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RATE ACCELERATIONS IN THE HYDROLYSIS OF SALICYLIC PHOSPHATES UNDER MICELLAR CONDITIONS - A STRUCTURE REACTIVITY STUDY

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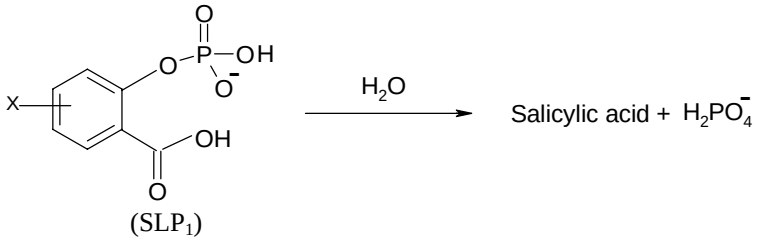
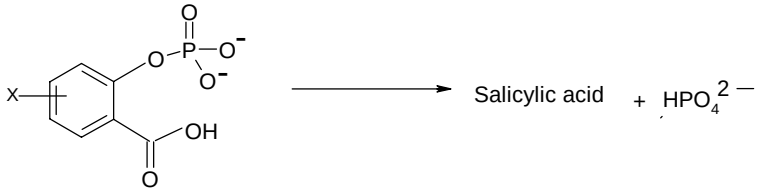
ABSTRACT

Spontaneous hydrolysis of salicylic phosphate is too sluggish even at elevated temperatures in aqueous buffer media. However, the reaction is dramatically accelerated under micellar conditions even at room temperature. This procedure worked efficiently in SDS (sodium dodecyl sulfate) and TX (Triton-X-100) media even at room temperature. Menger-Portnoy's enzymatic model and Piszkiewicz co-operativity model were used to explain the mechanism of hydrolysis under micellar conditions.



(SLP_1)

Route 1



(SLP_2)

Route 2

Key words: Acceleration, Hydrolysis, Salicyclic phosphates, Micellar.

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INTRODUCTION

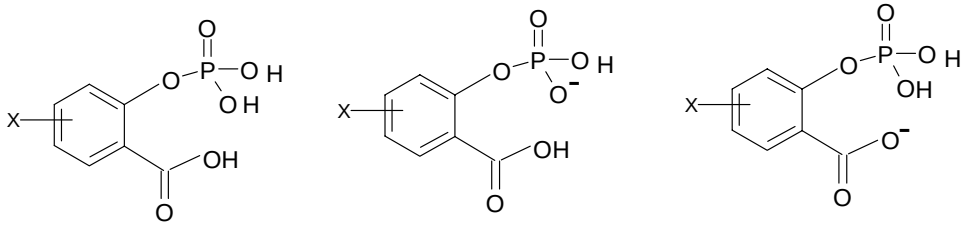
Phosphate groups are present in proteins, nucleic acids, and other bimolecular systems that play a key role in the living world with a special reference to the transfer of phosphoric groups in the biochemical processes[1-3]. It is also well known that metal ions catalyze the enzymic and non-enzymic hydrolysis of large number of phosphate esters[4-10]. It is understood that metal ions enhance the rate of hydrolysis through complex formation in which neutralization of charge, enhancement of nucleophilicity, polarization of P-O bonds and alignment of reactants facilitate the reaction. In view of this, the study of hydrolysis of organic phosphates has become the subject of research interest to chemists and biologists during the past few decades[4-13].

During the past few decades there has been an upsurge in exploiting the utility of non-ionic, cationic and anionic surfactants as catalysts in a variety of biologically important reactions owing to their analogous behavior with enzymes[14-20]. In recent past we have been focusing our attention on the use of micelles as catalysts in different types of organic reactions[17-18]. Micellar mediated organic reactions are of great interest because of the relationship in close parallelism with enzyme processes[14]. However, such studies with the phosphate hydrolysis appeared to be scarce in literature. Nevertheless, spontaneous hydrolysis of salicylic phosphate has been studied earlier by Chanley et al.[9-12] in aqueous buffer media, where it is reported that rate of hydrolysis was too slow. In the present study, we have reported kinetics of hydrolysis of salicylic phosphate under micellar conditions to gain an insight into the mechanistic aspects.

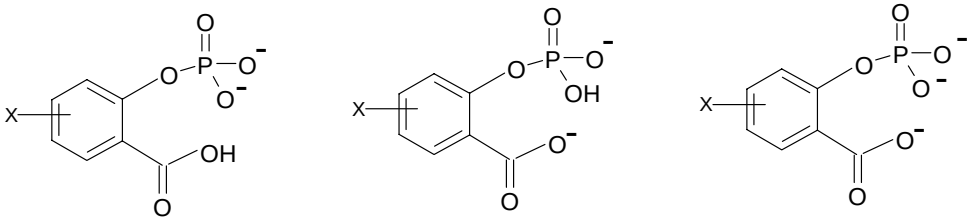
EXPERIMENTAL

Salicylic phosphates have been prepared according to literature procedures and recrystallized before use. All the other chemicals are either Aldrich or E-Merck grade samples. Progress of the hydrolysis of phosphate ester was followed by estimating the amount of phosphoric acid (free phosphate) liberated during the course of hydrolysis as a function of time, according to literature reports[13]. 2 mL aliquots of the reaction solution were transferred into a 10 mL volumetric flask containing 5 mL ice cold water and kept for 5-6 hrs at ice cold temperature. Extremely low temperature of ice arrests the progress of hydrolysis completely. The above solution is then, neutralized by the addition of NH_3 and then 1.0 mL of 0.2% $MgCO_3$ suspension and 1.0 mL of 5% $CaCl_2$ solution. Calcium phosphate precipitates out after sometime. In this procedure $MgCO_3$ acts as entrainer. The precipitate thus obtained was separated by centrifugation and dissolved in 60% $HClO_4$. To this solution 1.0 mL of 5% ammonium molybdate and 0.5 mL of ANS (1-amino-4-naphthol

sulfonic acid) reagent were added and the solution was made up to 10 mL with distilled water. The flask was then placed in a constant temperature bath at 25°C for 10 minutes for the development of blue color. The absorbance of the solution was immediately measured at 660 nm. The phosphate content has been computed from standard calibration curve constructed by using standard KH_2PO_4 solutions of various concentrations.



A (SLP_0) B (SLP_1) C (SLP_1)



D (SLP_2) E (SLP_2) F (SLP_3)

RESULTS AND DISCUSSION

Kinetic and mechanistic features of native reaction

The rate of hydrolysis of salicylic phosphate (SLP) has been found to be too sluggish even at elevated temperatures under normal conditions. Yet it has been found to depend on pH and followed first order kinetics. The k' vs pH profile indicated a hill-type curve with maximum hydrolysis around 4.8 to 5.8 pH, as shown in Fig. 1.

In aqueous solutions salicyl phosphate (SLP) exists as undissociated (SLP_0) and a variety of dissociated forms (SLP_1, SLP_2, SLP_3) according to the following equilibria. These species are shown in the structures (A to F).

$$SLP_0 \overset{K_1}{=\!=} SLP_1 + H^+ ; (SLP_1) = \frac{K_1 (SLP_0)}{(H^+)} \quad \ldots(1)$$

$$SLP_1 \overset{K_2}{=\!=} SLP_2 + H^+ ; (SLP_2) = \frac{K_2 (SLP_1)}{(H^+)} \quad \ldots(2)$$

$$SLP_2 \overset{K_3}{\rightleftharpoons} SLP_3 + H^+ ; (SLP_3) = \frac{K_3 (SLP_2)}{(H^+)} \quad \ldots(3)$$

In steps (1) to (3) K_1, K_2 and K_3 represent the dissociation constants of SLP species. The observed rate of hydrolysis, therefore, can be taken up as the algebraic sum of contribution of the above species.

$$\text{The rate of hydrolysis} = k'[SLP]_T$$

$$k'[SLP]_T = k_0 [SLP_0] + k_1[SLP_1] + k_2[SLP_2] + k_3[SLP_3]$$

$$\text{(or)} \quad k' = k_0M_0 + k_1M_1 + k_2M_2 + k_3M_3$$

In the above equation, k' = observed rate constant; k_0, k_1, k_2 and k_3 are specific rate constants for the species SLP_0, SLP_1, SLP_2 and SLP_3 respectively. M_o, M_1, M_2 and M_3 are the corresponding mole fractions of SLP_o, SLP_1, SLP_2 and SLP_3, respectively.

$$M_0 = [SLP_0] / [SLP]_T = [H^+]^3/Y$$

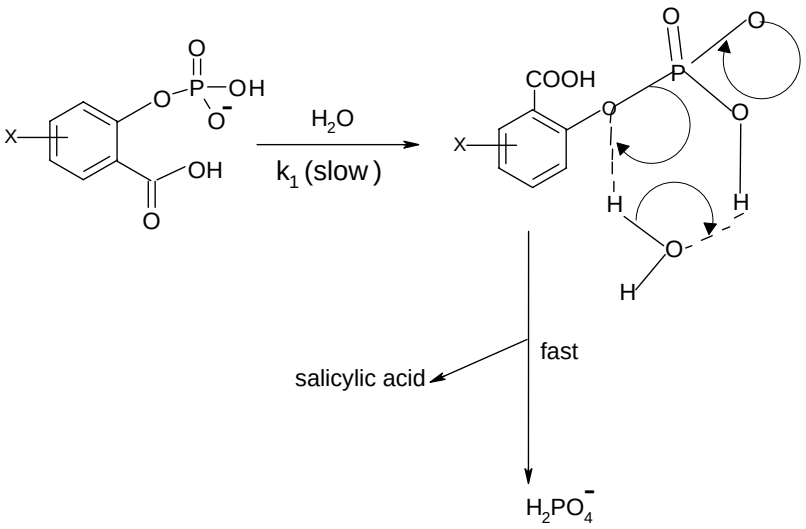
$$M_1 = [SLP_1] / [SLP]_T = K_1[H^+]^2/Y$$

$$M_2 = [SLP_2] / [SLP]_T = K_1K_2[H^+]/Y$$

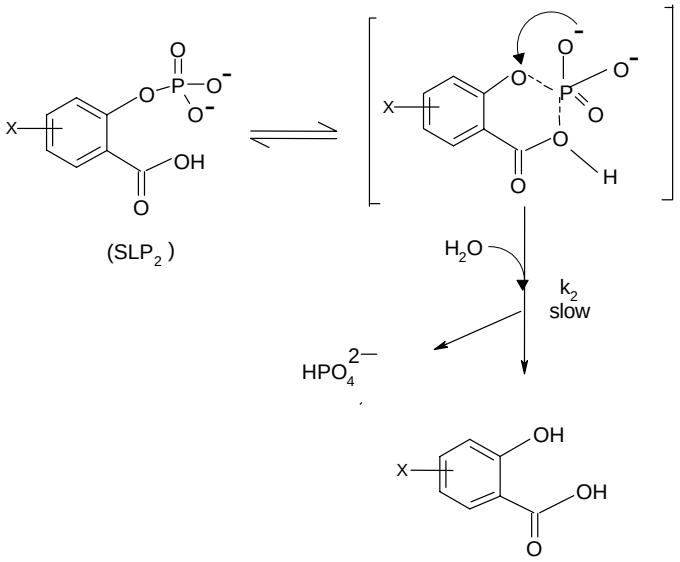
$$M_3 = [SLP_3] / [SLP]_T = K_1K_2K_3/Y$$

and $$Y = ([H^+]^3 + K_1[H^+]^2 + K_1K_2[H^+] + K_1K_2K_3)$$

Substitution of dissociation constants in various expressions at a known pH provided mole fractions M_o, M_1, M_2 and M_3 accordingly. The values of mole fractions presented in Table 1 reveal that in the pH range 2 to 11, concentration of SLP_0 is less than 10% while concentration of SLP_3 is appreciable above pH = 8. It was, therefore, reasonable to consider that the observed rate constant (k') is mainly the sum of SLP_1 and SLP_2 contributions, which are predominating at 2.30 and 5.67 respectively. However, SLP_3 appears to be important over the other species above 5.67 pH. The specific rate constants k_1, k_2 and k_3 values have been computed at various temperatures and the corresponding activation parameters have been compiled in Table 2. The data presented in Table 2 reveal that entropies of activation are more negative for SLP_2 indicating the formation of a relatively more rigid transition state than for SLP_1 species. **Scheme 1** represents the most plausible mechanism for the hydrolysis of Salicylic Phosphate in low pH range (below 3.10 pH) where SLP is mostly in the form of SLP_1.



Scheme 1: Hydrolysis of (SLP_1) species



Scheme 2: Hydrolysis of (SLP_2) species

As cited in earlier part of the text the concentration of SLP_2 gradually increases above 3.10 pH. The enthalpy of activation (ΔH_2) for the hydrolysis of SLP_2 is found to be far less over the other two steps. This trend indicates that rate of hydrolysis of SLP_2 is faster than the hydrolysis of other SLP species. The observed entropy of activation (ΔS_2) value is more negative compared to the other steps of hydrolysis. This could be probably explained

due the formation of a dianionic cyclic intermediate formed from the rearrangement of SLP_2 species. The cyclic dianionic intermediate thus formed could possibly bind with water through inter molecular hydrogen bonding prior to the decomposition. The mechanism of hydrolysis of SLP_2 is shown in **Scheme 2**.

In order to gain a further insight into the mechanism of hydrolysis of SLP, the reaction has been studied in the presence of anionic (sodium dodecyl sulphate-SDS) and non-ionic (triton-X 100-Tx) surfactants. However, such a study with cetyl trimethyl ammonium bromide (CTAB, a cationic micelle) was not fruitful because of the formation of a precipitate immediately after the addition of CTAB to SLP solution.

Table 1: Mole fractions of salicylic phosphate in aqueous buffer media

pH	$SLP_o(M_o)$	$SLP_1(M_1)$	$SLP_2(M_2)$	$SLP_3(M_3)$
2.30	0.004	0.880	0.025	-
3.00	0.019	0.890	0.120	-
3.25	-	0.560	0.440	-
4.00	-	0.070	0.910	0.020
5.65	-	0.010	0.890	0.100
6.93	-	-	0.360	0.640
7.70	-	-	0.007	0.900

Table 2: Specific rate constants and activation parameters of hydrolysis of salicylic phosphate in aqueous medium

Specific rate constants (s^{-1}) (303K)	$\Delta H^{\#}$ $kJmol^{-1}$	$\Delta G^{\#}$ $kJmol^{-1}$	$\Delta S^{\#}$ $JK^{-1}mol^{-1}$
$10^7k_1 = 4.73$	70.8	111	- 132
$10^7k_2 = 9.60$	57.1	109	- 171
$10^7k_3 = 4.00$	79.1	112	- 109

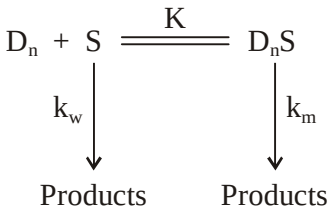
Kinetics and mechanism of SDS mediated hydrolysis of SLP

Variation of [SDS] on rate of hydrolysis has been extensively studied under different pH conditions using phthalate buffers. Below pH 3.80 rate of hydrolysis was substantially inhibited with an increase in [SDS], while above pH 3.80 rate of hydrolysis was significantly

catalyzed. As typical cases the rate data are presented in Table 3 and Fig. 2. Data presented in Tables 1 and 3 show that below pH 3.00, mono-anionic species of SLP are significant and in the range of pH 3.00 to 4.00 both mono-anionic and di-anionic species are present in significant proportions. The observed inhibition of SDS could be probably explained as the unfavorable electrostatic repulsions between negatively charged SLP_1 species (indicated as S in the following Scheme) and negatively charged surface of SDS. The mechanism in the presence of SDS in this range could be reasonably explained by the cooperatively model suggested by Piszkiewicz[20].

Table 3: Effect of pH and [SDS] on the rate of hydrolysis of salicylic phosphate

10^3[SDS] mol dm^{-3}	$10^6 k_\phi$ (sec^{-1}) at pH									
	2.20		2.60		3.80		4.20		5.80	
	303K	313K	303K	313K	303K	313K	303K	313K	303K	313K
2	3.57	6.46	6.14	11.2	8.46	15.6	8.26	14.2	13.0	23.8
4	3.03	6.13	5.98	9.87	7.86	13.8	8.50	14.8	13.3	25.5
8	3.03	6.10	5.91	9.75	7.35	13.6	8.82	15.6	13.2	25.2
10	2.74	5.94	5.85	9.58	7.18	13.1	8.90	15.8	13.0	24.8
12	2.69	5.92	5.81	9.54	7.04	12.9	8.96	16.0	12.8	24.2
14	2.65	5.90	5.80	9.52	6.90	12.8	9.01	16.1	12.5	23.7
16	2.64	5.90	5.80	9.50	6.78	12.7	9.03	16.3	12.2	23.3
18	2.64	5.89	5.77	9.48	6.68	12.6	9.03	16.3	12.1	23.2
22	2.62	5.87	5.57	9.46	6.48	12.5	9.05	16.5	12.1	23.2



Rate law for this scheme could be given as,

$$\log \{\frac{k_\phi - k_w}{k_m - k_\phi}\} = n \log [D] + \log K$$

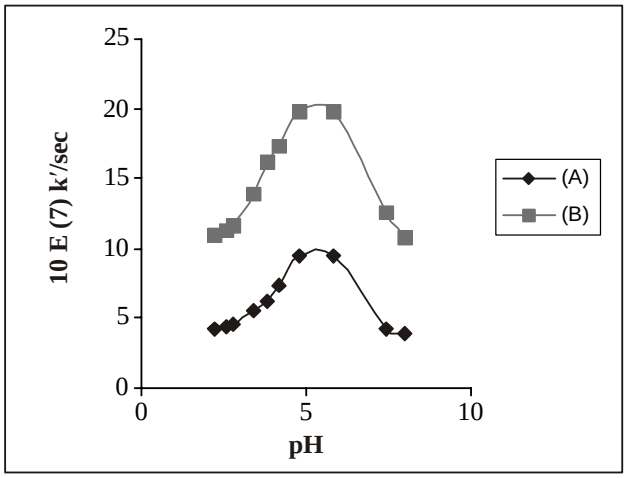


Fig. 1: Effect of pH on hydrolysis of salicylic phosphate, Temp = (A) 303K (B) 313K

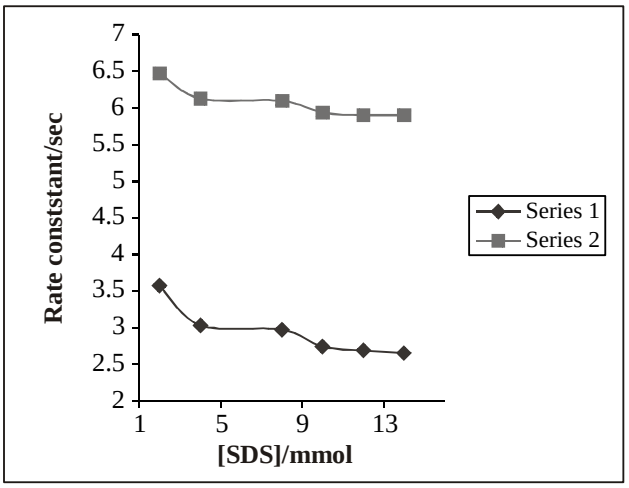


Figure-2: Effect of pH and [SDS] on the rate of hydrolysis of salicylic phosphate, Seires-1: pH = 2.20, Temp =303K; Seires-2: pH = 2.60, Temp =303K

According to the above equation plots of log $[(k_\phi - k_w)/(k_m - k_\phi)]$ as a function of log[D] have been found to be linear. Minimum values of observed rate constants k' in the presence of micelles were taken as k_m values. Binding constants K were evaluated at different temperatures and corresponding thermodynamic parameters were also presented in Table 5. Observed catalysis for SLP hydrolysis above pH 4.00 could be better explained due to favorable hydrophobic interactions although the electrostatic repulsions between dianionic species of SLP and negatively charged surface of micelle are expected to prevail. Considerably greater rate enhancement at higher pH and retardation in lower pH could also

be reasonably attributed to saturation of the anionic micelles by hydronium ions at higher acidities there by rendering them catalytically ineffective at higher acidities[14]. Mechanism in the SDS in this range could be explained due to cooperative model. Micelle substrate binding constants were evaluated by using the following equation as cited by Menger and Portnoy[19].

$$\{\frac{k_\phi - k_w}{k_m - k_\phi}\} = \frac{k_w + k_m K C_D}{1 + K C_D}$$

The values of k_m and K are presented in Tables 5 & 6.

Kinetics and mechanism of triton X-100 mediated reaction

Kinetic data presented in Table 4 revealed that rate of hydrolysis decreased with an increase in [Tx] below pH 4.00, while the rates are enhanced with [Tx] above pH 4.0. Although the surface of Tx-100 micelle is neutral, the oxygen of polyoxy ethylene group could create a negative surface that could develop an electrostatic repulsion with the mono ionic SLP species (which is the main species in the pH range below 4.00) causing observed rate inhibition. Cooperativity model [20] has been used to explain the observed trends. The observed rate accelerations above 4.00 pH, could be due to hydrophobic interactions, which appear to over weigh the electrostatic repulsions. Kinetic and thermodynamic parameters have been evaluated by considering Menger's [19] model applicable for micellar catalysis.

Table 4: Effect of pH and [Triton-X] on the rate of hydrolysis of salicylic phosphate

[Triton-X] % (v/v)	$10^6 k_\phi$ (sec^{-1}) at pH									
	2.20		2.60		3.80		4.20		5.80	
	303K	313K	303K	313K	303K	313K	303K	313K	303K	313K
0.15	9.40	19.3	5.86	12.2	4.77	11.6	5.73	12.7	5.72	12.3
0.25	9.36	19.3	5.84	12.1	4.94	11.6	5.80	12.9	5.72	12.4
0.45	9.13	19.7	5.35	11.8	5.53	11.8	6.17	13.2	5.73	12.7
0.55	7.67	15.9	4.82	9.32	5.83	12.4	6.78	13.9	5.75	12.8
0.65	6.99	14.0	4.50	9.12	6.08	13.2	7.02	15.1	5.81	12.8
0.75	6.66	13.4	4.36	9.06	6.18	13.3	7.17	15.5	5.81	12.9
0.85	6.44	13.2	4.27	9.06	6.23	13.4	7.27	15.8	5.82	12.9
0.95	6.28	13.1	4.24	9.04	6.24	13.5	7.32	16.0	5.82	13.0

Table 5: Binding constants (K) and thermodynamic parameters of SLP

pH	K (303K)	ΔH kJmol^{-1}	ΔG kJmol^{-1}	ΔS JK^{-1}mol^{-1}
(A) SDS system				
12.20	19.4	-25.6	-7.44	-59.9
2.60	4.71	10.0	-3.89	46.0
3.80	16.8	6.40	-7.09	2.00
4.20	74.6	4.58	-39.7	146
(B) Triton-X system				
12.80	0.735	0.430	0.773	-1.130
3.40	0.572	-0.704	1.40	-6.95
4.20	32.5	-25.7	-20.31	-17.8
5.80	3.8	-63.9	-14.9	260

Table 6: Rate constants and activation parameters of SLP

pH	k (303K)	$\Delta H^{\#}$ kJmol^{-1}	$\Delta G^{\#}$ kJmol^{-1}	$\Delta S^{\#}$ JK^{-1}mol^{-1}
(A) SDS system				
14.20	8.69	41.9	103	203
(B) Triton-X system				
14.20	0.618	58.6	110	170
4.80	0.729	58.8	109	168
5.80	0.820	59.9	110	166

Structure-reactivity study

It is of interest to note that structural variation in the phenyl ring of salicylic phosphate affect the rate of hydrolysis to a greater extent according to the trend : 5-nitro > H > 5-methyl, in SDS as well as Tx media, although rate of hydrolysis was not altered to any significant extent in aqueous buffer media (Tables 7 & 8). The observed trend shows that the rate of hydrolysis favored by the introduction of electron with drawing groups in the ring due to inductive (-I) effect.

Table 7: Hydrolysis of 5-methyl salicylic phosphate

pH	Rate constant 10^5 k (sec^{-1})		$\Delta H^{\#}$ $kJmol^{-1}$	$\Delta G^{\#}$ $kJmol^{-1}$	$\Delta S^{\#}$ $JK^{-1}mol^{-1}$
	(303K)	(313K)			
			(A) 10^3[SDS] = 8 mol dm^{-3}		
12.20	1.40	3.63	72.3	102	99.3
2.40	1.37	3.00	58.9	102	143
2.60	1.31	2.87	59.3	102	142
2.80	1.37	2.96	57.9	102	146
4.60	1.63	3.80	64.1	102	125
5.40	18.7	37.1	51.3	95.8	147
	10^4 k(sec^{-1})		(B) [Triton-X] = 0.55%		
12.80	3.37	7.03	55.5	94.4	128
4.40	46.5	88.1	47.7	87.8	132
4.80	1.81	3.78	55.5	95.9	133
5.40	113	220	50.0	85.5	117
5.60	11.6	24.3	56.0	91.2	117

Table 8: Hydrolysis of 5-nitro salicylic phosphate

pH	Rate constant 10^5 k (sec^{-1})		$\Delta H^{\#}$ $kJmol^{-1}$	$\Delta G^{\#}$ $kJmol^{-1}$	$\Delta S^{\#}$ $JK^{-1}mol^{-1}$
	(303K)	(313K)			
			(A) 10^3[SDS] = 8.00 mol dm^{-3}		
12.20	2.28	5.43	65.7	107	136
2.40	2.47	5.63	62.4	106	146
2.60	2.03	4.89	66.6	107	134
2.80	1.86	4.01	57.7	107	164
4.60	13.2	27.1	54.2	102	159
5.40	39.3	80.2	56.2	99.8	143
	10^4k(sec^{-1})		(B) [Triton-X] = 0.55%		
12.80	2.84	5.83	54.0	94.8	134
3.40	2.90	6.01	54.8	94.7	131
4.80	5.61	12.4	60.0	93.1	109
5.40	6.49	14.2	59.3	92.7	110
5.80	8.17	17.2	56.3	92.1	118

CONCLUSION

Rates of phosphate (SLP) hydrolysis have been found to be pH dependent in both native and micellar mediated reactions. Hydrolysis reactions of salicylic phosphate (SLP) are dramatically altered by anionic (SDS) and non-ionic (TX) micelles indicating that rate of hydrolysis is highly sensitive to both hydrophobic and electrostatic interactions. In micellar media rate hydrolysis was found to depend on the structure of salicylic phosphate even though such variation could not affect the rate of hydrolysis to any significant extent in aqueous medium.

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