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Kinetics of Oxidation of Oxyhaemoglobin by Benzoquinone

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Kinetics of the oxidation of oxyhaemoglobin by benzoquinone has been studied by spectrophotometrically. The specific rate of reaction is 3.07×10^2 M^{-1} s^{-1} at 25 ºC (pH 6). The kinetic parameters such as frequency factor, energy of activation and entropy of activation are found to be 1.206×10^6 s^{-1}, 20.51 kJ mol^{-1} and -136.79 JK^{-1} mol^{-1}, respectively. The effect of pH and the probable mechanism for the reaction are also studied.

Key Words: Kinetics, Oxidation, Oxyhaemoglobin, Benzoquinone.

INTRODUCTION

Quinones acting as redox agents and reactivity towards thiols, play critical role in therapeutic and toxicological properties[1]. Recently, the covalent bonding of quinones to blood proteins albumin and haemoglobin has been characterized[2-4]. Oxyhaemoglobin reacts with 2-methyl-1,4-napthaquinone to form methemoglobin[5] and many other addition and substraction reactions of quinones have been extensively studied.

In present study, the kinetics of oxidation of oxyhaemoglobin by benzoquinone was carried out. The specific reaction rate at various concentrations of benzoquinone were determined. The oxidation reaction was carried out at various temperatures to determine kinetic parameters. The most probable mechanism for this oxidation reaction was also proposed.

EXPERIMENTAL

Preparation of buffer solutions

Phosphate buffer: (a) **Potassium dihydrogen phosphate:** 1.055 g of potassium dihydrogen phosphate was dissolved in minimum quantity of double distilled water and finally diluted to 250 mL with double distilled water to get 0.1 M stock solution. (b) **Disodium hydrogen phosphate:** 8.900 g of disodium hydrogen phosphate was dissolved in minimum double distilled water and then diluted to 250 mL with double distilled water to get 0.1 M stock solution.

Equal volume of buffer solutions (a) and (b) were mixed and pH of the mixture was measured (pH = 6.86). The pH of the buffer solution was adjusted with dilute phosphoric acid and dilutes sodium hydroxide solution to get pH 5, 6, 7, 8 and 9, respectively.

Saline solution: Stock solution of saline having concentration of 0.3 M was prepared by dissolving 8.77 g of sodium chloride in minimum quantity of double distilled water and finally diluted to 500 mL to get 0.3 M stock solution.

Preparation of oxyhemoglobin solution

Hemolysis: 5 mL of fresh blood with anticoagulant was taken in a clean and glass centrifuge tube which was covered with polythene paper. The blood was then centrifuged for 5 min at 1000 rpm. The supernatant plasma protein was removed carefully. The sediment containing the red blood cells was washed by adding cold saline containing the anti-coagulant and then centrifuged for 5 min at 1000 rpm. The centrifuged was continued until the supernatant did not give white precipitate with 30 % trichloroacetic acid solution, showing the complete removal of plasma proteins. The sediment was washed two more times by centrifugation and the supernatant solution was discarded.

The sediment was treated with 10 mL of double distilled water and stirred well. The red cells were agitated with clean glass rod and shaken well. The solution was then kept for one hour at room temperature. The red blood cells were ruptured due to osmosis and the haemoglobin came out of the cells. After 1 h the solution was centrifuged for about 20 min at 3000 rpm. The supernatant solution of oxyhaemoglobin was collected and diluted to 50 mL with double distilled water. This solution of oxyhaemoglobin was preserved in ice.

Stock solution of haemoglobin was prepared and its concentration was determined.

Benzoquinone solution: Stock solution of benzoquinone of 5.8×10^{-4} M was prepared by dissolving 9.4×10^{-3} g of benzoquinone in required buffer solution and finally diluted with same to 100 mL.

Kinetic measurements: Kinetic measurements were carried out by taking out 6 mL of oxyhemoglobin (10.4×10^{-4} M) in one test tube and 6 mL of benzoquinone (5.2×10^{-4} M) + 8 mL sodium chloride (0.3 M) in another test tube. Both the test tubes were kept in thermostat for 0.5 h to attain the experimental temperature. Then two solution of the test tube were mixed together and simultaneously time was noted. The concentration of benzoquinone, oxyhaemoglobin and saline were 1.56×10^{-4} M, 1.56×10^{-4} M and 0.12 M, respectively in the reaction system. The absorbance was recorded at 548 nm using UV-visible spectrophotometer, at time intervals of 5 s.

Specific reaction rate (k): The reciprocal of the concentration of unreacted oxyhaemoglobin were plotted *versus* time. The slope of this linear curve gave the specific rate of reaction. Such determinations were made with various concentrations of oxyhaemoglobin and benzoquinone ranging from 1.56×10^{-4} to 3.12×10^{-4} M and 0.78×10^{-4} to 1.56×10^{-4} M, respectively at 25 ºC.

Effect of temperature: In order to evaluate the effect of temperature on the reaction, the specific reaction rates were determined at temperature ranging from 25 to 42 ºC.

Effect of pH: To verify the effect of pH on the oxidation of oxyhaemoglobin by benzoquinone, the specific reaction rate were measured at pH 5, 6, 7, 8 and 9 at 25 ºC.

RESULTS AND DISCUSSION

From the measurement of absorption at various interval of time, the graph of absorption *versus* time gives the concentration of oxyhaemoglobin unreacted. The reciprocal of this unreacted oxyhaemoglobin was plotted *vs.* time. A straight line curve is obtained, which indicates that the oxidation of oxyhaemoglobin by benzoquinone is a second order reaction. The slope of this curve was the specific reaction rate.

Frequent kinetic measurements under the identical experimental condition gives the specific reaction rate which agrees within the error ± 3 %.

The specific reaction rates of the reaction evaluated at various temperature ranging from 25 to 42 ºC (Table-1). The activation parameters such as frequency factor, energy of activation and entropy of activation are determined (Table-2). The variations of specific reaction rate of reaction with pH at 25 ºC are presented in Table-3. At pH 5 and at temperature 25 ºC, the specific reaction rate is 3.86×10^{2} M^{-1} s^{-1}, while the frequency factor and entropy of activation are 8.7646×10^{5} s^{-1} and -139.42 JK^{-1} mol^{-1}, respectively.

TABLE-1
KINETICS OF OXIDATION OF OXYHAEMOGLOBIN BY BENZOQUINONE: EFFECT OF TEMPERATURE
Buffer solution: Phosphate; pH: 6.00; Concentration of oxyhaemoglobin: 1.56×10^{-4} M; Concentration of benzoquinone: 1.56×10^{-4} M

Temperature (ºC)	Specific reaction rate ($k/10^{2}$ M^{-1} s^{-1})
25	3.07
30	3.46
37	4.23
42	4.78

TABLE-2
KINETICS OF OXIDATION OF OXYHAEMOGLOBIN BY BENZOQUINONE. ACTIVATION PARAMETERS:
Concentration of oxyhaemoglobin: 1.56×10^{-4} M; Concentration of sodium nitrite: 1.56×10^{-4} M; Ionic strength: 0.12 M; pH (phosphate buffer): 6.00; Wavelength of maximum absorbance: 548 nm

Activation parameters	Value	Unit
Energy of activation (Ea)	20.51×10^{3}	J mol^{-1}
Frequency factor (A)	1.206×10^{6}	s^{-1}
Entropy of activation ($\Delta S^{\neq}$)	-136.79	JK^{-1} mol^{-1}

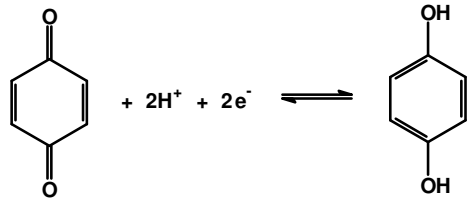
TABLE-3
KINETICS OF OXIDATION OF OXYHAEMOGLOBIN BY BENZOQUINONE: VARIATION OF SPECIFIC REACTION RATE k WITH pH
Buffer solution: Phosphate; Temperature: 25 ºC

pH	[oxyhaemoglobin] / 10^{-4} M	[Benzoquinone] / 10^{-4} M	k / 10^{2} M^{-1} s^{-1}
5	1.56	1.56	3.86
6	1.56	1.56	3.07
7	1.56	1.56	2.46
8	1.56	1.56	1.95
9	1.56	1.56	1.56

At pH 6, 7, 8 and 9 the specific reaction rates are 3.07×10^{2}, 2.46×10^{2}, 1.95×10^{2} and 1.56×10^{2} M^{-1} s^{-1}, respectively. Inspite of this, the energy of activation is same over the pH range.

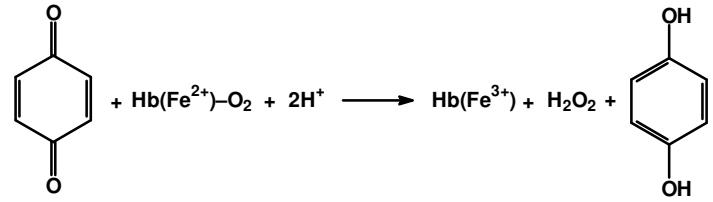
An interesting observation of the reaction is its high sensitivity to pH of reaction medium. The specific reaction rate significantly increases with decreasing pH of reaction medium.

In this context, it is relevant to consider the following equilibrium.



At lower pH value, the relative concentration of benzoquinone is lower than that at higher pH value, so also the specific reaction rate is higher at lower pH values than at higher pH values. Therefore, it is quite obvious that, the benzoquinone form is the oxidizing entity.

From the above observation, the probable oxidation of oxyhaemoglobin by benzoquinone follows the following reaction scheme.



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