

same range of angles, corresponding to a radius of gyration of 18–19 Å. It is obvious that the acid hydrolysis had degraded the humate considerably (as also indicated by gel filtration in an earlier study²). The detailed picture of this degradation depends on the explanation finally given to the occurrence of more than one straight line in the Guinier plot of the original humate. The hydrolyzed humate will be studied further with small angle X-ray scattering.

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The Transient State Kinetics of Horse Liver Alcohol Dehydrogenase*

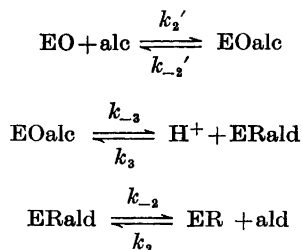
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The rapid mixing technique has made it possible to study the "pre-steady" state kinetics of enzyme reactions. When Bernard *et al.*¹ reported two distinct rate steps for the conversion of aromatic aldehydes into aromatic alcohols, we presumed that the initial rapid step at the "pre-steady" state is due to the rapid transformation of "ERald" to "EOalc" followed by the rapid dissociation of EOalc to EO + alc as shown in the following.**

* This communication was presented at the Wenner-Gren Symposium on "Structure and Function of Oxidation Reduction Enzymes", Stockholm, August 1970.

** Abbreviations: E, LADH; R, NADH; O, NAD⁺; ald, aldehyde; alc, alcohol.



Therefore, experiments were carried out to determine the rate constants of the individual steps using our "stopped-flow" spectrofluorimeter.

The solutions of E + R or E + O (E ≪ R or E ≪ O) were rapidly mixed with varying concentrations of different substrates in glycine-NaOH buffer, pH 9.0. A rapid appearance of the strong fluorescence of bound NADH is followed by a relatively slow appearance of the much weaker fluorescence of free NADH in the case of EO + ethanol or benzylalcohol (ER → E + R). A very rapid decrease of the ER fluorescence is followed by a slow decrease of the free R fluorescence in the case of the aldehydes being the substrates. ER and ERald seem to have the same fluorescence intensity as judged from "stopped flow" experiments in which ER was mixed with increasing concentrations of aldehydes. The total deflections after mixing kept reasonably constant until so high concentrations of aldehyde were reached that appreciable hydride transfer occurred during the "dead time" before observation.

A plot of the reciprocal of the initial rates of the rapid appearance of ER and ERald fluorescence against the reciprocal of the ethanol or benzylalcohol concentrations is shown in Fig. 1. The rate constants from the maximum velocities are calculated to be 100 sec⁻¹ and 6.4 sec⁻¹ for ethanol and benzylalcohol, respectively. The disappearance of the bound NADH fluorescence caused by acetaldehyde or benzaldehyde was too fast to be followed accurately. However, the approximate rate constants were estimated to be 300–500 sec⁻¹ for both cases. These rate constants should correspond to k_3 or k_{-3} which represent the rate of the hydride transfer. k_2 or k_2' can also be obtained from the slope of the plot shown in Fig. 1. From determinations of the dissociation constants of ERald or EOalc from the kinetics of the product inhibition,² it is possible to calculate k_{-2} or k_{-2}' . The data of the rate constants were

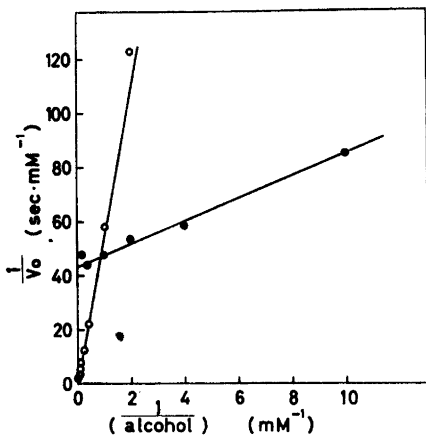


Fig. 1. A reciprocal plot of the initial rate at the "pre-steady" state against concentration of the substrate.

The solution of LADH ($4 \mu\text{N}$) + NAD^+ ($100 \mu\text{M}$) was rapidly mixed with various concentrations of ethanol (○) or benzylalcohol (●) in 0.1 M glycine-NaOH buffer, pH 9.0 at 23°C .

summarized in Table 1. The "burst" of the oxidation of ER by acetaldehyde or benzaldehyde corresponded to the stoi-

Table 1. Rate constants of the LADH reactions at pH 9.0.

Rate constants	Ethanol	Benzylalcohol
k_2' ($\mu\text{M}^{-1}\text{sec}^{-1}$)	0.0045	0.065
k_{-1}' (sec^{-1})	20	—
k_{-3} (sec^{-1})	100	6.4

chiometric formation of one mole of bound NAD^+ per one active site of LADH while the reduction of EO by ethanol or benzylalcohol corresponded to less than one mole of bound NADH per one active site of LADH. However, the stoichiometry of $\text{EO} + \text{alc}$ as judged from the size of the "burst reaction" is rather uncertain, since the size of the "burst reaction" is quenched in the presence of large excess of alcohols. Our value $k_{-3} = 100 \text{ sec}^{-1}$ at pH 9 is in essential agreement with J. Shore's 130 sec^{-1} at pH 7.³

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Electrolytic Cleavage of β -Ketosulfones

II.* Cleavage of Some α -Substituted β -Ketosulfones

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In Part I of the present series,¹ the electrolytic reductive cleavage of some β -ketosulfones to give ketones was described. Of the eight different β -ketosulfones studied in Part I,¹ all except one that had an α -methyl group, were unsubstituted in the α -position. In order to investigate the generality of the reaction, the work has now been extended to include some further β -ketosulfones carrying various substituents in the α -position, namely, methyl, ethyl, benzyl, and carbethoxymethyl. The substituted β -ketosulfones have been prepared by the ion pair extraction technique.²

The electrolytic reductive cleavage of the substituted compounds has been carried out using the same conditions as before¹ (mercury cathode, aqueous dimethylformamide, pH 7.8, undivided cell). In the work-up of the benzyl ketones, however, ether instead of pentane was used. This demanded more washing with water in order to

* For Part I, see Ref. 1.

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