

tides agreed both in kind and quantity with those <sup>8,9</sup> that would be obtained from hemoglobin that had not been treated with enzyme.

The results of the present investigation bear upon several as yet unanswered questions about the action of proteolytic enzymes on proteins. (1) They show that certain enzymes act vigorously on hemoglobin, a protein which is probably as "native" or "undenatured" as any. (2) They indicate that the type of reaction is the so-called "one-by-one" type <sup>10</sup>; that is, at any time after the action of the enzyme has begun, a molecule has either reacted to the fullest possible extent or it has not reacted at all. The identity of the non-precipitable products at various stages of reaction and the normal nature of the N-terminal DNP-peptides of the trichloroacetic acid precipitate are strong evidence for this conclusion. (3) They show that, if an enzyme must denature a protein prior to cleaving it or if it reacts only with the denatured protein of a reversible equilibrium between denatured and undenatured protein <sup>11</sup>, then, the denaturation is of a different type than that produced by heat because the products from "undenatured" and heat-denatured hemoglobin differ.

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## Kinetics of the Reaction between $\beta$ -Sulfinylpropionic Acids and Iodide Ion in Acid Solution

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The rate of the reaction:  $RSOR' + 2H^+ + 2I^- \rightarrow RSR' + I_2 + H_2O$  where  $R = HOCOCH_2CH_2-$ ,  $R' = HOCOCH_2CH_2-$ , and  $CH_3CH_2CH_2-$ , has been studied at 25°C in solutions of perchloric acid and perchloric acid-sodium perchlorate. With hydrogen- and iodide ions in excess the reaction is kinetically of the first order, *i.e.*, one obtains the rate expression:

$$k = \frac{1}{t} \ln \frac{a}{a-x}$$

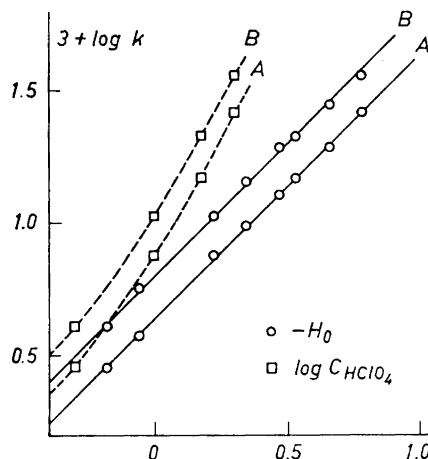
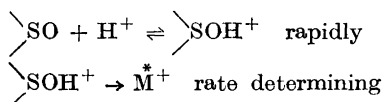


Fig. 1. Log  $k$  as a function of  $-H_0$  (unbroken lines) and of  $\log C_{HClO_4}$  (dashed lines) for the reaction for A and B, respectively, with iodide ion in acid solution at 25°C. (The dashed curves refer to perchloric acid solutions without any added sodium perchlorate.)

Table 1. The rate of the reaction for  $\text{HOCOCH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2\text{COOH}$  (A) and  $\text{CH}_3\text{CH}_2\text{CH}_2\text{SOCH}_2\text{CH}_2\text{COOH}$  (B), respectively, with iodide ion in acid solution at 25°C.

$C_{\text{HClO}_4}$ , M	$C_{\text{NaClO}_4}$ , M	10% $k$ min <sup>-1</sup>		$-H_0$	$-(\log k + H_0)$	
		A	B		A	B
2.00	0	26.2	36.2	0.78	2.36	2.22
1.50	0	14.8	21.1	0.53	2.36	2.21
1.00	0	7.56	10.6 <sub>5</sub>	0.22	2.34	2.19
0.50	0	2.86	4.07	-0.18	2.36	2.21
1.50	0.50	19.4	27.8	0.66	2.37	2.22
1.00	1.00	12.7	19.2	0.47	2.37	2.19
1.00	0.50	9.76	14.4	0.34 <sub>5</sub>	2.36	2.19
0.50	0.50	3.73	5.67	-0.06	2.37	2.19

where  $a$  and  $a-x$  are the concentrations of the sulfinyl compound at the start of the experiment and after  $t$  minutes, respectively<sup>1</sup>. In Table 1 the observed rate constants  $k$  (in min<sup>-1</sup>) are correlated to the acidity function  $H_0$  as defined by Hammett<sup>2,3</sup>. Hereby a fairly good linearity between  $\log k$  and  $-H_0$  with unit slope has been obtained (Fig. 1), which according to the Zucker-Hammett hypothesis<sup>4</sup> means that the first step of the reaction consists of a rapid proton transfer to the oxygen atom in the sulfinyl group:



The activated complex  $\text{M}^*$  then reacts rapidly with a nucleophilic reagent such as an iodide ion.

**Materials and method.** The sulfinyl acids have been obtained through oxidation of the corresponding sulfide acids in acetone solution with perhydrol in an excess of 10%. The sulfide acids were prepared in the following manner:

(a)  $\beta$ -Thiodipropionic acid from  $\beta$ -chloropropionic acid and sodium sulfide in water solution<sup>1</sup>.

(b)  $\beta$ -Propylmercaptopropionic acid from  $\beta$ -mercaptopropionic acid, sodium hydroxide and  $n$ -propylbromide in water-ethanol solution<sup>5</sup>. By using 70 g  $\beta$ -mercaptopropionic acid 89.5 g (91%)  $\beta$ -propylmercaptopropionic acid was obtained, b.p. 154°/20 mm Hg,  $n_{\text{D}}^{20} = 1.4803$ ,  $d_4^{20} = 1.0735$ . (Found: C 48.3; H 8.07; S 21.4;  $M_{\text{R}_D} = 39.24$ . Calc. for  $\text{C}_6\text{H}_{12}\text{O}_2\text{S}$ : C 48.6; H 8.16; S 21.6;  $M_{\text{R}_D} = 39.41$ .)

The sulfinyl acids could easily be purified through recrystallization from water.

The melting point of  $\beta$ -propylsulfinylpropionic acid (about 75°) was not sharp, probably due to a disproportionation at this temperature. (Found: C 43.9; H 7.40; S 19.4; equiv. wt 164.6. Calc. for  $\text{C}_6\text{H}_{10}\text{O}_3\text{S}$ : C 43.9; H 7.37; S 19.5; equiv. wt 164.2.)

The kinetic experiments have been made at  $25.0 \pm 0.05^\circ\text{C}$ . An oxygen-free solution containing known quantities of sodium iodide, sodium perchlorate and perchloric acid was pre-heated to the temperature mentioned. At the time  $t = 0$  a certain quantity of a solution composed of sulfinyl acid, perchloric acid and sodium perchlorate was rapidly put down into it. This quantity had been carefully measured by means of an injection syringe. Thus the reaction mixture at this time had the following composition:  $C_{\text{sulfinyl acid}} = 5.00$  mM,  $C_{\text{iodide}} = 200$  mM,  $C_{\text{HClO}_4}$  and  $C_{\text{NaClO}_4}$  according to Table 1.

At the time  $t$  the reaction was stopped in such a way that the reaction mixture was rapidly cooled by addition of finely crushed ice. After that the liberated iodine could be titrated with sodium thiosulfate.

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