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# IMIDAZOLE AS A CATALYST IN ACYL TRANSFER

## A MODEL ENZYME SYSTEM FOR PHYSIOLOGICAL TRANSACETYLATION

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**Abstract**—The transfer of an acyl group from glucose penta-acetate to methanol, n-amylamine, and n-amyl mercaptan with imidazole catalysis has been observed and studied with the aid of PMR spectroscopy. Through rate studies of the reaction between glucose penta-acetate and methanol in the presence of imidazole, and as a result of the observation of imidazole-catalyzed epimerization of $\beta$- to $\alpha$-glucose penta-acetate, a mechanism for the transacetylation reaction has been proposed. The possibility that this mechanism can be applied to enzyme catalyzed physiological transacetylations is discussed.

### INTRODUCTION

EFFORTS to elucidate enzymatic catalysis have resulted in numerous and varied experimental approaches. The present study was made with the purpose of determining and supporting a mechanism for participation of the histidine moiety of protein enzymes in enzymatic catalysis by utilizing a model system[1] involving imidazole and a new substrate. Studies of the ability of imidazole, as a model of the active moiety of histidine, to catalyze the hydrolysis of acetate have been made previously.[2] In this investigation PMR spectroscopy was used to study the reaction involving imidazole as a model enzyme and $\alpha$- and $\beta$-glucose penta-acetate, as substrate, and methanol, n-amyl mercaptan, and n-amylamine as model receptors. The reaction studied was that of imidazole-catalyzed acyl transfer between the penta-acetate and the methanol, mercaptan, or amine, the majority of the work being done with $\alpha$-glucose penta-acetate and methanol. PMR provided an effective method of following the course of the reaction. A non-aqueous solvent, deuterochloroform ($CDCl_3$), was used. Other work claiming physiological significance but performed with non-physiological solvents has been criticized on this basis; however, we do not consider these objections to be valid. A recent PMR study, for example, has emphasized the compatibility of spectral observations on DNA fragments carried out in water and in dimethyl sulfoxide.[3]

The results obtained in this study indicate that imidazole catalyses the transfer of an acetyl group from glucose penta-acetate to methanol, to mercaptan, and to amine by direct participation as transient n-acetyl imidazole. (See references 2 and 9.) The

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[1] F. H. Westheimer, *Enzyme Models* in *The Enzymes* (2nd Edition) Vol. 1, p. 259. Academic Press, New York (1959).

[2] M. L. Bender, *Chem. Revs.* **60**, 53 (1960); M. L. Bender and B. W. Turnquest, *J. Amer. Chem. Soc.* **79**, 1663 (1957).

[3] See for example, L. Gattin and J. C. Davis, Jr., *J. Amer. Chem. Soc.* **84**, 4464 (1962).

possibility of the use of this reaction as a method of accomplishing acetylation should be perspicuous. The physiological significance arises from the possibility of applying the reaction with its proposed mechanism to enzymatic catalysis of transacylation and transphosphorylation. This paper presents the proposed mechanism with supporting experimental evidence as well as a brief resume of potential physiological applicability. It will be apparent that much additional investigation can and will be based on this initial work.

## EXPERIMENTAL

A Varian Model A-60 spectrometer operating at 60 Mc/sec was used throughout this study. All the chemical shifts are expressed in cycles per second (cps) at 60 Mc/sec from tetramethylsilane (TMS) as internal reference. Various concentrations of the several reactants were used in the course of the experiments, but solution effects were kept at a minimum by the consistent use of dilute concentrations. Rate studies were made of the methanol-penta-acetate reaction by obtaining the area under the methanol methyl proton peak at intervals and comparing it with the area under the internal TMS peak. These areas were obtained by using the integrator of the Varian A-60 recorder. The $\beta$-glucose penta-acetate was prepared by first grinding together 5 g dry glucose and 4 g anhydrous sodium acetate. The mixture was then added to 25 ml acetic anhydride and heated on a steam bath for about 2½ hr. The reaction mixture was then poured into about 300 ml ice water containing a few pieces of crushed ice. The solid product was collected, washed with cold water, and recrystallized from ethyl alcohol. The final product had a m.p. of 130·8–131·6°. The $\alpha$-glucose penta-acetate was prepared from the $\beta$-isomer by mixing with the acidic "isomerizing reagent" of Hudson and Montgomery.[4,5]

## RESULTS AND DISCUSSION

The PMR spectrum of glucose penta-acetate was observed to change with time when penta-acetate, imidazole, and methanol were mixed in $CDCl_3$ solution. The initial and final PMR spectra of the mixture when $\alpha$-glucose penta-acetate was used appear in Fig. 1. Peak assignments[6] are also presented in this Fig. It will be noted that the reaction is interpreted as the exchange of an acyl group between glucose penta-acetate and methanol producing $\alpha$-hemiacetal tetra-acetate and methyl acetate. This is shown clearly by the shift of the methanol methyl peak from −195 cps to −220 cps as a result of substitution of the more electronegative acyl group for the —OH group; by the disappearance of the axial acetate methyl peak at −131 cps with the simultaneous appearance of three additional protons in the −122 cps acetate proton region; and by the shift of the equatorial proton doublet at −377 cps upfield as a result of the substitution of the less electronegative —OH on the adjacent carbon atom.

The peaks due to the C—H protons of imidazole did not change in intensity or position. Apparently, imidazole undergoes no change in the over-all reaction. There was, however, no change in the spectrum of $\alpha$-glucose penta-acetate when methanol is added without imidazole. In the rate studies for this transacetylation reaction it was found that when the imidazole concentration was held constant the reaction was second-order with respect to methanol and $\alpha$-glucose penta-acetate (see Fig. 2). Likewise, it was observed that the rate was not affected when the molar concentration ratio of imidazole–methanol–penta-acetate was varied from 1–1–1 to 4–1–1 (see Fig. 3, B and C). However, when the ratio was changed to 0·5–1–1, there

[4] E. Montgomery and C. S. Hudson, *J. Amer. Chem. Soc.* **56**, 2463 (1934).

[5] The authors would like to acknowledge the assistance of Dr. M. T. Clark in this phase of the experimental work.

[6] R. U. Lemieux, *J. Amer. Chem. Soc.* **79**, 1005 (1957).

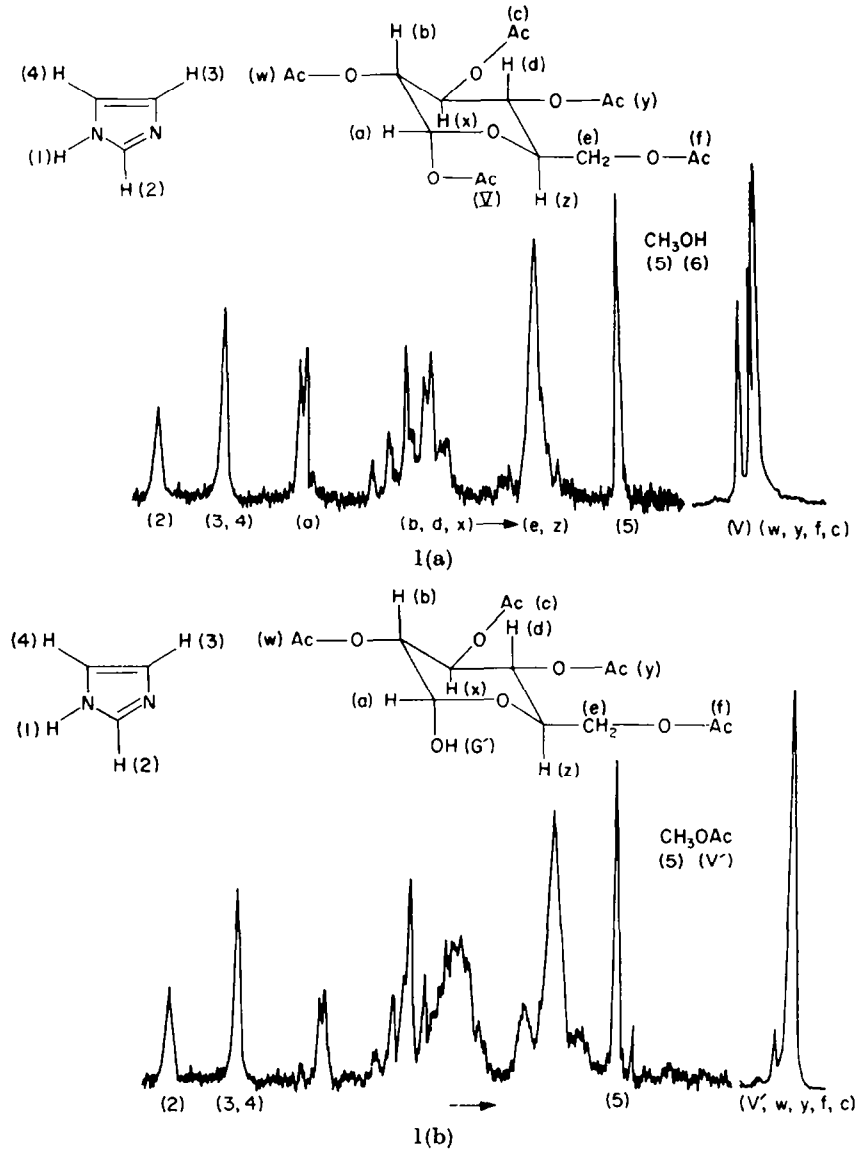



FIG. 1. (a) PMR spectrum of α-glucose penta-acetate–methanol–imidazole mixture immediately after mixing with assignment of peaks. (b) PMR spectrum of penta-acetate–methanol–imidazole mixture after reaction with assignment of peaks. Arrows indicate direction of increasing magnetic field strength. Upfield portion, i.e., the acetate methyl proton region, of both spectra was obtained at 1/10 spectrum amplitude of low field portion.

was a significant drop in the rate of the reaction (see Fig. 3). When the ratio was decreased further to 0·1–1–1, the rate was too slow to be measured accurately, using the A-60 integrator. This data indicates that between the molar concentration ratios of 0·5–1–1 and 1–1–1 that concentration of imidazole is reached above which an increase in imidazole concentration does not affect the rate of the reaction and below

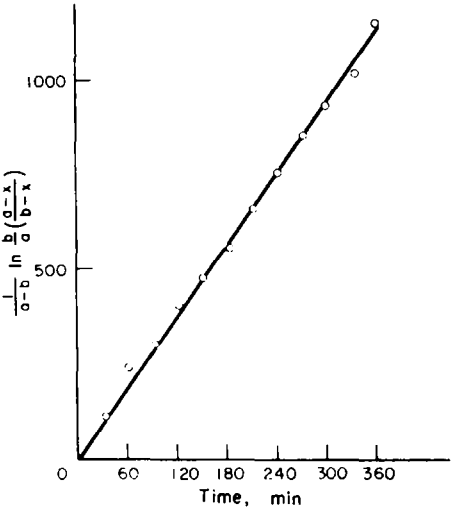



FIG. 2. Plot of $\frac{1}{a-b}$ in $\frac{b}{a}\frac{a-x}{b-x}$ vs. reaction time in min where a is the initial molar concentration of α-glucose pentaacetate, b the initial molar concentration of methanol, and x the molar concentration of methyl acetate at time t.

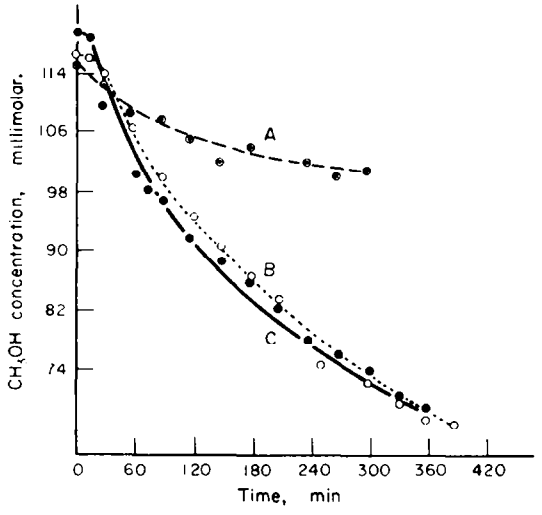



FIG. 3. Plot of concentration of methanol in moles/gm $CDCl_3$ vs. reaction time in min. Ratios of imidazole/methanol/penta-acetate are (A) 0·5/1/1, (B) 1/1/1, and (C) 4/1/1.

which its concentration plays an important role in the reaction kinetics. At this concentration ratio the maximum velocity of the reaction is reached. This would be the expected effect if imidazole participated in the reaction merely as a catalyst.

When 2-methylimidazole was substituted for imidazole, it was found that the reaction rate was approximately the same or perhaps slightly slower than the imidazole-catalyzed reaction. Since 2-methylimidazole is more basic than imidazole, the $pK_a'$s of imidazole and 2-methylimidazole being 6·95 and 7·86, respectively,[7] it would be expected that the reaction rate would have been increased if the catalytic action of the imidazole moiety was merely due to its basicity. Since the rate was not increased, it can be inferred that the catalytic action of the imidazole in the transacetylation reaction is due to a different type of participation.

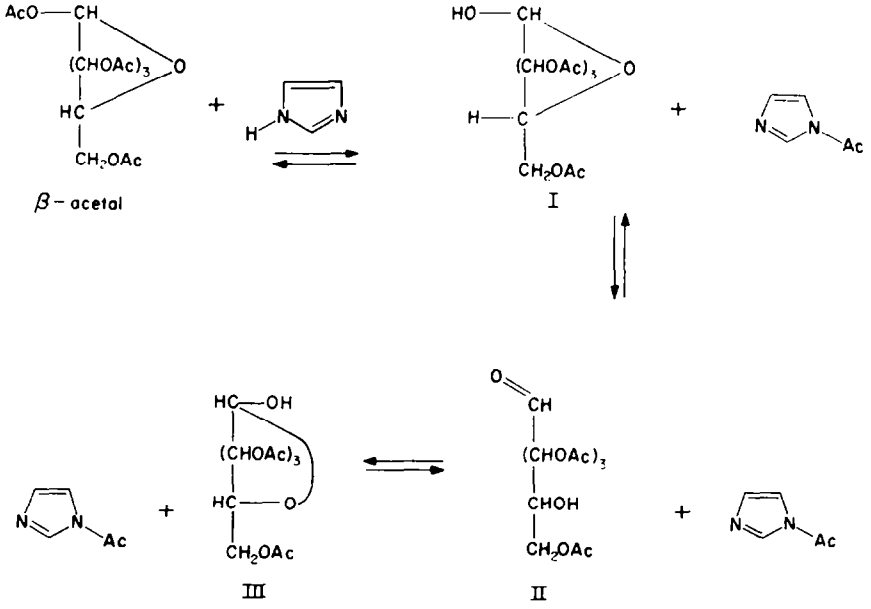
When β-glucose penta-acetate was substituted for the α-isomer, the rate of the

[7] K. Hofman, *The Chemistry of Heterocyclic Compounds: Imidazole and Its Derivatives* Part 1, p. 15. Interscience, New York (1953).

reaction was initially slightly faster but then became essentially the same as the rate of the reaction of the α-isomer. The PMR spectrum of the reaction mixture after the reaction was completed was the same when either the α- or the β-isomer were used as substrate. Since the PMR spectra of the α- and β-glucose penta-acetates are sufficiently different to permit a distinction between the two isomeric forms, this indicates that the final product is an equilibrium mixture of the tetraacetylated α- and β-hemiacetals.

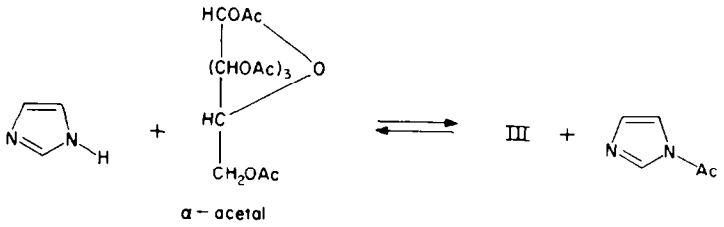
When α-glucose pentacetate was placed in $CDCl_3$ solution with imidazole, there was no apparent change in the PMR spectrum. When the β-isomer and imidazole were dissolved in $CDCl_3$, a reaction occurred, the final products being imidazole and α-glucose penta-acetate. In the absence of imidazole, there was no reaction. Since both penta-acetate isomers are in the acetal form, epimerization or equilibration of the α- and β-isomers, cannot occur unless the acetal linkage is broken. It must be postulated, therefore, that the imidazole is being acetylated to form N-acetyl imidazole, thereby releasing the β-hemiacetal which can now undergo equilibration, via aldehyde II, to the α-hemiacetal III. Hemiacetal III can then be actylated by N-acetyl imidazole to give α-glucose penta-acetate. Apparently, since there is no measurable quantity of β-glucose penta-acetate present in the equilibrium mixture, the α-isomer is the more stable form.[8]

The above reaction is considered as proof that imidazole reacts directly as a nucleophilic reagent in the transacetylation reaction, reacting with first one substrate to become N-acetyl imidazole and thence delivering the acyl group to a receptor molecule.[9] The following mechanism is demanded by the above epimerization and is compatible with the observed kinetics:




[8] C. R. Noller, *Chemistry of Organic Compounds* p. 377. W. B. Saunders Company, Philadelphia, Pennsylvania (1957).

[9] Compare T. C. Bruice and R. Lapinski, *J. Amer. Chem. Soc.* **80**, 2265 (1958); L. L. Ingraham, *Biochemical Mechanism* John Wiley, p. 41. New York (1962).




The physiological significance of this reaction lies in the possibility of applying this proposed mechanism to the study of enzymatic catalysis. Enzymes almost universally contain an imidazole moiety in the form of the amino acid histidine linked in the enzyme protein by a peptide bond. It would seem very likely that the imidazole moiety of the histidine group of trans-acetylase, for example, could act as a catalyst via the proposed mechanism, serving as the temporary carrier of an acyl group (*vide infra*). Although no work has yet been done with the transfer of phosphate groups, a similar mechanisn for transfer of such groups in physiological reactions might be cautiously proposed.

We found that imidazole also catalyzes the transfer of an acyl group from α-glucose penta-acetate to primary amines and to mercaptans. The possibilities of the use of the imidazole catalyzed formation of acetylamines and acetylmercaptans is again obvious. This also broadens the biochemical possibilities. Coenzyme A contains a sulfhydryl group which serves as a center of acetyl attachment. Acetyl coenzyme A, in the presence of an enzyme, transfers this acyl group to choline whose primary hydroxyl group accepts the acyl, forming ester. Further the enzyme thiotrans-acetylase is involved in the transfer of an acyl from acetyl lipoic acid to coenzyme A. The participation of an imidazole moiety in these enzymes is an interesting possibility.

Finally, it was found that when histamine is placed in $CDCl_3$ solution with glucose penta-acetate, acyl transfer occurs between the penta-acetate and the primary amino group of histamine. Since transacetylation normally does not occur when imidazole is not present, it is supposed that the imidazole group of histamine catalyzes the transfer either intra- or inter-molecularly.

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