made in a mixture of H_2O–D_2O indicated the participation of OH^- ion in the formation of transition state. The reaction fails to initiate polymerization of acrylonitrile. Activation parameters for the composite reaction were deduced from Arrhenius plots. In case of uncatalyzed reactions, the reaction constants involved in the proposed scheme were deduced. Under the identical set of experimental conditions, the kinetics of Os(VIII) catalyzed oxidation of biotin by CAT in alkaline medium has been compared with uncatalyzed reactions, revealing that the catalyzed reactions are eight fold faster than the uncatalyzed reactions. Hence, Os(VIII) acts as an efficient catalyst for the oxidation of biotin by CAT in alkaline medium. The catalytic constant (K_C) has been calculated at different temperatures and the values of activation parameters with respect to catalyst have been evaluated from the plots of $\log K_C$ versus $1/T$. Some spectroscopic evidence for the formation of 1:1 complex between oxidant and Os(VIII) has been obtained. $CH_3C_6H_4SO_2NHCl$ has been postulated as the reactive oxidizing species in both the cases. The observed experimental results have been explained by plausible mechanisms and the related rate laws have been deduced for both catalyzed and uncatalyzed reactions.

Keywords: Os(VIII) catalysis; Oxidation kinetics; Chloramine-T; Biotin

1. Introduction

The chemistry of organic sulfonyl haloamines has evoked considerable interest, as they are sources of haloanium cations, hypohalite species and *N*-anions which act both as nucleophiles and electrophiles [1]. The prominent member of this class of compounds, sodium *N*-chloro-4-methyl benzenesulfonamide or commonly called chloramine-T (p-$CH_3C_6H_4SO_2NClNa.3H_2O$ and abbreviated as CAT) is a byproduct of saccharin manufacture. Although the oxidation kinetics of several organic substrates with CAT has been extensively studied [1–8], a review of literature shows that a very little information is available on the oxidation kinetics of vitamins by CAT.

Biotin (2′-keto-3, 4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid), a member of the B-vitamin family, is an essential nutrient in human nutrition. It is commonly referred to as Vitamin H and present in yeast cells and liver [9]. Biotin is widely distributed in many foods, it plays a role in gene expression and

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in DNA replication and also in many carboxylation reactions. As a matter of fact, biotin is the essential coenzyme for carboxylating agent [9]. Biotin free and bound biotin are readily oxidized by oxidizing agents to form various oxy derivatives [10–12]. Biotin has been oxidized to biotin sulfone by H_2O_2 [11]. Exhaustive literature survey revealed that, no other reports on the oxidation of biotin by any other oxidants. Also none has examined the role of transition metal ions as catalysts in the oxidation of this substrate. Such studies are expected to highlight the interaction and reactivity of transition metals in this redox system. Therefore, we felt it would be worthwhile to investigate the oxidative behaviour of CAT and catalytic activity of Os(VIII) towards biotin substrate to explore the kinetic and mechanistic aspects of this redox reaction. Consequently the present work has been undertaken.

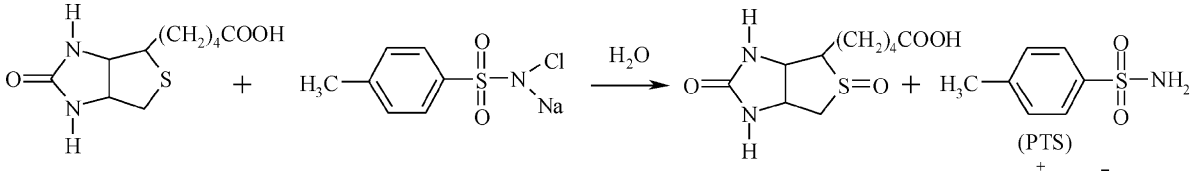
Transition metal ions have been extensively used as catalysts for affecting a variety of reactions [13–15]. In recent times, the studies on the use of transition metal ions either alone or as binary mixtures as catalysts in many redox reactions, have been gaining interest. Their oxidizing and catalytic activities are due to the existence of variable oxidation states, as a consequence of partly filled d or f orbitals. Osmium tetroxide (Os(VIII)) has been widely used as a homogeneous catalyst in various redox reactions particularly in alkaline medium [16–20]. The mechanism of catalysis is quite complicated due to the formation of different intermediate complexes, free radicals and different oxidizing states of Os(VIII). Although many complexes of Os(VIII) with various organic and inorganic substances have been reported, a literature survey shows a very few kinetic investigations on the oxidation reactions of biomolecules in general and on vitamins in particular involving Os(VIII) as a homogeneous catalyst.

The reactions of biotin with CAT in NaOH medium become facile in the presence of micro-quantity of Os(VIII) catalyst. In the present communication, we report for the first time the results of the detailed investigations on the kinetic and mechanistic aspects of Os(VIII) catalyzed and uncatalyzed oxidation of biotin by CAT in NaOH medium at 303 K. Objectives of the present study are to: (i) develop optimum conditions for the selective conversion of biotin to biotin sulfoxide, (ii) elucidate plausible mechanisms, (iii) deduce appropriate rate laws, (iv) identify the stoichiometry and products, (v) ascertain the various reactive species, (vi) find the catalytic efficiency of Os(VIII), (vii) determine the complex formation and (viii) compare the kinetic patterns and reactivity of Os(VIII) catalyzed and uncatalyzed oxidation.

2. Results and discussion

2.1. Stoichiometry

Reaction mixtures containing varying ratios of CAT to biotin in the presence of 1.4×10^{-3} mol dm^{-3} NaOH and 1.0×10^{-6} Os(VIII) (in case of catalyzed reaction) were equilibrated at 303 K for 24 h. Determination of unreacted CAT in reaction mixture showed that one mole of biotin consumed one mole of CAT for both Os(VIII) catalyzed and uncatalyzed reactions, confirming the following stoichiometry:



(1)

2.2. Product analysis

The reactions under stirred condition were allowed to progress for 4 h at 303 K in round bottomed flasks. After completion of the reaction, the reaction products were neutralized with HCl and subjected to spot tests and chromatographic analysis (TLC technique), which revealed the formation of biotin sulfoxide and *p*-toluenesulfonamide as the oxidation product and reduction product of biotin and CAT, respectively. The reduction product of CAT, *p*-toluenesulfonamide (PTS; $TsNH_2$), was extracted with ethyl acetate and was detected [21] by thin layer chromatography using petroleum ether:chloroform:1-butanol (2:2:1, v/v/v) as the solvent system and iodine as the spray reagent ($R_f = 0.905$). Further it was confirmed by its melting point 138 °C (lit. mp 137–140 °C). The oxidation product of biotin, biotin sulfoxide has been separated by lyophilization technique and recrystalized from acetic acid–ethanol mixture. These two products were further confirmed by GC–MS analysis. The GC–MS data was obtained on a 17A Shimadzu gas chromatograph with a QP-5050 Shimadzu mass spectrometer. The mass spectrum was obtained using the electron impact ionization technique. The mass spectrum showed a molecular ion peak at 171 and 260 amu clearly confirming *p*-toluenesulfonamide and biotin sulfoxide, respectively (Figs. 1 and 2). Other peaks observed in mass spectra can be interpreted in accordance with the observed structures. It was also observed that there was no further oxidation of biotin sulfoxide under present kinetic conditions.

The kinetics of oxidation of Os(VIII) catalyzed and uncatalyzed oxidation of biotin by CAT was investigated at several initial concentrations of the reactants in NaOH medium at 303 K.

2.3. Kinetics of uncatalyzed and Os(VIII) catalyzed oxidation of biotin

At constant $[Biotin]_0$, [NaOH] and temperature, where $[Biotin]_0 \gg [CAT]_0$, plots of log[CAT] versus time were linear ($r > 0.9918$), indicating a first-order dependence of rate on

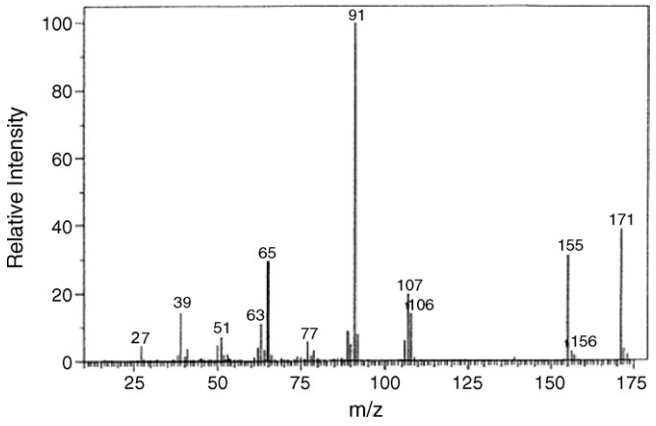


Fig. 1. GC–mass spectrum of *p*-toluenesulfonamide with its molecular ion peak at 171 amu.

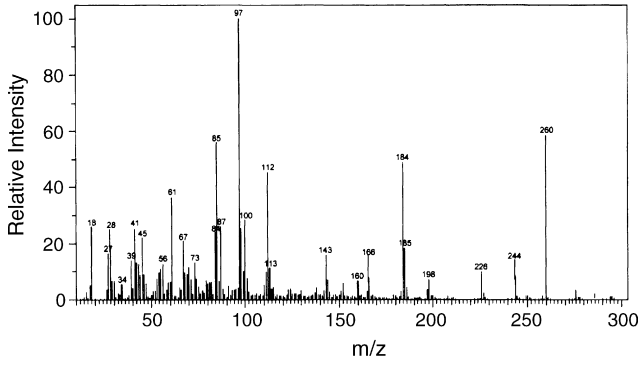


Fig. 2. GC–mass spectrum of biotin sulfoxide with its molecular ion peak at 260 amu.

$[CAT]_0$ for both Os(VIII) catalyzed and uncatlyzed reactions. The pseudo first-order rate constants, k', obtained are listed in Tables 1 and 6. Further, the values of k' are unaltered with variation of $[CAT]_0$, confirming first-order dependence on $[CAT]_0$ in both the cases. The rate increased with increase in $[Biotin]_0$ (Table 1) for uncatalyzed reaction and a plot of $\log k'$ versus log[Biotin] was linear ($r = 0.9961$) with a slope of 0.66, indicating a fractional-order dependence on $[Biotin]_0$. Further, a plot of k' versus [Biotin] was also linear ($r = 0.9902$) with a y-intercept, confirming the fractional-order dependence on $[Biotin]_0$. But the order in $[Biotin]_0$ was found to be zero in case of Os(VIII) catalyzed reactions (Table 6). The rate of the reaction decreased with increase in [NaOH] (Table 1 and Table 6) in both the cases. The log–log plots of rates versus [NaOH] ($r > 0.9902$), showed inverse fractional-order dependence for uncatalyzed reaction (Table 1) and inverse first-order dependence in case of Os(VIII) catalysis (Table 6). The rate was increased with increase in [Os(VIII)] (Table 6) and a log–log plot yields a unit slope, indicating a first-order dependence of reaction rate on [Os(VIII)].

Table 2
Effect of varying ionic strength (I) of the medium on the rate of reaction

[I] (mol dm^{-3})	k' ($\times 10^4$ s^{-1})	
	Uncatalyzed[a]	Os(VIII) catalyzed[b]
0.10	3.40	20.1
0.20	2.90	19.9
0.30	2.41	20.0
0.40	2.00	20.3
0.50	1.75	20.2

[a] $[CAT]_0 = 2.00 \times 10^{-4}$ mol dm^{-3}; $[Biotin]_0 = 2.00 \times 10^{-3}$ mol dm^{-3}; $[NaOH] = 1.40 \times 10^{-3}$ mol dm^{-3}; $T = 303$ K.
[b] $[CAT]_0 = 2.00 \times 10^{-4}$ mol dm^{-3}; $[Biotin]_0 = 2.00 \times 10^{-3}$ mol dm^{-3}; $[NaOH] = 1.40 \times 10^{-3}$ mol dm^{-3}; $[OsO_4] = 1.00 \times 10^{-6}$ mol dm^{-3}; $T = 303$ K.

The effect of ionic strength (I) of the medium on the rate was carried out in a range of 0.10 to 0.50 mol dm^{-3} using $NaClO_4$ solution, keeping the other experimental conditions constant. The rate was found to decrease with increase in ionic strength of the medium (I; Table 2) and a plot of $\log k'$ versus $I^{1/2}$ gave a straight line ($r = 0.9999$) with a negative slope of 0.65 for uncatalyzed reactions. But variation of ionic strength showed negligible effect on the rate in Os(VIII) catalysis. Hence, only in case of uncatalyzed reactions, the ionic strength of the medium was maintained at high concentration of 0.30 mol dm^{-3} of $NaClO_4$ for kinetic runs in order to swamp the reaction. The effect of dielectric constant (D) of the solvent medium of the reaction

Table 1
Effect of varying oxidant, substrate and alkali concentrations on the rate of uncatalyzed reaction

$[CAT]_0$ ($\times 10^4$ mol dm^{-3})	$[Biotin]_0$ ($\times 10^3$ mol dm^{-3})	[NaOH] ($\times 10^3$ mol dm^{-3})	k' ($\times 10^4$ s^{-1})
0.60	2.00	1.40	2.50
1.00	2.00	1.40	2.35
2.00	2.00	1.40	2.41
4.00	2.00	1.40	2.46
6.00	2.00	1.40	2.39
2.00	0.60	1.40	1.00
2.00	1.00	1.40	1.46
2.00	2.00	1.40	2.41
2.00	4.00	1.40	4.00
2.00	6.00	1.40	6.01
2.00	2.00	0.40	5.14
2.00	2.00	0.70	3.55
2.00	2.00	1.40	2.41
2.00	2.00	3.00	1.42
2.00	2.00	5.00	0.98

$[I] = 0.30$ mol dm^{-3}; $T = 303$ K.

Table 3
Effect of varying dielectric constant (D) of the medium on the rate of reaction

MeOH (%, v/v)	D	k' ($\times 10^4$ s^{-1})	
		Uncatalyzed[a]	Os(VIII) catalyzed[b]
0	76.73	2.41	20.0
10	72.37	3.21	14.2
20	67.48	4.49	10.0
30	62.71	7.12	5.72

[a] $[CAT]_0 = 2.00 \times 10^{-4}$ mol dm^{-3}; $[Biotin]_0 = 2.00 \times 10^{-3}$ mol dm^{-3}; $[NaOH] = 1.40 \times 10^{-3}$ mol dm^{-3}; $[I] = 0.30$ mol dm^{-3}; $T = 303$ K.
[b] $[CAT]_0 = 2.00 \times 10^{-4}$ mol dm^{-3}; $[Biotin]_0 = 2.00 \times 10^{-3}$ mol dm^{-3}; $[NaOH] = 1.40 \times 10^{-3}$ mol dm^{-3}; $[OsO_4] = 1.00 \times 10^{-6}$ mol dm^{-3}; $T = 303$ K.

mixture was studied by varying the percentage of methanol (0–30% v/v). The rates were found to increase with increase in methanol content (Table 3) in case of uncatalyzed reaction and a plot of $\log k'$ versus $1/D$ was linear ($r = 0.9912$) with a positive slope. But in case of Os(VIII) catalysis, the slope of such a plot ($r = 0.9916$) was negative (Table 3). The values of D for MeOH-H_2O mixtures reported in the literature [22] were employed. Blank experiments with methanol indicated that the oxidation of methanol under the present experimental conditions was negligible.

The reactions were studied at different temperatures (293–313 K), keeping other experimental conditions constant. From the Arrhenius plots of $\log k'$ versus $1/T$ ($r > 0.9990$), values of activation parameters (E_a, $\Delta H^{\neq}$, $\Delta S^{\neq}$, $\Delta G^{\neq}$ and $\log A$) for the overall reaction were evaluated in each case. These results are summarized in Table 4. The solvent isotope effect $k'(H_2O)/k'(D_2O)$ was found to be 1.67 and 1.64 for uncatalyzed and Os(VIII) catalyzed reactions respectively. Proton inventory studies were performed by carrying out the reaction in H_2O–D_2O mixtures in both the cases and the results are given in Table 5. The rate remained constant with the addition of the reduction product of CAT, *p*-toluenesulfonamide ($TsNH_2$; 4.0×10^{-4} to 8.0×10^{-4} mol dm^{-3}) and halide ions (NaCl or NaBr; 2.0×10^{-2} to 8.0×10^{-2} mol dm^{-3}) in both the cases. Uncatalyzed and Os(VIII) catalyzed mixtures could not initiate polymerization in acrylamide solution, demonstrating the absence of free radical species in the reactions.

Table 4
Temperature dependence and activation parameters for uncatalyzed and Os(VIII) catalyzed oxidation of biotin by CAT in alkaline medium

Temperature (K)	$10^4\,k'$ (s^{-1})		$K_C \times 10^3$
	uncatalyzed[a]	Os(VIII) catalyzed[b]	
293	0.95	11.2	1.00
298	1.78	15.9	1.41
303	2.41	20.0	1.79
308	3.56	28.1	2.45
313	5.26	38.1	3.28
E_a kJ mol^{-1}	70.0	41.8	37.6
$\Delta H^{\neq}$ kJ mol^{-1}	66.9 ± 0.61	38.9 ± 0.72	35.2 ± 0.90
$\Delta G^{\neq}$ kJ mol^{-1}	95.0 ± 0.09	89.9 ± 0.19	36.9 ± 0.26
$\Delta S^{\neq}$ J K^{-1} mol^{-1}	−92.0 ± 0.69	−167 ± 0.21	−66.9 ± 0.54
$\log A$	8.40 ± 0.10	4.51 ± 0.26	9.74 ± 0.71

Also values of catalytic constants (K_C) at different temperatures and activation parameters calculated from K_C values.
[a] $[CAT]_0 = 2.00 \times 10^{-4}$ mol dm^{-3}; $[Biotin]_0 = 2.00 \times 10^{-3}$ mol dm^{-3}; $[NaOH] = 1.40 \times 10^{-3}$ mol dm^{-3}; $[I] = 0.30$ mol dm^{-3}.
[b] $[CAT]_0 = 2.00 \times 10^{-4}$ mol dm^{-3}; $[Biotin]_0 = 2.00 \times 10^{-3}$ mol dm^{-3}; $[NaOH] = 1.40 \times 10^{-3}$ mol dm^{-3}; $[OsO_4] = 1.00 \times 10^{-6}$ mol dm^{-3}.

Table 5
Proton inventory studies for the oxidation of biotin in H_2O–D_2O mixtures

Atom fraction of deuterium (n)	k'_n($\times 10^4$ s^{-1})	
	Uncatalyzed[a]	Os(VIII)catalyzed[b]
0.000	2.41	20.0
0.248	2.13	17.9
0.496	1.78	16.3
0.744	1.51	14.1
0.992	1.35	12.2

[a] $[CAT]_0 = 2.00 \times 10^{-4}$ mol dm^{-3}; $[Biotin]_0 = 2.00 \times 10^{-3}$ mol dm^{-3}; $[NaOH] = 1.40 \times 10^{-3}$ mol dm^{-3}; $[I] = 0.30$ mol dm^{-3}; $T = 303$ K.
[b] $[CAT]_0 = 2.00 \times 10^{-4}$ mol dm^{-3}; $[Biotin]_0 = 2.00 \times 10^{-3}$ mol dm^{-3}; $[NaOH] = 1.40 \times 10^{-3}$ mol dm^{-3}; $[OsO_4] = 1.00 \times 10^{-6}$ mol dm^{-3}; $T = 303$ K.

Chloramine-T behaves as an oxidizing agent in both acidic and alkaline media [1–4]. Chloramine-T is a strong electrolyte and depending upon the pH of the medium, it furnishes different types of reactive species [23–26]. The possible oxidizing species in acidified CAT solutions are the conjugate acid TsNHCl, the dichloramine $TsNCl_2$, the hypchlorous acid HOCl and possibly the protonated hypochlorous acid H_2O^+Cl. In alkaline solutions, $TsNCl_2$ does not exist, and the predominant species are $TsNCl^-$, OCl^-, TsNHCl and HOCl. Bishop and Jennings [23] have calculated the concentrations of various species of CAT present at different pH. Under the present experimental conditions, the concentration of OCl^- ion is small and it does not make any significant contribution to the oxidation of biotin. Hence, at the pH employed in the present studies, the dominant oxidizing species are TsNHCl, $TsNCl^-$ and HOCl.

2.4. Mechanism and rate law of uncatalyzed oxidation of biotin

In alkaline solution of CAT, $TsNCl_2$ does not exist. Under the present experimental conditions, the concentration of OCl^- ion is small [23] and it does not make any significant contribution to the oxidation of biotin. Hence the expected reactive species are TsNHCl, HOCl and $TsNCl^-$. Hardy and Johnston [25] have reported the following equilibrium in alkaline solutions of CAT:

$$TsNClNa + H_2O \rightleftharpoons TsNHCl + NaOH \quad (2)$$

The first-order dependence of rate on $[CAT]_0$, fractional-order dependence on $[Biotin]_0$ and inverse fractional-order on $[OH^-]$ clearly indicate the formation of TsNHCl as the active oxidizing species, which is likely formed by the hydrolysis of CAT in alkali retarding step. Spectroscopic evidence for the complex formation between oxidant and substrate was obtained from UV-VIS spectra of biotin, CAT and a mixture of both. Absorption maxima in aqueous medium appear at 290 nm for biotin, 223 nm for CAT and 282 nm for a mixture of both (Fig. 3.). A hypsochromic shift of 8 nm from 290 to 282 nm of biotin suggests that complexation occurs between CAT and biotin. In view of these findings,

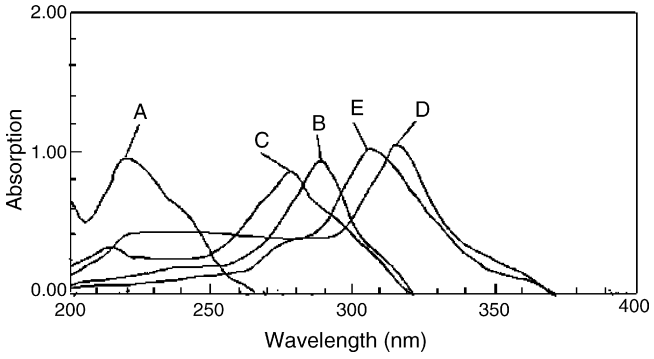


Fig. 3. UV–vis spectra of: (A) CAT, (B) biotin, (C) CAT + biotin, (D) Os(VIII) and (E) Os (VIII) + CAT.

it is likely that Scheme 1 is more probable for explaining the oxidation of biotin by CAT in alkaline medium.

A detailed mechanistic interpretation of biotin-CAT reaction in NaOH medium is presented in Scheme 2, in which the structures of complex intermediate species X and X' are given. In alkali retarding step, CAT undergoes hydrolysis to furnish TsNHCl as the reactive oxidizing species, which in turn reacts with biotin to give cationic complex intermediate X in the fast step. Further, in the rate limiting step, the intermediate species X gives another intermediate X' in presence of alkali, which is on rearrangement in the next fast step gives the final product, biotin sulfoxide.

If $[CAT]_t$ is the total concentration of CAT, then

$$[CAT]_t = [TsNClNa] + [TsNHCl] + [X] \tag{3}$$

for which rate law (4) can be derived:

$$\text{rate} = \frac{K_1K_2k_3[CAT]_t[Biotin][H_2O]}{[NaOH] + K_1[H_2O] + K_1K_2[Biotin][H_2O]} \tag{4}$$

The rate law (4) is in agreement with the experimental results.

Since rate $= k'[CAT]_t$, Eq. (4) can be transformed into Eqs. (5) and (6):

$$k' = \frac{K_1K_2k_3[CAT]_t[Biotin][H_2O]}{[NaOH] + K_1[H_2O] + K_1K_2[Biotin][H_2O]} \tag{5}$$

$$\frac{1}{k'} = \frac{[NaOH] + K_1}{K_1K_2k_3[Biotin]} + \frac{1}{k_3} \tag{6}$$

The double reciprocal plot ($1/k'$ against 1/[Biotin]) of Eq. (6) is found to be linear ($r = 0.9819$) and from this plot the value of decomposition constant k_3 has been evaluated and the value is found to be $1.66 \times 10^{-3}\ s^{-1}$. If K_1 is assumed to be small, the slope, $([NaOH] + K_1)/K_1K_2k_3$ of the Eq. (6) becomes $[NaOH]/K_1K_2k_3$. From this slope the value of K_1K_2 is calculated and value is found to be $0.33\ dm^3\ mol^{-1}$.

The proposed mechanism and the derived rate law are substantiated by the following experimental facts.

The mechanism proposed was supported by the observed solvent isotope effect, $k'\ (H_2O)/k'(D_2O) = 1.78$. For a reaction involving a fast equilibrium H^+ or OH^- ion transfer, the rate was found to increase in D_2O medium since D_3O^+ and OD^- are stronger acid and base respectively, than H^+ and OH^- ions [27,28]. In the present case, the observed solvent isotope effect is greater than unity, which is due to the greater basicity of OD^- compared to OH^-. Further, for chemical reactions carried out in H_2O and D_2O mixtures, the dependence of rate on atom fraction of deuterium (n) can be shown [29,30] by Eq. (7):

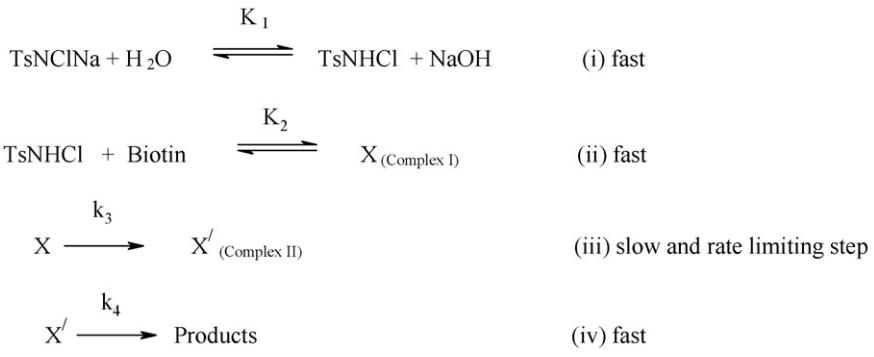
$$\frac{k'_n}{k'_0} = \frac{\prod_i^{TS}\pi(1 - n + n\Phi_i)}{\prod_j^{RS}\pi(1 - n + n\Phi_j)} \tag{7}$$

Here Φ_i and Φ_j are isotopic fractionation factors for isotopically exchangeable hydrogenic sites in the transition state (TS) and reactant state (RS), respectively. If the reaction proceeds through a single transition state, Eq. (7) takes the form as shown by Eqs. (8) and (9):

$$k'_n = k'_0(1 - n + n\Phi_j)^{-2} \tag{8}$$

$$\left(\frac{k'_0}{k'_n}\right)^{1/2} = [1 + n(\Phi_j - 1)] \tag{9}$$

Eq. (9) shows that a plot of $(k'_0/k'_n)^{1/2}$ versus n should be linear. In the present investigations, plot of $(k'_0/k'_n)^{1/2}$ versus n was found to be linear ($r = 0.9897$) with slope of $(\Phi_j - 1) = -0.25$ from which fractionation factor Φ_j of OH^- is 0.75. Kresge and Allred [31] have obtained a value of 0.80 from NMR studies for the isotopic fractionation factor of OH^- ion, which was confirmed by the works of Gold and Grist [32]. There is a fair agreement between the present value and the values reported [32–34] for the fractionation factor of OH^- ion.



Scheme 1.

Addition of methanol to the reaction mixture increased the reaction rate. The plot of $\log k'$ versus $1/D$ was linear, having a positive slope. The dependence of the rate constant on the dielectric constant of the medium is given [35] by the following equation:

$$\ln k' = \ln k'_0 - \left(\frac{(NZ_AZ_Be^2}{DRTr_{\neq}}\right) \quad (10)$$

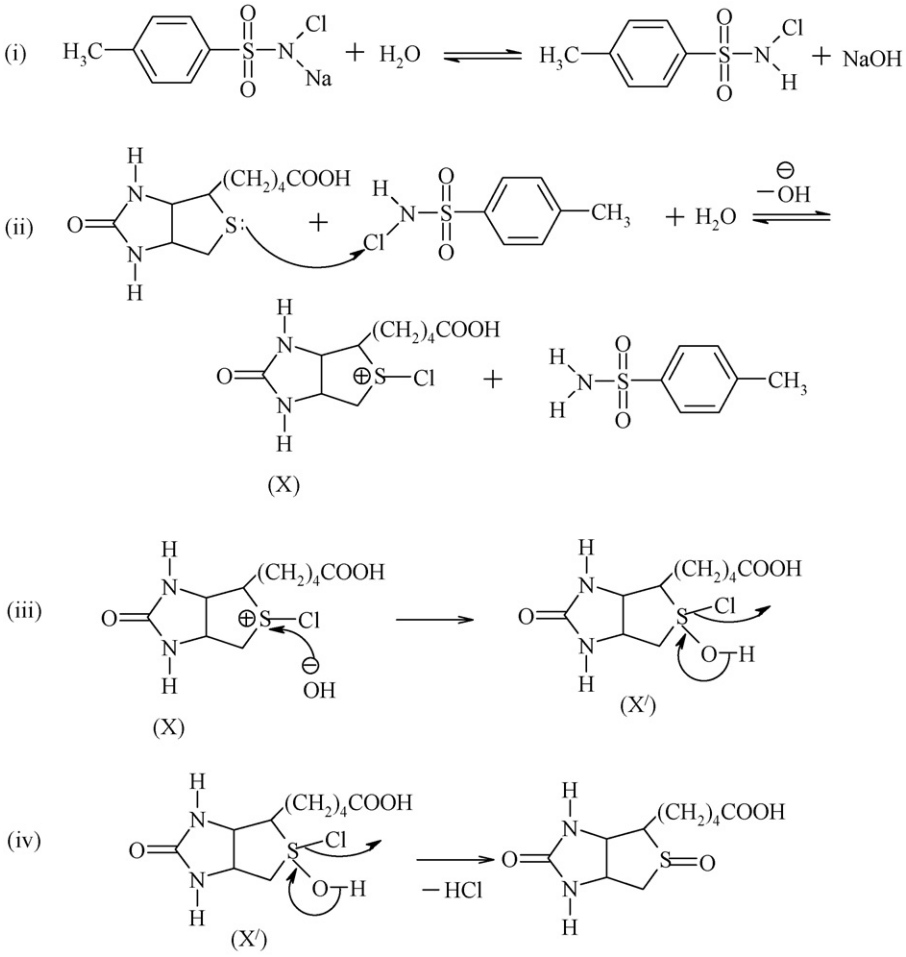
In this equation, k'_0 is the rate constant in a medium of infinite dielectric constant, Z_Ae and Z_Be are the total charges on the ions A and B, $r_{\neq}$ is the radius of the activated complex, R, T and N have their usual meanings. This equation predicts a linear plot of $\log k'$ against $1/D$ with a negative slope if the charges on the ions are of the same sign and a positive slope if they are of opposite sign. The positive dielectric effect observed in the present studies (Table 3) clearly supports [35] the involvement of dissimilar charges in the rate limiting step of the proposed mechanism (Scheme 2).

The ionic strength (I) effect on the reaction rates has been described according to the theory of Bronsted and Bjerrum [36], which postulates the reaction through the formation of an activated complex. According to this theory, the effect of ionic strength on the rate for a reaction involving two ions is given by the relationship:

$$\log k = \log k_0 + 1.02Z_AZ_BI^{1/2} \quad (11)$$

Here Z_A and Z_B are the valency of the ions A and B, and k and k_0 are the rate constants in the presence and absence of the added electrolyte, respectively. A plot of $\log k'$ against $I^{1/2}$ should be linear with a slope of $1.02Z_AZ_B$. If Z_A and Z_B have similar signs, the quantity Z_AZ_B is positive and the rate increases with the ionic strength, having a positive slope, while if the ions have dissimilar charges, the quantity Z_AZ_B is negative and the rate would decrease with the increase in ionic strength, having a negative slope. In the present case, a primary salt effect is observed as the rate decreases with increase in ionic strength of medium [36], supporting the involvement of ions of opposite sign in the rate limiting step (Scheme 2). The Debye–Huckel plot ($\log k$ against $I^{1/2}$) gave straight line with a slope of -0.65. In the present system a positive ion and a negative ion are involved in the rate-limiting step (Scheme 2) and the expected slope of -1 has not been found. This may be due to the fact that the ionic strength employed is beyond the formal Debye–Huckel limiting range. Alternatively, there could be formation of ion pairs in concentrated solutions, as suggested by Bjerrum [36].

The negligible effect of halide ions on the rate indicates that neither interahalogen nor free chlorine was formed prior to the



Scheme 2. Uncatalyzed oxidation of biotin.

$$\mathrm{TsNClNa} + \mathrm{H_2O} \overset{K_5}{\rightleftharpoons} \mathrm{TsNHCl} + \mathrm{NaOH} \qquad \text{(i) fast}$$

$$\mathrm{TsNHCl} + \mathrm{OsO_4} \xrightarrow{k_6} \mathrm{X''_{(Complex\ III)}} \qquad \text{(ii) slow and rate limiting step}$$

$$\mathrm{X''} + \mathrm{Biotin} \xrightarrow{k_7} \mathrm{Products} \qquad \text{(iii) fast}$$

Scheme 3.

rate limiting step. This is in conformity with the proposed mechanism. The mechanism is further supported by the high enthalpy of activation and positive Gibb's free energy of activation indicates that the transition state is solvated. The high negative entropy of activation shows the formation of a more ordered transition state with less degrees of freedom.

2.5. Mechanism and rate law of Os(VIII) catalyzed oxidation of biotin

Kinetics of first-order dependence on $[CAT]_0$, inverse first-order on [NaOH] and rate independent on $[Biotin]_0$ confirms TsNHCl is the probable oxidizing species in the Os(VIII) catalyzed oxidation of biotin in alkaline medium.

It has been shown that osmium has a stable +8 oxidation state [13,37–38] and exists in the following equilibria in alkaline solutions:

$$\mathrm{OsO_4} + \mathrm{OH^-} + \mathrm{H_2O} \rightleftharpoons [\mathrm{OsO_4(OH)H_2O}]^- \tag{12}$$

$$[\mathrm{OsO_4(OH)H_2O}]^- + \mathrm{OH^-} \rightleftharpoons [\mathrm{OsO_4(OH)_2}]^{2-} + \mathrm{H_2O} \tag{13}$$

The complexes $[OsO_4(OH)(H_2O)]^-$ and $[OsO_4(OH)_2]^{2-}$ which can be reduced to $[OsO_2(OH)_4]^{2-}$, with octahedral geometries are less likely to form species of higher coordination with the oxidant. It is more realistic to postulate that OsO_4, which has tetrahedral geometry, as the active catalyst species can effectively form a complex with the oxidant species. Furthermore, the observed kinetic results indicate that the intermediate complex formed from OsO_4 and CAT serves as an oxidant and the possible oxidizing species here would be TsNHCl.

The existence of a complex between the catalyst and oxidant was evidenced from the UV–visible spectra of both Os(VIII) and Os(VIII)–CAT mixture, in which the hypsochromic shift of Os(VIII) from 319 to 315 nm was observed, indicating the formation of a complex. Such type of oxidant–catalyst complex formation has also been reported in other studies [39,40].

Further, for a general equilibrium (14):

$$\mathrm{M} + n\mathrm{S} \overset{K}{\rightleftharpoons} (\mathrm{MS}_n) \tag{14}$$

between two metal species, M and MS_n, having different extinction coefficients, Ardon [41] has derived the following Eq. (15):

$$\frac{1}{\Delta A} = \frac{1}{[\mathrm{S}]^n}\left\{\frac{1}{\Delta E[\mathrm{M_{total}}]K}\right\} + \frac{1}{\Delta E[\mathrm{M_{total}}]} \tag{15}$$

where K is the formation constant of the complex, [S] the concentration of CAT, ΔE the difference in extinction coefficient between two metal species, $[M]_{total}$ the total concentration of metal species and ΔA is the absorbance difference of solution in absence of S and one that contains a certain concentration of S represented by [S]. Eq. (15) is valid provided that [S] is many times greater than $[M]_{total}$ that the amount of S tied up in the complex is negligible or it is subtracted from the initial concentration of S. According to Eq. (15), a plot of $1/\Delta A$ versus 1/[S] or $1/[S]^2$ should be linear with an intercept in the case of 1:1 or 1:2 type of complex formation between M and S. The ratio of intercept to slope of this linear plot gives the value of K.

Os(VIII) in NaOH medium containing CAT showed an absorption peak at 315 (λ_{max} for the complex). The complex formation studies were made at this λ_{max} of 315 nm. In a set of experiments, the solutions were prepared by taking different amounts of CAT (0.6×10^{-4} to 6.0×10^{-4} mol dm^{-3}) at constant amounts of Os(VIII) (1.0×10^{-6} mol dm^{-3}) and NaOH (1.4×10^{-3} mol dm^{-3}). The absorbance of these solutions was measured at 315 nm. The absorbance of the solution in the absence of CAT was also measured at the same wavelength. The difference of these absorbance values (with and without CAT) gives the differential absorbance, ΔA. A plot of $1/\Delta A$ versus 1/[CAT] was linear ($r = 0.9921$) with an intercept suggesting the formation of a 1:1 complex between Os(VIII) catalyst and CAT oxidant. Further, the plot of $\log(1/\Delta A)$ versus $\log(1/[CAT])$ was also linear ($r = 0.9892$). From the slope and intercept of the plot $1/\Delta A$ versus 1/[CAT], the value of the formation constant, K, of the complex was evaluated; it was found to be 1.05×10^2.

Based on the preceding discussion, a detailed mechanistic interpretation (Scheme 3) for the Os(VIII) catalyzed biotin–CAT reaction in alkaline medium is proposed to account for the observed kinetics.

In Scheme 3, X'' represents the complex intermediate species whose structure is shown in Scheme 4, where a detailed mechanistic interpretation of Os(VIII) catalyzed biotin–CAT reaction in alkaline medium is depicted. In the rate limiting step, the oxidizing species, TsNHCl of CAT forms a complex intermediate (X'') with the catalyst Os(VIII). So formed catalyst–oxidant complex brings about the oxidation of biotin to yield biotin sulfoxide as the final product.

From rate limiting step (ii) of Scheme 3:

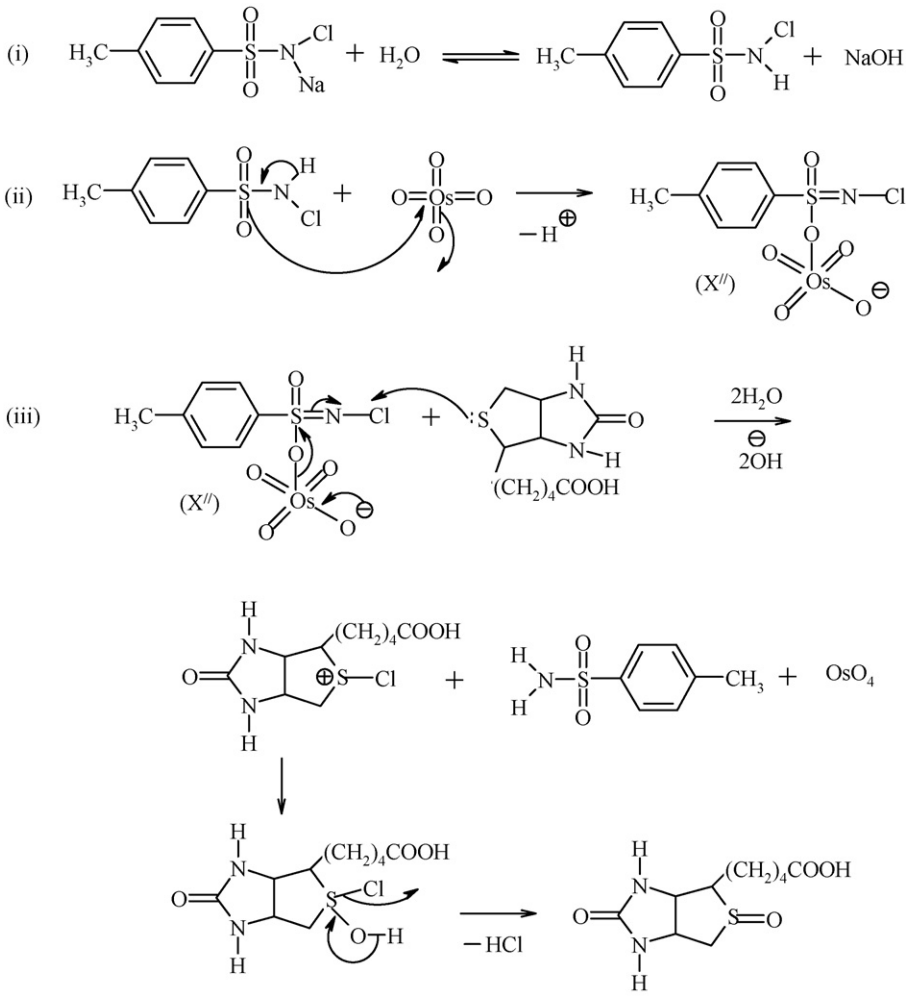
$$\text{rate} = k_6[\mathrm{TsNHCl}] + [\mathrm{OsO_4}] \tag{16}$$

Solving for [TsNHCl] and substituting its value in Eq. (16), the following rate law (17) is obtained:

$$\text{rate} = \frac{K_5 k_6 [\mathrm{CAT}][\mathrm{OsO_4}][\mathrm{H_2O}]}{[\mathrm{NaOH}]} \quad (\text{Since TsNClNa} = \mathrm{CAT}) \tag{17}$$

The proposed Scheme 3 and the derived rate law (17) are substantiated by the experimental observations discussed below.

The solvent isotope effect of $k'(H_2O)/k'(D_2O) > 1$ is observed in Os(VIII) catalyzed reaction. This is generally correlated with



Scheme 4. Os(VIII) catalyzed oxidation of biotin.

the greater basicity of OD^- as compared to OH^- [27,28] In the present investigation, a plot of $(k_0'/k_n')^{1/2}$ versus n is found to be linear ($r = 0.9954$) with a slope $(\Phi_j - 1) = -0.27$ for which Φ_j of OH^- is 0.73. There is a good agreement between the present value and the values reported for the fractionation factor of OH^- ion [31–34]. Rate of reaction decreases with decreasing methanol content. This observed solvent effect leads to the reported conclusion [42], that the decrease in methanol content showed a decrease of reaction rate for the reaction involving a negative ion and dipolar molecule as shown in Scheme 4, which substantiates the proposed mechanism.

It was felt reasonable to compare the reactivity of catalyzed oxidation of biotin towards CAT in the absence of Os(VIII) catalyst under identical experimental conditions in order to evaluate the catalytic efficiency of Os(VIII). The reaction rate of Os(VIII) catalyzed reaction was found to increase about 8 times than the uncatalyzed reaction. This was also confirmed by the activation parameters calculated (Table 4). Thus the observed rates of oxidation in the presence of Os(VIII) catalyst justify the need of a catalyst for a facile oxidation of the biotin by CAT. The activation parameters evaluated for the catalyzed and uncatalyzed reactions explain the catalytic effect on the reaction. The catalyst Os(VIII) forms a complex (X'') with the oxidant, which increases the oxidizing property of the CAT than without Os(VIII). Further, the catalyst Os(VIII) favorably modifies the reaction path by lowering the energy of activation (Table 4).

2.6. Catalytic activity of Os(VIII)

It has been pointed out by Moelwyn-Hughes [43] that, even in presence of the catalyst, the uncatalyzed reactions also proceed simultaneously, so that

$$k_1 = k_0 + K_C[\text{catalyst}]^x \tag{18}$$

Here k_1 is the observed pseudo first-order rate constant obtained in the presence of Os(VIII) catalyst, k_0 is the pseudo first-order rate constant for the uncatalyzed reaction, K_C is the catalytic constant and x is the order of the reaction with respect to [Os(VIII)]. In the present investigations, 'x' value was found to be unity. Then the value of K_C is calculated using the Eq. (19):

$$K_C = \frac{k_1 - k_0}{[\text{Os(VIII)}]} \tag{19}$$

Table 6
Effect of varying oxidant, substrate, alkali and Os(VIII) concentrations on the rate of Os(VIII) catalyzed reaction

$[CAT]_0$ ($\times 10^4$ mol dm^{-3})	$[Biotin]_0$ ($\times 10^3$ mol dm^{-3})	[NaOH] ($\times 10^3$ mol dm^{-3})	$[OsO_4]$ ($\times 10^6$ mol dm^{-3})	k' ($\times 10^3$ s^{-1})
0.60	2.00	1.40	1.00	2.01
1.00	2.00	1.40	1.00	2.16
2.00	2.00	1.40	1.00	2.00
4.00	2.00	1.40	1.00	1.99
6.00	2.00	1.40	1.00	2.11
2.00	0.60	1.40	1.00	2.02
2.00	1.00	1.40	1.00	2.14
2.00	2.00	1.40	1.00	2.00
2.00	4.00	1.40	1.00	2.09
2.00	6.00	1.40	1.00	1.99
2.00	2.00	0.40	1.00	8.70
2.00	2.00	0.70	1.00	4.51
2.00	2.00	1.40	1.00	2.00
2.00	2.00	3.00	1.00	0.81
2.00	2.00	5.00	1.00	0.41
2.00	2.00	1.40	0.25	0.51
2.00	2.00	1.40	0.50	1.01
2.00	2.00	1.40	1.00	2.00
2.00	2.00	1.40	2.00	4.12
2.00	2.00	1.40	4.00	8.02

$T = 303$ K.

The values of K_C have been evaluated at different temperatures (293–313 K) and K_C was found to vary with temperature. Further, plot of log K_C versus $1/T$ was linear ($r = 0.9901$) and the values of energy of activation and other activation parameters were computed and are summarized in Table 4.

3. Conclusion

Oxidation of biotin to biotin sulfoxide has been performed efficiently by chloramine-T in alkaline medium in presence and absence of Os(VIII) catalyst. The kinetic and mechanistic behaviour of catalyzed and uncatalyzed oxidation of biotin are found to be different. The 1:1 stoichiometry has been found and biotin sulfoxide, the oxidation product, was identified by mass spectral analysis in both the cases. Activation parameters were evaluated for both catalyzed and uncatalyzed reactions. Catalytic constants and activation parameters with reference to Os(VIII) catalyst have been computed. Os(VIII) catalyzed reactions showed rates about 8 fold faster than the uncatalyzed reactions. Proof for the formation of a 1:1 complex between CAT and Os(VIII) has been provided. The observed results have been explained by plausible mechanisms and the related rate laws have been deduced. It can be concluded that Os(VIII) acts as an efficient catalyst in the selective oxidation of biotin to biotin sulfoxide by chloramine-T in alkaline medium.

4. Experimental

4.1. Materials

Chloramine-T (Merck) was purified by the method of Morris et al. [24]. An aqueous solution of the compound was standardized by the iodometric method and preserved in brown bottles to prevent its photochemical deterioration. D-Biotin (SRL, India) was used as received. Aqueous solution of desired strength was prepared freshly each time. Solvent isotope studies were made in D_2O (99.4%) supplied by Bhabha Atomic Research Centre, Mumbai, India. Reagent grade chemicals and double distilled water were used throughout.

4.2. Kinetic procedure

The kinetic runs were performed under pseudo first-order conditions with $[Biotin]_0 \gg [CAT]_0$ at constant temperature 30 ± 0.1 °C by measuring the rate of disappearance of CAT iodometrically as discussed earlier [21]. The course of the reaction was studied for more than two half-lives. The pseudo first-order rate constants (k') calculated from the linear plots of log[CAT] versus time were reproducible within ±2–5%. Regression coefficients, 'r' were evaluated.

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