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Nature of the Amino Acid Catalysis of the Glucose Mutarotation Reaction

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ABSTRACT

*Because they possess both an acid and a base function it is to be expected (and is widely held) that amino acids, particularly histidine, are catalysts for the mutarotation of reducing sugars. However, 0·014*M *histidine increased the mutarotation rate of glucose by only 3–4% although the molar ratio of amino acid to sugar was about 1:10. The limited action on either* α-D- *or* β-D-*glucose was nonstereospecific with respect to the amino acid enantiomer employed. The contribution of the amino acid to the slightly increased glucose mutarotation rate appears to be due to a general increase in buffer concentration.*

INTRODUCTION

The mutarotation of reducing sugars is catalyzed by acids and bases, and both are necessary (Pigman & Isbell, 1968). As water is an ampholyte, it serves as an excellent solvent for spontaneous catalysis of the phenomenon. Whenever the solvent employed possesses only the function of a base (e.g. pyridine), the reaction becomes autocatalytic (Hill & Shallenberger, 1969) as the sugars themselves are ampholytes and serve as the acid in this case.

As the amino acids possess both an acid and a base function (i.e. they are 'zwitterionic') it is to be expected that these substances are effective catalysts for the mutarotation reaction. In support of this expectation it

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has been reported (Westheimer, 1937) that amino acids are in fact catalysts for the mutarotation of glucose, particularly 0·014M histidine which 'at pH 6 is a better catalyst than the same concentration of a strong acid'. This finding prompted Bentley & Bhate (1960) to explore the possibility that either histidine or histylhistidine might be the active site for the enzyme mutarotase. The results they obtained, however, could not be distinguished from the spontaneous mutarotation found in water and D_2O alone.

With respect to the ability of different amino acids to catalyze glucose mutarotation it was found (Westheimer, 1937) that there is little difference between bases with a net negative charge, bases with a net negative charge but also containing dipolar ion, bases with no net charge, and bases with a positive charge. From these results it might be concluded that the effect of amino acids on the rate of reducing sugar mutarotation is due either to a general effect of employing a buffer or to increasing the buffer concentration. A second, but remote, possibility is that the effect is stereomeric. As the present author has an interest in the stereochemistry of sugar–amino acid interactions (cf. Shallenberger, 1983) a study was initiated to establish the possible differential effect of D- and L-histidine on the rate of mutarotation of the anomeric glucoses in the presence and absence of a buffer. The results obtained serve as the basis of this report.

MATERIALS AND METHODS

The crystalline sugars employed (α- and β-D-glucose) were dried overnight in a vacuum oven at 40 °C and then cooled and stored in a desiccator over P_2O_5. Their optical rotatory constants in water ($c = 2$, $M = 0{\cdot}11$) are used as data in the Results section and agree well with literature values. The L-histidine monochloromonohydrate used had $[\alpha]_D^{15} = +4{\cdot}3°$ and the D-enantiomer had $-5{\cdot}4°$.

For buffered systems, the proper proportion of 0·2M acetic acid and 0·2M sodium acetate was used to prepare 0·1M acetate buffer, pH 4·0 (actual, 3·95). This buffer (or water only in unbuffered systems), at the appropriate temperature, was used to prepare 2·0 % (w/v) solutions of the sugars. The sugar solutions were then quickly transferred to a 2-dm jacketed polarimeter tube. Thereafter, the polarimeter automatically monitored the course of the mutarotation reaction.

The polarimeter used was a Rudolph Research (Fairfield, NJ) Autopol

III equipped with a strip chart recorder. The polarimeter tube was kept at the desired temperature by circulating an ethylene glycol–water mixture through the jacket of the tube using an American Instrument Co. (Silver Spring, MD) constant temperature laboratory bath equipped with a circulating system.

CALCULATIONS

For α-D-glucose, the equilibrium rotation (obtained after 24 h) was subtracted from all readings (observed rotations) and the regressions of the logarithms of the difference between observed and final rotations ($\ln \Delta$) was computed. The slope of the regression equation so obtained is k_m for the first-order equilibrium mutarotation coefficient.

In the equilibrium expression

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B$$

A is α-D-glucopyranose and B is β-D-glucopyranose. For the data reported herein k_m, or $k_1 + k_2$ is multiplied by 10^3 to give small whole decimal values, and is the rate expression employed.

The specific rotations for the sugars reported herein are corrected to account for the contribution of the histidine.

RESULTS

Preliminary studies were conducted at 28 °C without temperature control to re-establish the general course of the α-D-glucose mutarotation in water only. The preliminary studies also included the glucose mutarotation in the presence of unbuffered 0·014M D- and L-histidine, unbuffered 0·014M D- and L-asparagine and 0·015M HCl. All treatments yielded mutarotation curves suggesting that there was very little difference, among the various treatments, on the course of the glucose mutarotation even though pH, for example, differed widely (1·5–5·9); the molar ratio of amino acid to sugar was 0·126:1.

As the initial report to the effect that the glucose mutarotation reaction is catalyzed by amino acids employed a temperature of 18 °C, further studies were made at 15 °C (±0·1 °). The results of a first-order kinetic

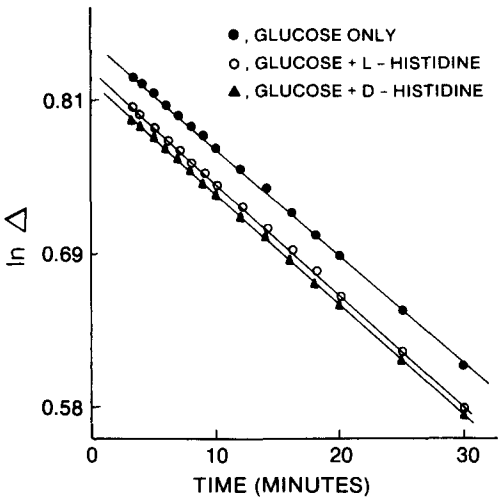

Fig. 1. First-order kinetic plot for the mutarotation of α-D-glucose at 15 °C, buffered at pH 4, and in the presence of D- and L-histidine (0·014M), also buffered at pH 4.

plot of the α-D-glucose mutarotation in acetate buffer at pH 4·0 and in the presence of D- and L-histidine at 15 °C are shown in Fig. 1. The fit of the data to first-order reaction kinetics is ideal (*r* greater than 0·999). This is true also for all subsequent data obtained. The plots show very little difference in mutarotation rate.

The rate constants for the mutarotations along with the initial and final specific rotations are shown in Table 1. They indicate that D- and L-histidine increased the α-D-glucose mutarotation rate by 3–4%, with a corresponding 3–4% decrease in the initial specific rotation and a 3–4% decrease in the final specific rotation. The differential effect between the enantiomeric histidines is negligible.

When approaching the glucose mutarotation equilibrium from the other direction by starting with β-D-glucose in the presence of the acetate buffer, results of the same order were obtained. The mutarotation rate was increased by 3% in the presence of either D- or L-histidine, and the initial and final specific rotations were diminished by 3%.

On repeating the above studies at 15 °C in the absence of buffer, the results were again essentially the same. These data are also given in Table 1. However, in the presence of acetate buffer, the overall α-D-glucose rate constants are 6% higher and those of β-D-glucose are 17% higher than those obtained in the absence of buffer.

TABLE 1

Glucose Mutarotation Rate Constants and the Initial and Equilibrium Specific Rotations at 15 °C in the Presence and Absence of Buffer (pH 4·0)

System	*Buffer present*			*Buffer absent*		
	Initial $[\alpha]_D$	*Final* $[\alpha]_D$	*Rate* $(k_1 + k_2 \times 10^3)$	*Initial* $[\alpha]_D$	*Final* $[\alpha]_D$	*Rate* $(k_1 + k_2 \times 10^3)$
α-D-glucose only	+112·1°	+53·6°	8·15	+112·4°	+54·6°	7·60
Plus 0·014M L-histidine	+109·5°	+51·8°	8·45	+110·5°	+53·6°	7·95
Plus 0·014M D-histidine	+108·0°	+50·9°	8·36	+111·9°	+54·6°	7·92
β-D-glucose only	+18·6°	+52·4°	7·92	+18·7°	+52·6°	6·68
Plus 0·014M L-histidine	+17·9°	+51·4°	8·19	+17·8°	+53·6°	6·89
Plus 0·014M D-histidine	+18·3°	+52·1°	8·18	+18·2°	+53·2°	7·00

DISCUSSION

The preliminary results obtained at 28 °C in the unbuffered solutions were surprising. In retrospect, they should not have been since it is known (Nelson & Beegle, 1919) that the glucose mutarotation rate is constant over the pH range 2–7, and if 0·014M histidine were to be as strong a mutarotation catalyst as a strong acid at the same concentration, then HCl at 0·015M, or at a pH even less than 2, could not be a very effective catalyst (cf. Danehy & Pigman, 1951). Furthermore, the general effect of increasing buffer concentrations on the mutarotation rate of glucose is well established. The data of Los *et al.* (1956) show that each 0·1M increase in the buffer concentration will increase the $k_1 + k_2 \times 10^3$ rate constant (k_m) for α-D-glucose by 5·8 units at 25 °C. At a temperature 10 °C lower (15 °C) increases of 0·51 units and 1·24 units, caused by the 0·1M acetate buffer, were found for α-D-glucose and β-D-glucose, respectively. In an attempt to put the results presented in this paper in proper perspective, it can be calculated from the data of Hudson & Dale (1917) that $k_1 + k_2 \times 10^3$ (k_m) for α-D-glucose will vary by 0·7 units for each degree of temperature change. Thus the magnitude for the differences in rate constants reported herein might also have been obtained by a mere change of 1 °C in temperature.

It is concluded therefore that the increased mutarotation rate of α- and β-D-glucose by amino acids is rather slight and largely due to a general effect of increasing the buffer concentration of the system. This general effect is enhanced again by addition of a traditional buffer. Presumably the general effect is upon the structure of water, as it is nonstereospecific, and differences between different amino acids of the homologous series are presumably due to their differential buffering capacity and other intrinsic differences, such as hydrophobicity.

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