[CONTRIBUTION FROM THE KEDZIE CHEMICAL LABORATORY, MICHIGAN STATE UNIVERSITY]

# Kinetics of the Amino Acid-catalyzed Dealdolization of Diacetone Alcohol[1]

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RECEIVED MAY 6, 1957

Velocity determinations have been carried out for the dealdolization of diacetone alcohol in three amino acid buffers. The data indicate that, other than hydroxide ion, the amino acid anion catalyzes this reaction by acting as an amine. Cupric ion inhibits the glycinate-catalyzed reaction, apparently by formation of the $Cu(glycinate)_3^-$ ion which is probably catalytically inactive.

Catalysis of the aldol condensation by amino acids is particularly interesting in connection with certain carbohydrate transformations. For example, it appears that this may be one of the principal effects by amino acids during their interaction with reducing sugars in what is known as the Maillard reaction.[2] In addition to this, the possibility of similarity between this catalysis by amino acids and that by certain of the aldolases should not be ignored.

The previous investigations[3-5] of amino acid- and peptide-catalyzed aldolizations did not include precise rate measurements for any of these reactions. Neither did they identify the species which are mainly responsible for the catalytic activity. Since this information was desired in order to substantiate work in this Laboratory on the Maillard reaction, as well as to provide a possible clue to the mechanism of enzyme-catalyzed aldol condensations, the problem has been reinvestigated kinetically. The present writing describes catalysis of the dealdolization of diacetone alcohol by glycine, DL-$\alpha$-alanine and $\beta$-alanine buffers. It also describes the effect of cupric and magnesium ions on catalysis by the glycine–glycinate system.

## Experimental

**Materials.**—Eastman Kodak diacetone alcohol was redistilled before each run, b.p. 60–62° (14 mm.). Ammonia-free glycine (Eastman) was recrystallized from a methanol–water mixture (21% methanol by volume). DL-$\alpha$-Alanine (Pfanstiehl Chemical Co.) and $\beta$-alanine (Eastman) were recrystallized from ethanol–water mixtures. All other reagents were C.P. quality.

**Procedure.**—The procedure for velocity determinations was essentially the same as that employed by Westheimer and Cohen[6] in their investigation of the amine-catalyzed dealdolization of diacetone alcohol. Carbon dioxide-free water was used for the buffer solutions which were prepared by adding the appropriate amount of sodium hydroxide to the amino acid. Unless indicated otherwise, the ionic strength of the reaction mixtures was adjusted to 1.0 by addition of sodium chloride. The reaction velocities were measured dilatometrically at 18.60° in a thermostat which maintained this temperature during actual measurements to within 0.002°. The observed rate constants for the reaction, which was strictly first order in diacetone alcohol, were evaluated by Guggenheim's method[7] using decadic logarithms.

(1) This work was carried out under contract with the Quartermaster Food and Container Institute for the Armed Forces. The views presented here are the authors'.

(2) L. C. Maillard, *Compt. rend.*, **154**, 66 (1912).

(3) F. G. Fischer and A. Marschall, *Ber.*, **64B**, 2825 (1931).

(4) W. Langenbeck and G. Borth, *ibid.*, **75B**, 951 (1942).

(5) E. V. Budnitskaya, *Biokhimiya*, **6**, 146 (1941).

(6) F. H. Westheimer and H. Cohen, THIS JOURNAL, **60**, 90 (1938).

(7) E. A. Guggenheim, *Phil. Mag.*, [7] **2**, 538 (1926).

## Results and Discussion

Observed rate constants obtained for different concentrations and ratios of amino acid buffers are given in Table I.

TABLE I

OBSERVED AND SPECIFIC RATE CONSTANTS FOR REACTIONS CATALYZED BY AMINO ACID ANIONS AND AMINES

| System | Amino acid anion, $M$ | Amino acid, $M$ | $k' \times 10^5$, min.$^{-1}$ | $k_A \times 10^4$, mole$^{-1}$ min.$^{-1}$ |
|---|---|---|---|---|
| Glycine | 0.10 | 0.10 | 18.1[a] | |
| | .25 | .25 | 44.2[a] | |
| | .30 | .30 | 53.3 | |
| | .40 | .40 | 73.7 | |
| | .50 | .50 | 94.9 | |
| | .20 | .067 | 36.8 | |
| | .40 | .133 | 74.0 | |
| | .45 | .150 | 86.0 | |
| | | | | 18.5 |
| DL-$\alpha$-Alanine | 0.20 | 0.20 | 15.5 | |
| | .30 | .30 | 25.3 | |
| | .40 | .40 | 32.5 | |
| | | | | 8.1 |
| $\beta$-Alanine | 0.10 | 0.10 | 29.2 | |
| | .20 | .20 | 58.5 | |
| | .30 | .30 | 80.0 | |
| | | | | 26.4 |
| Methylamine | | | | 133[b] |
| Dimethylamine | | | | 8.8[b] |

[a] Ionic strength = 0.5; for all other runs $\mu$ = 1.0. [b] Data of Westheimer and Cohen.[6]

Westheimer and Cohen[6] observed that in the dealdolization of diacetone alcohol catalyzed by primary and secondary amines, the catalytic species are hydroxyl ion and the molecular amine. No catalysis by the substituted ammonium ion in the buffers used for their experiments was detected. By analogy, the observed rate constants ($k'$) should fit the following expression, since the principal species arising from the amino acids in these

$$k' = k_{OH^-}[OH^-] + k_A[A] \qquad (1)$$

systems are the anion (A) and the zwitterion. This has been realized in the present experiments, for, as shown in Fig. 1, plots of $k'$ against amino acid anion concentration are linear. Moreover, the slopes are identical for two different ratios of the glycine buffers. Hence, these catalytic effects are very probably also examples of what has been termed specific amine catalysis. Values for the specific rate constants for these amino acid-catalyzed reactions are given in Table I together with values obtained at 18.05° by Westheimer for methylamine and dimethylamine. Comparison of

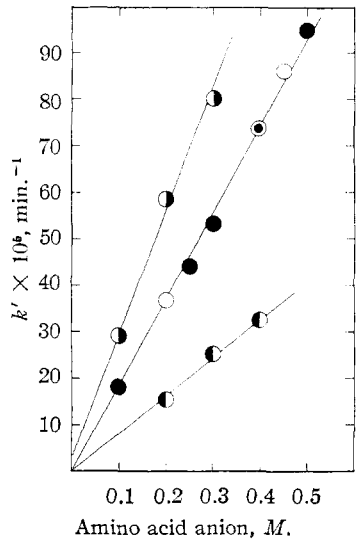


Fig. 1.—Observed rate constants at 18.60° in different amino acid buffers: ◑, β-alanine; ●, glycine ([glycinate]/[glycine] = 1); ○, glycine ([glycinate]/[glycine] = 3); ◐, DL-α-alanine.

these data reveals that these amino acid anions are somewhat poorer catalysts than is methylamine. From this it follows that no pronounced effect on the catalysis by the amino group arises from the presence of a neighboring carboxyl group.

In view of the known effects by heavy metal ions on the activity of certain aldolase preparations, it seemed desirable to test the influence of added metal ions on the present systems. Magnesium and cupric ions (added as the chlorides) were chosen for experiments with the glycine system inasmuch as of the several glycine complexes investigated by Albert[8] those formed by $Mg^{++}$ and $Cu^{++}$ are, respectively, the least and most stable. The results given in Table II show that the presence of $Mg^{++}$ depresses the rate of the glycinate-catalyzed reaction only slightly. On the other hand, the diminution in reaction rate when $Cu^{++}$ was introduced into this mixture should be regarded as significant. Moreover, if it is assumed that the $Cu(glycinate)_3^-$ ion, which has been determined polarographically[9] as the predominating cupric complex under the conditions of the present experiments, is catalytically inactive, it becomes possible to calculate the magnitude of this effect by $Cu^{++}$ on the glycinate-catalyzed reaction. The agreement between $k'$ where $Cu^{++}$ was added to the glycine–glycinate system and that computed on the basis of Keefer's dissociation constant for the $Cu(glycinate)_3^-$ ion[9] is quite good (Table II).

(8) A. Albert, *Biochem. J.*, **47**, 531 (1950).
(9) R. M. Keefer, THIS JOURNAL, **68**, 2329 (1946).

TABLE II

EFFECT OF METAL IONS ON THE GLYCINATE-CATALYZED REACTION

| | Metal ion, $M$ | Amino acid anion, $M$ | Amino acid, $M$ | $k' \times 10^5$, min.$^{-1}$ Obsd. | Calcd. |
|---|---|---|---|---|---|
| $Mg^{++}$ | 0.01 | 0.30 | 0.30 | 51.5 | .. |
| $Cu^{++}$ | 0.01 | 0.30 | 0.30 | 48.3 | 48.0 |

The results of these experiments shed little new light on the mechanism of catalysis by the aldolases. Nevertheless, it should be pointed out that the action of skeletal muscle aldolase may be analogous to that of the amine catalysts since it may involve the reaction of an amino group with the carbonyl group of dihydroxyacetone phosphate. Such a mechanism is in conformity with the following known facts about muscle aldolase, the latter two being of lesser significance for the present argument: (1) the marked inhibition of the enzyme by $Cu^{++}$ and $Ag^+$, this effect being of greater magnitude than that produced by $Hg^{++}$,[10] (2) the enzyme's rather high content of basic amino acids,[11] and (3) an optimum *p*H of 7.2 for the enzyme[12] as compared with an isoelectric point of 5.7.[13,14] It is also conceivable that such an interaction of an amino group on the enzyme and the carbonyl group of dihydroxyacetone phosphate can account for the aldolase-catalyzed specific exchange of hydrogen on dihydroxyacetone phosphate with tritium of the solvent observed by Rose and Rieder.[15] Further work on this problem is contemplated.

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(11) S. F. Velick and Ethyl Ronzoni, *J. Biol. Chem.*, **173**, 627 (1948).
(12) A. L. Dounce, Shirley R. Barnett and G. T. Beyer, *ibid.*, **185**, 769 (1950).
(13) J. F. Taylor, Arda A. Green and Gerty T. Cori, *ibid.*, **173**, 591 (1948).
(14) The data for both the *p*H optimum and the isoelectric point given here were obtained with aldolase from rat skeletal muscle.
(15) I. A. Rose and S. V. Rieder, THIS JOURNAL, **77**, 5764 (1955).