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Kinetic and Mechanism Studies of the Reaction Between L-Tyrosine and Iodine on the Basis of UV-Vis Spectrophotometric Method

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Abstract: This study concern to the kinetic and mechanism studies of interaction between L- tyrosine (L-T) and iodine upon UV-Vis spectrophotometric measurements. The results showed that the reaction is first order respect to each reactant by using the initial rate method. Also, the rate constant of the reaction at two different temperature 25.0 and 15.0°C were determined. Then the resulting activation functions such as E_a, $\Delta H_{\#}$, $\Delta G_{\#}$, $\Delta S_{\#}$ and frequency factor, A, were calculated. The resulted values at 25.0°C are: $E_a = 36.82$ kJ mol^{-1}, $A = 2.032\times10^6$ L mol^{-1} sec^{-1}, $\Delta G^{\circ}_{\#} = 73.17$ kJ mol^{-1}, $\Delta H^{\circ}_{\#} = 34.34$ kJ mol^{-1} and $\Delta S^{\circ}_{\#} = -132.4$ J mol^{-1} K^{-1}.

Key words: L-Tyrosine, iodine, rate constant, activation function, Arrhenious parameters

INTRODUCTION

Proteins are very important kind of bio-molecules and nowadays the interactions of small molecules with proteins have aroused great interest in supra-molecular chemistry. The study of interaction between supra compound such as organic dyes, drugs and quantitative analysis of proteins, especially the micro determination of proteins, is one of the basis requisites in biochemical assays and clinical tests. Many methods such as spectrophotometry (Guo *et al*., 2000; Gao *et al*., 2001; Guo and Xu, 2002), fluorometry (You *et al*., 1997; Ma *et al*., 1996; Ci and Chen, 1996) and light scattering techniques (Ma *et al*., 1997a, b; Cao *et al*., 2001; Yao *et al*., 1999) are suitable for studying the subject.

As we know, free Iodine reacts with the protein of bacteria (presumably by iodinating tyrosine residues) and thus kills the bacterium organism (Klebanoff, 1967). At pH = 6.8, iodine reacts with tyrosine as well as with cysteine. Cysteine and tryptophane are the only amino acids with reducing groups which are known to react with dilute iodine at pH = 3.2 (Anson, 1940). Iodine deficiency is currently the most preventable cause of the worlds cretinism, brain damage and thyroid disorders. Biologically, iodine is most essential in the synthesis of thyroid hormones, which serve in the differentiation, growth, metabolism and physiological function of virtually all tissues (Yen, 2001). In the thyroids colloid, iodide is not only collected but also concentrated. After concentration, iodide is oxidized to I^+ by the enzyme thyroid peroxidase (TPO) in the presence of H_2O_2. The oxidized iodine is then bound to tyrosine residues of the protein thyroglobulin to produces monoiodothyrosine (MIT) and diiodothyrosine (DIT). Thyroglobulin (Tg) acts as the substrate for thyroid hormone (T_3, T_4) biosynthesis (Mallet *et al*., 1995) (Fig. 1).

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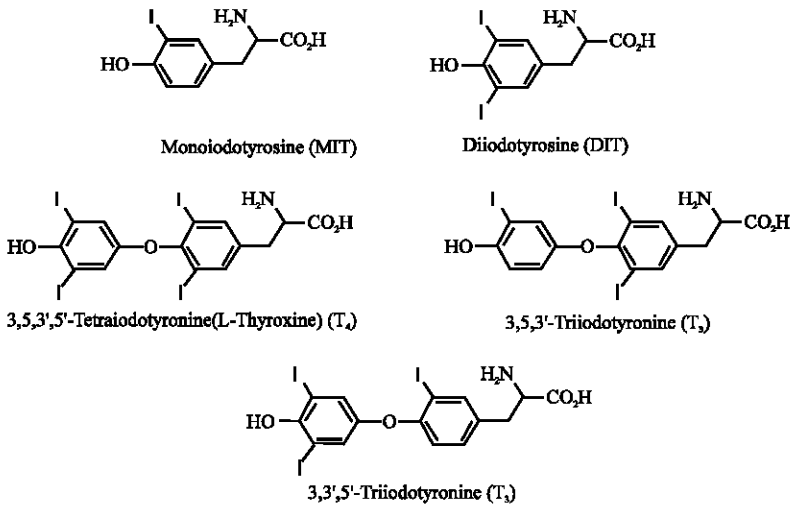


Fig. 1: Structures of thyroid hormones and precursors

MATERIALS AND METHODS

Material and Instrumentation

L-tyrosine, iodine, acetic acid, sodium acetate and potassium iodide with high purities were purchased from Merk Company and were used without further purification. A Shimadzu UV-2101 PC (Japan) spectrophotometer was used for recording the UV-Vis kinetic data.

RESULTS AND DISCUSSION

The kinetic of the reaction between L-tyrosine (L-T) and iodine at pH = 5.6 (using 0.02 M solution of sodium acetate as a buffer) and λ = 350 nm was studied at two different constant temperature 15.0 and 25.0°C. During adding tyrosine solution (20.0 mM) to iodine solution (0.6 mM), the absorbance of iodine solution decreased gradually, indicating that a binding reaction or complex formation takes place between tyrosine and iodine.

In order to insure the applicability of Beer's law, the absorbance of iodine solutions were tested over a concentration range of 1.45×10^{-4}-4.5×10^{-4} M by the independent experiment. The plot of absorbance-concentration of iodine solution is linear over the above concentration range insuring the applicability of Beer's law (Fig. 2). In Fig. 3, the absorption spectra of different iodine solutions are shown. The maximum of each bond lies near 290 to 350 nm.

The order of reaction with respect to each reactant was determined using the initial rate method. At the first, the reaction was spectrophotometrically monitored for nearly constant concentration of tyrosine (20 mM) but the different concentrations of iodine: 0.60, 0.70, 0.80 and 0.975 mM, respectively, at λ = 350 nm. Again, the same procedure was repeated for each of the following nearly constant concentrations of L- tyrosine: 25.0, 30.0 and 35.0 mM, respectively. For each of the above concentration of L- tyrosine, the reaction progress curve with respect to each iodine solution was recorded and the absorbance versus time was then drawn (Fig. 4).

The initial rate respect to each above concentration of L-T+I_2 was determined as follows:

$$\text{Rate} = -d[I_2]/dt = k[I_2]^n[\text{L-T}]^m \quad (1)$$

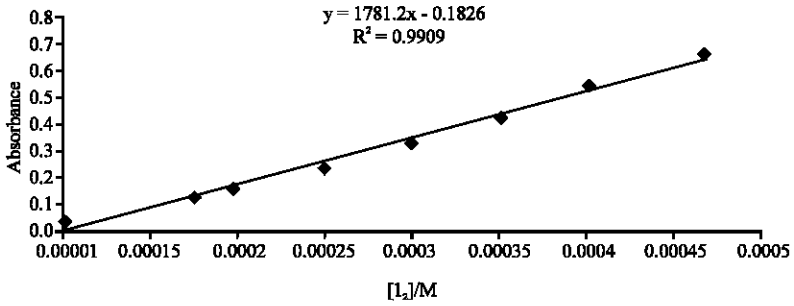


Fig. 2: Plot of absorbance vs. concentration of iodine solution at λ = 350 nm

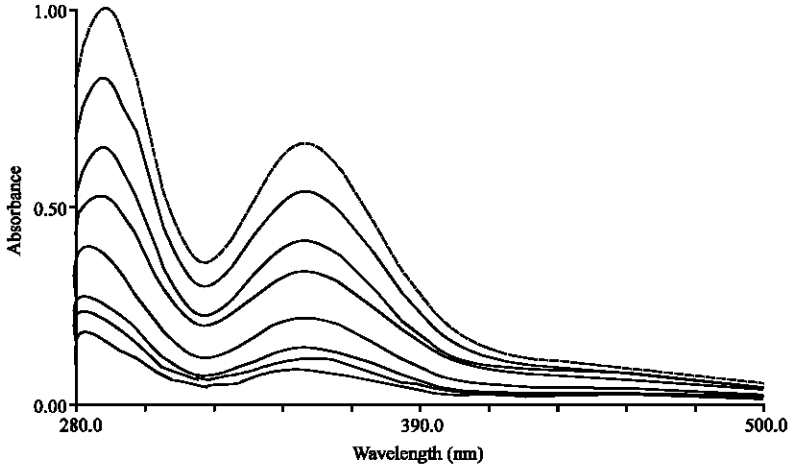


Fig. 3: The UV-Vis absorption spectra of iodine solution in different concentrations

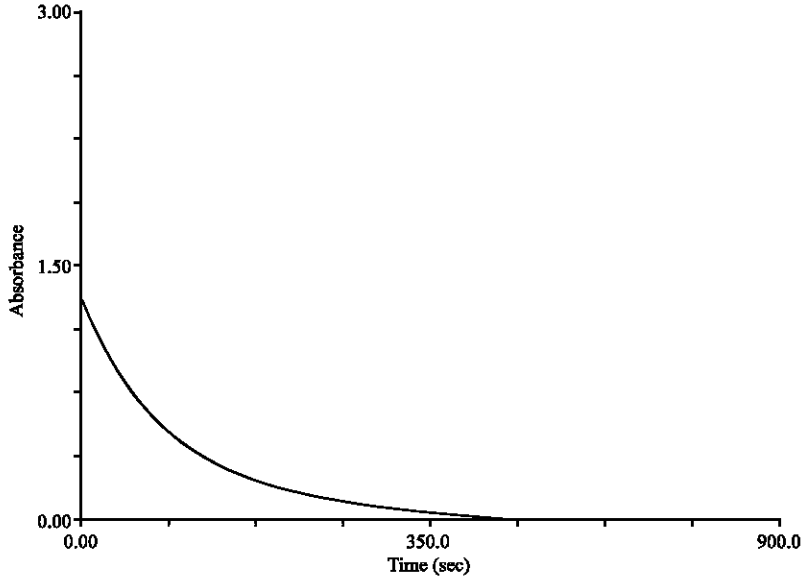


Fig. 4: Plot of absorbance vs. time for L-T+I_2 system

Table 1: Values of n and lnk' at 15.0°C

Iteration	n	ln k'
1	1.04	-4.88
2	1.05	-4.67
3	1.03	-4.50
4	1.03	-4.30

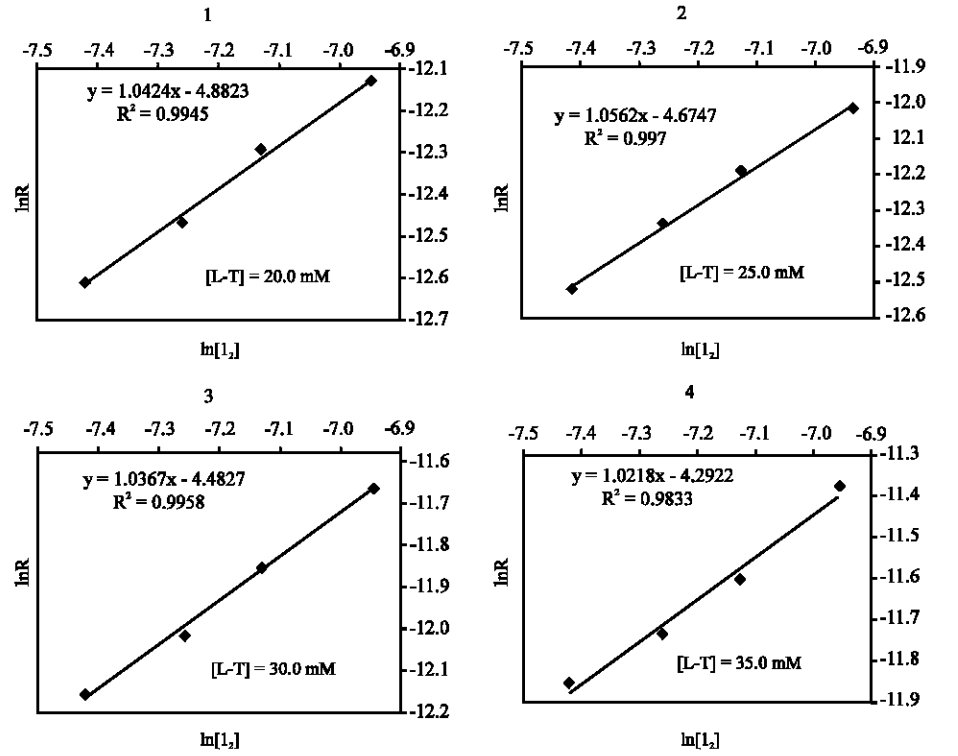


Fig. 5: Plots of (ln R) vs. ln [I_2] at 288.15 K (15°C) and various concentrations of L-T where: (1): [L-T] = 20.0 mM, [I_2] = 0.60, 0.70, 0.80, 0.975 mM; (2): [L-T] = 25.0 mM, [I_2] = 0.60, 0.70, 0.80, 0.975 mM; (3): [L-T] = 30.0 mM, [I_2] = 0.60, 0.70, 0.80, 0.975 mM; (4): [L-T] = 35.0 mM, [I_2] = 0.60, 0.70, 0.80, 0.975 mM

where, k is the rate constant and n and m are the order of reaction with respect to I_2 and L-T, respectively. Or

$$\text{Rate} = k'[I_2]^n, \text{ where } k' = k[\text{L-T}]^m \cong \text{constant} \quad (2)$$

So,

$$\ln(\text{Rate}) = \ln k' + n\ln[I_2] \quad (3)$$

From the linear plot of ln(Rate) versus ln[I_2], one can determine the slope, n and intercept, ln k' (Table 1, Fig. 5).

Now from the plot of ln k' versus ln[L-T] at 288.15K(Fig. 6) we can easily evaluate the order of the reaction with respect to L-T and ln k at 288K; slope = m; intercept = lnk; k = rate constant

All steps of the experiment and calculations were repeated at 298.15 (25°C). The results are showed in Fig. 7 and Table 2.

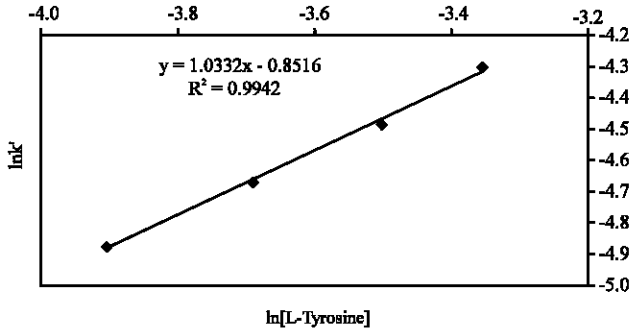


Fig. 6: Plot of ln k' versus ln[L-T], slope = m = 1.05 and intercept = lnk = -0.85

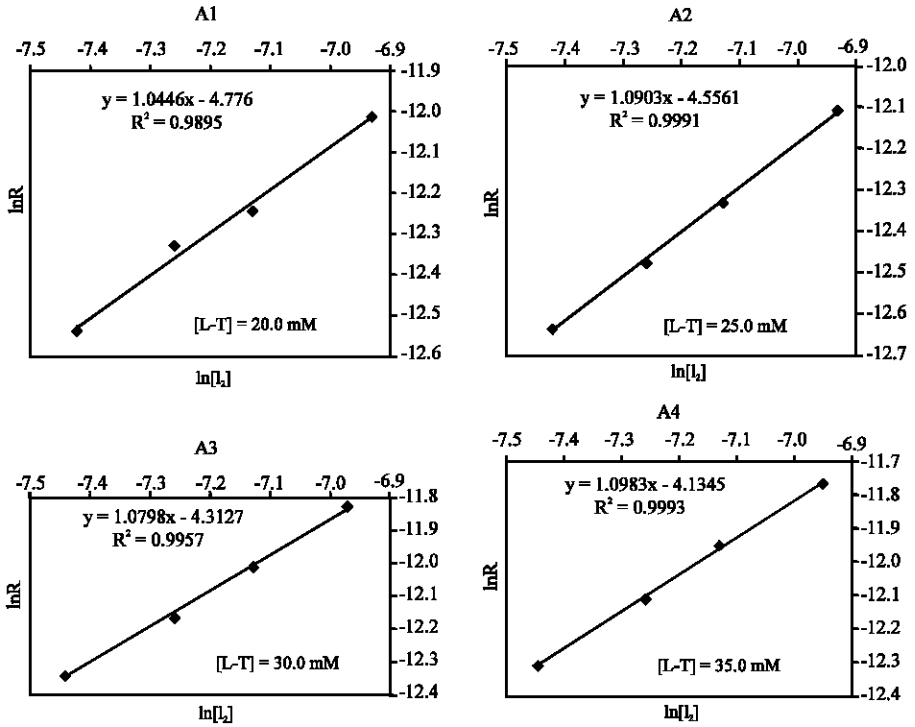


Fig. 7: Plots of ln (Rate) vs. ln[I_2] at 298.15 (25.0°C). (A1): [L-T] = 20.0 mM, [I_2] = 0.60, 0.70, 0.80, 0.975 mM; (A2): [L-T] = 25.0 mM, [I_2] = 0.60, 0.70, 0.80, 0.975 mM; (A3): [L-T] = 30.0 mM, [I_2] = 0.60, 0.70, 0.80, 0.975 mM; (A4): [L-T] = 35.0 mM, [I_2] = 0.60, 0.70, 0.80, 0.975 mM

Table 2: The values of n and lnk' at 25.0°C

Iteration	n	lnk'
1	1.05	-4.77
2	1.09	-4.56
3	1.08	-4.32
4	1.10	-4.13

From the plot of ln k' vs. ln[L-T] (Fig. 8) the order of the reaction with respect to L-T, m and rate constant, k, can be obtained at 298.15 K, slope = m and intercept = ln k.

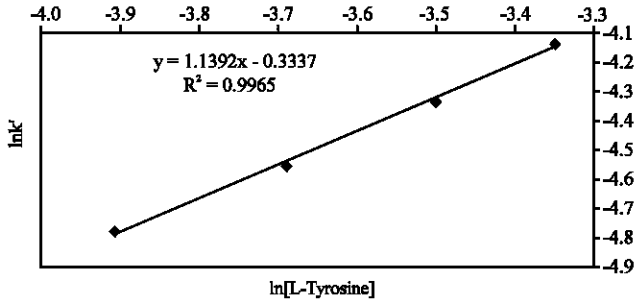


Fig. 8: Plot of ln k' vs. ln[L-T] at 298.15 K, slope = m = 1.16, intercept = lnk = -0.33

Finally the resulting activation functions of the reaction, such as activation energy, E_a, activation enthalpy, $\Delta H_{\#}$, activation entropy, $\Delta S_{\#}$ activation Gibbs free energy, $\Delta G_{\#}$and frequency factor, A, were calculated at 298.15 K as follows:

$$\ln(k_2/k_1) = E_a/R(1/T_1 - 1/T_2)$$

Where:

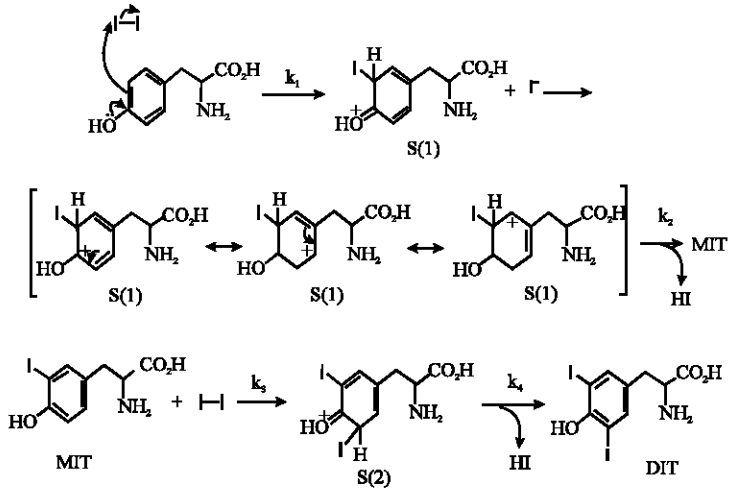
T_1 = 288.15 K
k_1 = 0.43 L mol^{-1} sec^{-1}
T_2 = 298.15 K
k_2 = 0.72 L mol^{-1} sec^{-1}

The resulting activation functions are as follow:

E_a = 36.82 kJ mol^{-1}
$\Delta H_{\#}$ = 34.34 kJ mol^{-1}
$\Delta S_{\#}$ = -132.4 J mol^{-1} K^{-1}
$\Delta G_{\#}$ = 73.17 kJ mol^{-1}
A = 2.032×10^{6} L mol^{-1} sec^{-1}

Proposed Mechanism

On the bases of the resulted rate equation, one may propose the following mechanism for the considered reaction:



Assuming that the final step is rate determining and applying the steady state approximation for all steps, one can conclude the overall rate law for the reaction as: Rate = $k_1[I_2][L\text{-}T]$ that is the same as the experimental rate law.

CONCLUSION

This study reports the results of kinetic and mechanistic investigation of the reaction of L-tyrosine with iodine in the acetate buffer solution of pH = 5.6. The experimental results revealed an overall second-order reaction, which is first-order respect to each reactant. The resulting Arrhenious parameters, A and E_a, activation functions, $\Delta H_{\#}$, $\Delta S_{\#}$ and $\Delta G_{\#}$ were determined at 298.15 K. Finally, an approximate mechanism that reproduces the experimental rate law has been proposed.

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