Buffer Effects in the Nonenzymatic Transamination of L-Alanine and Pyridoxal

By F. P. SIEGEL and M. I. BLAKE

The kinetics of the reaction between L-alanine and pyridoxal at 100° in various acetate–acetic acid, formate–formic acid, and pivalate–pivalic acid buffers were evaluated. The reaction was found to be general acid and base catalyzed.

THE PYRIDOXAL-mediated transamination reaction is an important reversible biological process which occurs between certain amino and keto acids. The mechanism of enzymatic and nonenzymatic transamination has been reviewed by Snell *et al.* (1, 2). Vernon (3) studied the kinetics of the reaction of pyridoxamine and pyruvate at 25° and pH 10. Blake *et al.* (4) reported the general acid catalyzed reaction of L-alanine and pyridoxal at 100° in an acetic acid–acetate buffer system. The authors indicated, however, that simple catalysis did not prevail and that further experiments were required. Bruice and Topping (5–8) studied the imidazole-catalyzed reaction between α-aminophenylacetic acid and pyridoxal. The rate-limiting step was proposed to be the abstraction of a proton from the α-carbon of the intermediate imine. This was substantiated by Blake *et al.* (4) in their study of the L-alanine-pyridoxal system by comparing the rates of L-protio-alanine with L-deuterio-alanine. Similar studies relating to L-deuterio-glutamic acid are in press (9).

Bruice and Topping (6) proposed a concerted general acid and base mechanism as operative in their imidazole-catalyzed system. This paper explores the L-alanine–pyridoxal system concerning the possible existence of both general acid and base catalysis.

EXPERIMENTAL

The rates of transamination between 0.2 *M* L-alanine and 0.01 *M* pyridoxal were carried out at 100° in 1:2, 1:1, and 2:1 sodium acetate–acetic acid, sodium formate–formic acid, and sodium pivalate–pivalic acid buffer systems.

The buffer solutions were prepared at an ionic strength of 0.4. Sodium chloride was used to maintain the constant ionic strength. The buffers were prepared from weighed amounts of acetic acid and sodium acetate and by neutralization of weighed amounts of purified formic or pivalic acids with a standardized sodium hydroxide solution to produce the prescribed ratio of acid to salt.

The first-order reaction was followed by measuring the disappearance of pyridoxal with time

$$-\frac{d\,(\text{pyridoxal})}{dt} = K_1\,(\text{pyridoxal})$$

using the spectrophotometric procedure of Siegel and Blake (10), based on the formation of a yellow condensation product formed with acetone in the presence of base.

Received August 26, 1964, from the College of Pharmacy, University of Illinois, Chicago.
Accepted for publication September 25, 1964.
Presented to the Scientific Section, A.PH.A., New York City meeting, August 1964.

The reaction mixtures were prepared for each run from weighed quantities of L-alanine and pyridoxal hydrochloride dissolved in the appropriate buffer. The solution was transferred to 5-ml. neutraglas ampuls, sealed, and placed in a boiling water bath at 100° at zero time. At suitable intervals, ampuls were removed, plunged into ice water, and the contents then analyzed for pyridoxal at room temperature using a Coleman junior spectrophotometer.

RESULTS AND DISCUSSION

The first-order velocity constants are proportional to the concentration of acid species for all three buffer systems (Fig. 1).

Figure 2 shows that the first-order velocity constants are also proportional to the salt species concentration for all three buffer systems, an indication of probable simultaneous general acid-base catalysis.

The K for the hydrogen ion catalysis obtained from the data (see intercepts Figs. 1 and 2) agrees with previously obtained data for the unbuffered reaction at various pH values (4).

The catalytic constants for both the acid and the base species appear to be extremely close for each buffer system. This is demonstrated by the plot of K *versus* total [acid species] + [base species], shown by Figs. 3 and 4.

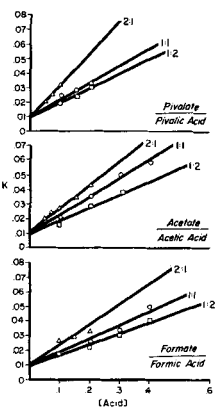


Fig. 1.—Plots of K *vs.* [acid] for the three buffer systems.

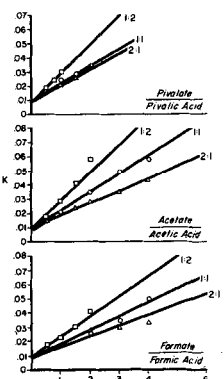


Fig. 2.—Plots of K *vs.* [base] for the three buffer systems.

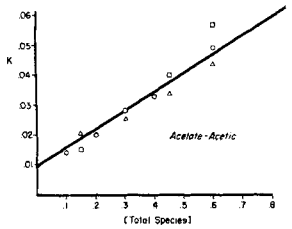


Fig. 3.—Plot of K *vs.* [acid] + [base].

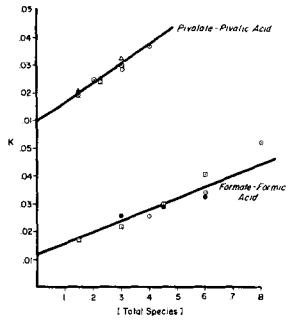
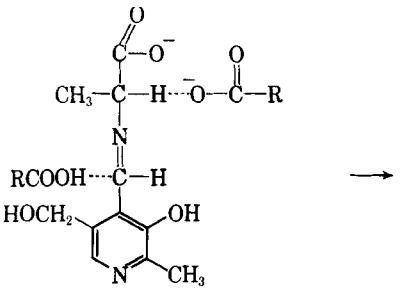
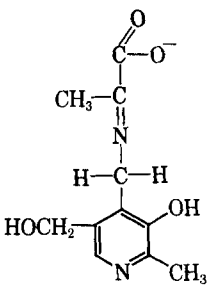


Fig. 4.—Plot of K *vs.* [acid] + [base].





Scheme I

The magnitude of catalysis follows in the order of formic acid < acetic acid < pivalic acid.

Thus, the appearance of equi-acid-base catalysis in the above transamination supports the likelihood of the simultaneous push–pull mechanism of Swain and Brown (11), proposed by Bruice and Topping (6).

Scheme I depicts the rate-limiting step in transamination showing the concerted removal of the α-hydrogen by the base species and the addition of a proton from the acid species.

REFERENCES

(1) Snell, E. E., and Jenkins, W. T., *J. Cellular Comp. Physiol.*, **54**, 161(1959).

(2) Snell, E. E., *et al.*, eds., "Chemical and Biological Aspects of Pyridoxal Catalysis," The Macmillan Co., New York, N. Y., 1963.

(3) Banks, B. E. C., Diamantis, A. A., and Vernon, C. A., *J. Chem. Soc.*, **1961**, 4235.

(4) Blake, M. I., *et al.*, *J. Am. Chem. Soc.*, **85**, 294(1963).

(5) Bruice, T. C., and Topping, R. M., *ibid.*, **84**, 2450 (1962).

(6) *Ibid.*, **85**, 1480(1963).

(7) *Ibid.*, **85**, 1488(1963).

(8) *Ibid.*, **85**, 1493(1963).

(9) Lin, S. L., Blake, M. I., and Siegel, F. P., THIS JOURNAL, in press.

(10) Siegel, F. P., and Blake, M. I., *Anal. Chem.*, **34**, 397 (1962).

(11) Swain, C. G., and Brown, J. F., *J. Am. Chem. Soc.*, **74**, 2534(1952).

Tissue Culture Method for Screening Toxicity of Plastic Materials to be Used in Medical Practice

By S. A. ROSENBLUTH, G. R. WEDDINGTON, W. L. GUESS, and J. AUTIAN

In the past, a number of methods has been used to evaluate toxicities of plastic items for use in medical and related fields. This paper describes a tissue culture method utilizing monolayers of strain L 929 mouse cells in modified Eagle's medium. Samples of plastics are placed in direct contact with cell monolayers. After 24 hours, cells adjacent to the plastic samples are examined for toxic manifestations. Controls include toxic and nontoxic plastic samples. Up to the present time, a large number of plastic samples has been screened by the tissue culture method, and the method has been found to be more sensitive, more rapid, and less expensive than the previously employed intramuscular implantation technique in rabbits.

ONE OF THE most successful tests used in screening of plastic items to be used in medical practice is the implantation method originated by Brewer and Bryant (1). This technique consists of implanting small strips of the plastic sample into the paravertebral muscle of rabbits for a period of 3 to 7 days, at which time the animals are sacrificed and the sites of implants examined both macroscopically and by histopathological methods. Such a method was employed to screen a number of plastic items used in medical practice (2).

Even though the intramuscular implantation technique in rabbits has proven useful to the authors and other investigators in screening plastic items,

Received August 26, 1964, from the Drug-Plastic Research Laboratory, College of Pharmacy, University of Texas, Austin.

Accepted for publication October 7, 1964.

Presented to the Scientific Section, A.PH.A., New York City meeting, August 1964.

This investigation was supported by grant CA-06120 from the National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

The authors are indebted to Dr. Royce Z. Lockart, Jr., Associate Professor of Microbiology, University of Texas, Austin, for technical advice.