DETERMINATION OF CONSECUTIVE REACTION RATE CONSTANTS BETWEEN GLYCINE AND ISOLEUCINE WITH *o*-PHTHALDIALDEHYDE AND 2-MERCAPTOETHANOL FROM SINGLE EXPERIMENT DATA

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Received November 18, 2010; Accepted March 22, 2011

ABSTRACT

Kinetics study of chemical reaction is important in producing optimum product, designing a reactor, analyzing a compound, and many other purposes. This work presents the result of study of consecutive pseudo first order reaction between glycine and isoleucine with o-phthaldialdehyde and 2-mercaptoethanol in borate buffer solution at pH=9.0. Continuous detection was used to follow the change of fluorescence intensity of formed isoindole during the reaction. Although concentrations of initial compound and its derivative were not known; by using iteration method, the reaction constants could be determined with high precision from single experiment data. Result of the work showed that reaction rate constants k_1 and k_2 for consecutive reaction of glycine with excess of o-phthaldialdehyde and 2-mercaptoethanol were $18.75x10^{-3}$ and $26.70x10^{-5}$ s^{-1}, respectively; whereas for isoleucine were $6.06x10^{-3}$ and $12.59x10^{-5}$ s^{-1}, respectively.

Keywords: *kinetics, consecutive reaction, iteration, glycine, isoleucine*

INTRODUCTION

Kinetics study is important for many purposes. Henriquez et al. [1] studied the kinetics profile associated with the reaction of phenol and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) to quantitatively measure the capacity of antioxidant. Simendinger and Balik [2] determined chemical reaction kinetics of sulfur dioxide and oxygen with unsaturated drying oils in attempt to quantify the relative reactivity of unsaturated drying oils with O_2 and mixture of SO_2 and O_2. Kerr et al. [3] followed chemical reaction kinetics of hydrolysis of disodium-*p*-nitrophenyl phosphate in frozen sugar and maltodextrin solutions to examine relation between hydrolysis and glass transition temperature in frozen food polymer solution. Bandstra et al. [4] studied relation between reaction kinetics and product distribution for the reduction of 2,4,6-trinotrotoluene by granular iron metal. Birtill [5] tried to design better equipment and better procedures for the efficient and informative testing of catalyst decay by studying measurement and modeling of the kinetics of catalyst decay in fixed beds. Bandstra and Tranyek [6] examined applicability of single-site rate equations for a reaction on inhomogeneous surface. Bi et al. [7] used reactive transport model and the kinetic iron model to characterize reactive and non-reactive sites on granular iron. These are several examples of chemical reaction kinetics studies with their different purposes where the last 3 examples related to the development of theory.

This paper presents a chemical reaction kinetic study of pseudo first order reaction between glycine and isoleucine in the excess of *o*-phthaldialdehyde (OPA) and 2-mercaptoethanol, in borate buffer solution pH 9.0 with the purpose to determine its reaction rate constants, k_1 and k_2. Continuous detection was used to follow the change of fluorescence intensity of formed isoindole during the course of reaction. This kinetic study is important in HPLC amino acids analysis because the sensitivity of the analysis depends on its derivatization time, whereas this variable is depended on kinetics parameters (reaction rate constants) all of amino acids analyzed. The method of HPLC amino acids analysis has more advantages compared with Kjeldahl method. Beside total protein can more accurately be calculated quantitatively from total amino acids, by HPLC analysis its amino acids as composer of protein can also be known qualitatively. This method can also be used for quantitative analysis of compounds containing primary amine in pharmaceutical preparations as shown by Izquierdo et al. [8], or in biological tissues as shown by Koros et al. [9], or in any other samples. Between several amino acids analysis methods, analysis using OPA derivatization is one of the most frequently used method. Main advantages of OPA as a fluorogenic derivatization reagent for analysis of amino acids and

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other compounds containing primary amine are a high formation rate for its fluorescent derivatives at room temperature, lack fluorescence of the reagents, high sensitivity of highly fluorescent 1-alkyl-thio-2-alkyl substituted isoindole formed [8], high selectivity due to specific excitation and emission wavelength should be used, and every amino acids have their specific retention time when HPLC analysis is used. Although Izquierdo et al. [8] and Beketov et al. [10] have tried to substitute 2-mercaptoethanol with another compound, the method of Roth [11] is still widely used due to substitution of 2-mercaptoethanol with another compound resulting longer derivatization reaction time, therefore, the kinetics study of the reaction as presented by Gui [12] and Meyer [13] is important.

In this work, reaction of glycine or isoleucine with OPA and 2-mercaptoethanol was conducted in the excess of the last two compounds; therefore the reaction kinetics will only depend on the amino acid concentration. For this condition, the reaction may be presented as:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \quad (1)$$

where A is glycine or isoleucine, B is its isoindole compound, and C is non-fluorescent product. According to reaction kinetics theory as described by Steinfeld et al. [14] or presented in many other references, if all reactions are first order and initial concentration $[B]_o = 0$, then it can be derived that concentration [B] at reaction time t is

$$[B] = \frac{[A]_o k_1}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right) \quad (2)$$

and

$$t_{max} = \frac{1}{k_1} \ln \frac{k_1 [A]_o}{k_2 [B]_{max}} \quad (3)$$

$[B]_{max}$ and t_{max} could be determined experimentally. Due to A and C are not fluorescence compound, and B is not stable compound, it is very difficult to determine proportional constant which relates between these compounds with their fluorescence intensity. Consequently, it is impossible to determine k_1 and k_2 directly from the equations already mentioned above. In this work, such difficulties were solved by introducing iterative technique.

Principle of the iteration method for determining k_1 and k_2 of consecutive reaction in this paper (when $[A]_o$ was not known) was that for several reaction times; [B] was calculated via equations (2) and (3) using expected values of $[A]_o$ and k_1, and then absolute values of the differences between [B] from calculation and [B] from experiment were added for all reaction times. The calculation was repeated for other values of $[A]_o$ and k_1, and then errors from all calculations were compared to find the lowest error which gave the best value of kinetics parameters.

EXPERIMENTAL SECTION

Materials

All Chemicals were p.a. grade. Borate buffer was made by dissolving 0.9530 g of $Na_2B_4O_7 \cdot 10H_2O$ in 50 mL aquabidest, and then 0.05 M HCl was added stepwise until pH = 9.0 was reached, finally aquabidest was added to produce 100.0 mL of solution. *o*-Phthaldialdehyde solution was made by dissolving 0.020 g of *o*-phthaldialdehyde in 2 mL ethanol and then 50 mL of pH 9.0 borate buffer and 1.5 mL 2-mercaptoethanol 5% in ethanol were added. Amino acid solutions were made by dissolving 0.46 mg and 0.80 mg of respectively glycine and isoleucine in 5 mL NaCl 1% solution**.**

Instrumentation

Hitachi F-4000 fluorescence spectrophotometer equipped with 1.0 cm thickness of quartz cell was used to follow fluorescence intensity of isoindole formed during the reactions. Both excitation and emission band pass was set at 10 nm, and response was taken every 2 sec.

Procedure

Detection of kinetics reaction

This experiment was started by determining wavelength, which gave maximum excitation (λ_{ex}), and maximum emission (λ_{em}) intensity. Fluorescence spectrophotometer was set as follow: scan speed was 120 nm/min; for determining maximum excitation wavelength, wavelength of emission was adjusted at zero; and for determining maximum emission wavelength, the wavelength of excitation was adjusted at zero. Then, 3 mL of OPA solution and 100 µL of amino acid solution were mixed in the cell. After about three min reaction time, solution was scanned from 220 nm to 800 nm. Next, the spectrophotometer was set at maximum λ_{ex} and maximum λ_{em}. Finally, 3 mL of OPA solution and 100 µL of amino acid solution were mixed in the cell, and time scanning was directly started and stopped after 10 min of reaction time.

RESULT AND DISCUSSION

Determination of k_1 and k_2 for consecutive reaction did not necessarily use iterative process when the decrease in concentrations of initial compound ([A]) and intermediate ([B]) could be detected separately during the course of reaction. In this case, k_1 could be calculated using eq. $[A] = [A]_o e^{-k_1 t}$, and then k_2 was measured from eq. (2). Consequently, twice experiments

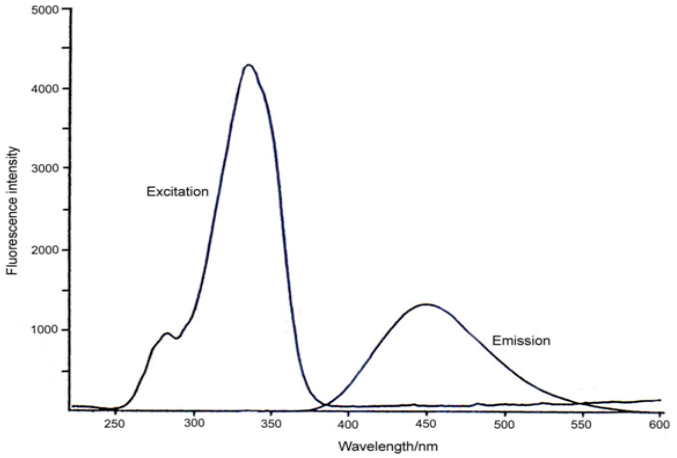

Fig 1. Excitation and emission fluorescence intensity of 1-alkyl-thio-2-alkyl substituted isoindole at various wavelength, determined after 3 minutes reaction time. Maximum excitation (λ_{ex}) = 335 nm and maximum emission (λ_{em}) = 450 nm

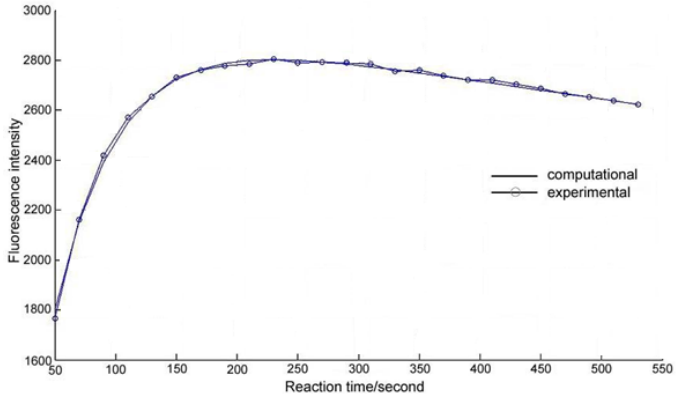

Fig 2. Plot of isoindol (an intermediate product of reaction between excess OPA and 2-mercaptoethanol with glycine) fluorescence intensity versus reaction time for both experiment and computational results

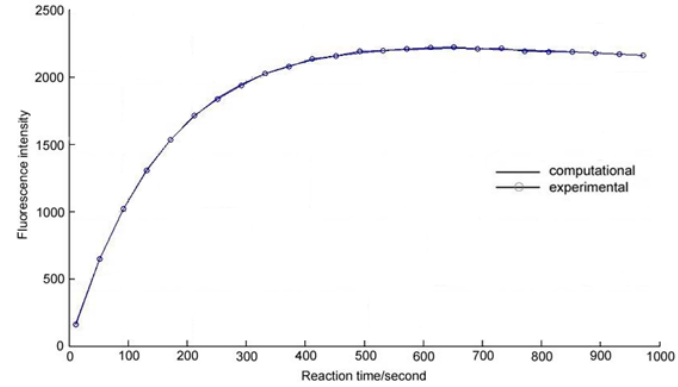

Fig 3 Plot of isoindol (an intermediate product of reaction between excess OPA and 2-mercaptoethanol with isoleucine) fluorescence intensity versus time for both experiment and computational results

must be done. However, as already described in previous section, in the case of derivatization of amino acids with OPA and 2-mercaptoethanol, both A and C are non-fluorescent compounds; therefore, only B can be detected by fluorometer. Hence, the experiment could only follow fluorescence intensity of B during the reaction process. As the result, when fluorometric detection method was used, k_1 and k_2 could only be calculated from the data of fluorescence intensity propagation of B, and it must be determined by iteration method.

Steinfeld et al. [14] has shown iterative process for determining reaction constants of reversible reaction. In different way this paper presents iterative process for pseudo first order consecutive reaction. Computer program for determining k_1 and k_2 in this work was based on principle that from initial expectation values of $[A]_o$ and $k_1(1)$; k_2 was calculated by using eq. (3) where the values of t_{max} and $[B]_{max}$ are known from experiment. Further, values of [B] at t_1, t_2, t_3, …, t_N are calculated via eq. (2). These values are noted as values of $B_{cal}(1)$, $B_{cal}(2)$, $B_{cal}(3)$, ..., $B_{cal}(N)$. Then, DELTA $B_{cal}(1)$ as an absolute value of the different between $B_{cal}(1)$ and $B_{exp}(1)$ was calculated. The same calculation was also conducted for DELTA $B_{cal}(2)$, DELTA $B_{cal}(3)$, ..., DELTA $B_{cal}(N)$. Further, ΣDELTA $B_{cal}(1)$ was calculated as the sum of all DELTA B_{cal}. This value was compared with sum of all B_{exp}, and noted as Error(1). The same calculation was done using $k_1(2) = k_1(1) + 0.001k_1(1)$ to produce ΣDELTA Error(2). This procedure was repeated until Error(M) was obtained. Further, all Error were compared to find $[A]_o$, k_1 and k_2 from the result giving the lowest error.

It was already known, at low concentration, the concentrations of A and B (unstable isoindole compound) are linearly proportional to their fluorescence intensities; however, it was difficult to determine the proportional constant. Hence, for iteration to determine k_1 and k_2, concentrations of both $[A]_o$ and [B] were replaced by their fluorescence intensities. This procedure is able to be done because [B] and $[A]_o$ have the same unit of mol/L, whereas k_1 and k_2-k_1 have the same unit of sec^{-1}. Hence, both left and right side of equation (4) as the result of conversion from equation (2) have no unit (dimensionless).

$$\frac{[B]}{[A]_o} = \frac{k_1}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right) \qquad (4)$$

Results of wavelength and time scanning are presented in Fig. 1 and Fig. 2, respectively. Fig. 1 showed that the isoindole derivative of amino acid has maximum excitation wavelength (λ_{ex}) at 335 nm and maximum emission wavelength (λ_{em}) at 450 nm. These

Table 1 Fluorescence intensity of 1-alkyl-thio-2-alkyl substituted isoindole as the result of reaction between excess of OPA and 2-mercaptoethanol with glycine, detected at various reaction time using λ_{ex} = 335 nm and emission λ_{em} = 450 nm

Reaction Time/ second	Fluorescence Intensity		
	Computation	Experiment	Difference
50	1799.1	1766.0	33.1
70	2153.4	2161.2	7.8
90	2392.1	2419.8	27.7
110	2551.2	2570.4	19.2
130	2655.8	2655.4	0.4
150	2722.8	2731.4	8.6
170	2764.1	2760.0	4.1
190	2787.8	2778.0	9.8
210	2799.3	2784.0	15.3
230	2802.5	2804.6	2.1
250	2800.0	2789.6	10.4
270	2793.7	2792.6	1.1
290	2784.7	2790.6	5.9
310	2773.9	2785.2	11.3
330	2761.9	2756.2	5.7
350	2749.1	2761.2	12.1
370	2735.8	2737.2	1.4
390	2722.1	2721.2	0.9
410	2708.2	2721.2	13.0
430	2694.2	2705.2	11.0
450	2680.2	2686.8	6.6
470	2666.1	2664.8	1.3
490	2652.1	2651.8	0.3
510	2638.0	2636.8	1.2
530	2624.0	2622.8	1.2
Total		66254.0	211.5
Error = (Total Differences/Total Experiments)x100%			0.32%

Table 2 Fluorescence intensity of 1-alkyl-thio-2-alkyl substituted isoindole as the result of reaction between excess of OPA and 2-mercaptoethanol with isoleucine, detected at various reaction time using λ_{ex} = 335 nm and emission λ_{em} = 450 nm

Reaction Time/ second	Fluorescence Intensity		
	Computation	Experiment	Difference
12	168.2	161.0	7.2
52	646.5	647.3	0.8
92	1019.2	1018.4	0.8
132	1309.2	1303.0	6.2
172	1534.1	1535.0	0.9
212	1708.1	1710.6	2.5
252	1842.1	1836.6	5.5
292	1944.8	1936.2	8.6
332	2022.8	2023.2	0.4
372	2081.6	2078.2	3.4
412	2125.2	2132.8	7.6
452	2156.9	2157.8	0.9
492	2179.4	2190.8	11.4
532	2194.6	2196.8	2.2
572	2204.1	2206.8	2.7
612	2209.1	2218.8	9.7
652	2210.6	2221.8	11.2
692	2209.4	2206.8	2.6
732	2206.1	2212.8	6.7
772	2201.1	2191.8	9.3
812	2194.9	2187.8	7.1
852	2187.6	2187.0	0.6
892	2179.6	2179.0	0.6
932	2170.9	2170.0	0.9
972	2161.8	2161.0	0.8
Total		47071.3	110.6
Error = (Total Differences/Total Experiments)x100%			0.23%

results agree very well compared with the result of previously reported studies [9,11].

In determining $[A]_o$, k_1 and k_2; iteration program as shown in Appendix 1 was used. Value of $[A]_o$ for glycine was chosen 2800-3200 with the increase of 10 units because maximum intensity of its isoindole was 2804.6; while for k_1 was chosen 0.01500-0.02500 with the increase 0.00001 unit. After iterative process using intensity data of the isoindole as shown in the third column in Table 1; 0.32% minimum error obtained to give values of $[A]_o$ = 2980, $k_1 = 18.75 \times 10^{-3}$ sec^{-1}, and $k_2 = 26.70 \times 10^{-5}$ sec^{-1}. This result was satisfied as the plot of fluorescence intensity versus time for both experiment and computational results are overlapping each other as shown in Fig. 2.

In determining $[A]_o$, k_1 and k_2 of isoleucine, value of $[A]_o$ was chosen 2000-2500 with the increase of 10 units because maximum intensity of its isoindole was 2221.8; while for k_1 was chosen 0.00000-0.01000 with the increase 0.00001 unit. After iterative process using intensity data of the isoindole as shown in the third column in Table 2; 0.23% minimum error was obtained to give values of $[A]_o$ = 2400, $k_1 = 6.06 \times 10^{-3}$ sec^{-1}, and $k_2 = 12.59 \times 10^{-5}$ sec^{-1}. This result was satisfied because the plot of fluorescence intensity versus time for both experiment and computational results are overlapping each other as shown in Fig. 3. Comparison of Fig. 2 to Fig. 3 showed that the degree of overlapping between experiment and computational results of isoleucine was better than that of glycine. This may be due to detection of fluorescence intensity of isoindole produced from isoleucine was relatively less noisy compared to isoindole obtained from glycine.

CONCLUSION

Iteration method could be used for the determination of consecutive reaction rate constants with high precision from the data of abundance of intermediate product at various reaction times. Reaction rate constants k_1 and k_2 for consecutive reaction of glycine with excess of *o*-phthaldialdehyde and 2-mercaptoethanol were 18.75×10^{-3} and 26.70×10^{-5} sec^{-1}, respectively; whereas for isoleucine were 6.06×10^{-3} and 12.59×10^{-5} sec^{-1}, respectively.

ACKNOWLEDGEMENT

Authors thank Erlin Ismawati, for her partial involvement in laboratory work. Acknowledgement is also directed to Prof. Dr. M. Utoro Yahya, M.Sc., who gave valuable suggestions and correction of the manuscript.

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