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Binding of Organic Monophosphates  
to the Dihydroxyacetone Phosphate Binding  
Sites of Rabbit Muscle Aldolase

by

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Major: Biochemistry

Minor: Chemistry

Submitted to the Faculty of the Graduate School in partial fulfillment  
of the requirements for the degree of Doctor of Philosophy in the  
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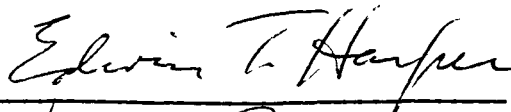
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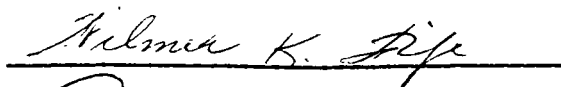
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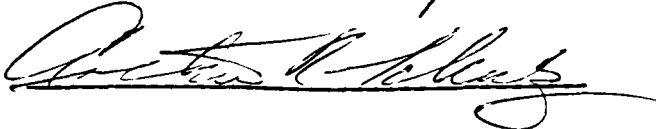
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## ABSTRACT:

Dissertation: Binding of Organic Monophosphates to the Dihydroxyacetone Phosphate Binding Sites of Rabbit Muscle Aldolase

The structural requirements of binding of organic monophosphate ester ligands to the dihydroxyacetone phosphate sites of rabbit muscle aldolase were studied by fluorescence quenching. Previous studies showed that phosphate esters which do not possess a carbonyl group are known only to bind noncovalently to aldolase. We found that this type of compound had the same affinity as inorganic phosphate for the enzyme (dissociation constant of about 1 mM), indicating that electrostatic interaction of the phosphate group was primarily responsible for formation of the noncovalent complex.

It is known that phosphate esters which contain a carbonyl group can form covalent complexes with aldolase by interaction with a lysine residue at the active site. We observed a linear correlation of the free energy of binding of carbonyl monophosphates with the free energy of hydration of these compounds. Based on this correlation, the free energy of carbinolamine formation for each compound was estimated. It appeared that the amount of carbinolamine formed was affected by electronic factors, electron-withdrawing substituents favoring formation of carbinolamine. Imine intermediates were formed by some compounds, but both 1-fluoro-3-hydroxyacetone phosphate and 1-chloro-3-hydroxyacetone phosphate, which contain the strongly electron-withdrawing chloro and fluoro groups, respectively, did not appear to readily form protonated imine complexes. The

electron-withdrawing substituents may have destabilized iminium ion formation so that the covalent complex primarily formed by both of these compounds was carbinolamine.

Based upon the above results, a free energy diagram was constructed describing the binding reaction of dihydroxyacetone phosphate. A value of 1 mM was assumed for the dissociation constant governing noncovalent complex formation and an equilibrium constant for the formation of carbinolamine was estimated from the linear plot described above. The free energy diagram revealed that dihydroxyacetone phosphate may be bound almost exclusively as imine and enamine to aldolase.

D-Erythrulose 1-phosphate was a slow, reversible inhibitor of aldolase. A kinetic analysis of the inactivation reaction of aldolase indicated the formation of two kinetically distinct complexes. Initially, a complex composed primarily of carbinolamine intermediate was formed rapidly, followed by a slow conversion of the initial complex to a complex composed of imine and enamine intermediates. A free energy diagram describing this binding reaction was constructed.

TABLE OF CONTENTS:

Page

GLOSSARY OF NAMES AND STRUCTURES ..... i  
LIST OF ABBREVIATIONS ..... ii  
List of Reaction Schemes ..... iii  
List of Figures ..... iv  
List of Tables ..... vi

INTRODUCTION 1

I. Statement of the Problem ..... 1  
II. Approach to the Problem ..... 2  
III. Literature Review of Rabbit Muscle Aldolase .... 3  
    A. Mechanism of the Aldolase Reaction ..... 4  
    B. Kinetic Parameters of the Aldolase-Catalyzed  
        Cleavage of Fructose 1,6-Bisphosphate ..... 7  
    C. Specificity of the Aldolase Reaction ..... 10  
        1. Specificity of Aldolase for the Anomers  
            of Fructose Bisphosphate ..... 10  
        2. Specificity of Aldolase for Substrates  
            other than Fructose Bisphosphate ..... 12  
    D. Carboxypeptidase-Treated Aldolase ..... 19  
    E. Fluorometric Studies of Aldolase ..... 22  
    F. Binding to Aldolase ..... 27  
        1. Number of Binding Sites ..... 27  
        2. Binding of Anions to Aldolase ..... 28  
        3. Binding of Dihydroxyacetone Phosphate  
            to Aldolase ..... 30  
        4. Binding of Monophosphates to Aldolase .. 37  
        5. Binding of Bisphosphates ..... 44

MATERIALS 47

I. Enzymes and Proteins ..... 47  
II. Materials for Chromatography ..... 47  
III. Substrates and Ligands ..... 47  
IV. Solvents and Reagents ..... 48  
V. Instruments ..... 49

METHODS	50
I. General Methods .....	50
A. Preparation of Solutions for Phosphate Analysis .....	50
B. Inorganic Phosphate Assay .....	50
C. Alkali-Labile Phosphate Assay .....	50
D. Total Phosphate Assay .....	51
E. Controls and Calculations .....	51
F. Thin Layer Chromatography .....	52
G. NMR Spectra of Barium Alkyl Phosphates .....	53
H. Conversion of Barium Salts of Organic Phosphates to Dicyclohexylammonium Salts ....	53
I. Preparation of Diphenyl Esters of Alcohols ..	54
J. Hydrogenolysis Procedures .....	54
II. Synthesis of Organic Phosphate Esters .....	55
A. Synthesis of Barium 2-Hydroxyethyl Phosphate	55
B. Synthesis of Barium Propyl Phosphate .....	57
C. Synthesis of Barium 2-Hydroxypropyl Phosphate	57
D. Synthesis of Barium 3-Hydroxypropyl Phosphate	58
E. Barium Methallyl Phosphate and Disodium Allyl Phosphate .....	59
F. Recrystallization of Barium Methallyl Phosphate to Remove Inorganic Phosphate .....	59
G. Synthesis of Barium Hydroxyacetone Phosphate	60
H. Synthesis of 1-Hydroxy-2-butanone Phosphate Dicyclohexylammonium Salt .....	62
I. Synthesis of L-1,3-Dihydroxy-2-butanone 1-Phosphate .....	64
1. Silver Oxide .....	65
2. Ethyl L-2-Benzyloxy-propanoate (II) ....	65
3. L-2-Benzyloxy-propanoic Acid (III) .....	66
4. L-2-Benzyloxy-propanoyl Chloride (IV) ..	67
5. L-1-Diazo-3-benzyloxy-2-butanone (V) ...	67
6. L-1-Hydroxy-3-benzyloxy-2-butanone (VI)	68
7. L-1-Hydroxy-3-benzyloxy-2-butanone Dimethyl Ketal (VII) .....	68

8.	Diphenyl L-1-Hydroxy-3-benzyloxy- 2,2-dimethoxy-butyl Phosphate (VIII) ...	69
9.	Diphenyl L-1,3-Dihydroxy- 2,2-dimethoxybutyl 1-Phosphate (IX) ....	70
10.	L-1,3-Dihydroxy-2-butanone 1-Phosphate Dimethylketal Dicyclohexylammonium Salt (X) .....	71
11.	L-1,3-Dihydroxy-2-butanone 1-Phosphate (XI) .....	72
J.	Synthesis of 1,4-Dihydroxy-2-butanone 1-Phosphate .....	73
III.	Preparation of Solutions of Ligands .....	76
IV.	Enzymes, Substrates, Enzyme Assays .....	77
A.	Preparation of Enzyme Solutions .....	77
B.	Preparation of D-Fructose 1-Phosphate Solution .....	78
C.	Preparation of L-Erythrulose 1-Phosphate ....	79
D.	Assay of Rabbit Muscle Aldolase .....	79
E.	Assay for the Determination of $K_m$ and $V_{max}$ of D-Fructose 1-Phosphate and L-Erythrulose 1-Phosphate .....	79
F.	Assay of the Rate of Cleavage of L-Erythrulose 1-Phosphate by Aldolase Using Alcohol Dehydrogenase as Coupling Enzyme .....	80
G.	Enzymatic Assay of L-1,3-Dihydroxy-2-butanone 1-Phosphate .....	81
V.	Ligand Binding to Aldolase .....	81
A.	Standardization of Pipets .....	81
B.	Determination of Dissociation Constants of Phosphates to Aldolase by the Quenching of Aldolase Fluorescence .....	81
C.	Purification of 8-Anilino-1-Naphthalenesulfonic Acid (ANSA) .....	84
D.	Determination of Ligand Binding to Rabbit Muscle Aldolase Using ANSA .....	85
E.	Binding of D-Erythrulose 1-Phosphate to	

Aldolase .....	86
VI. Detection of Enzyme-Bound Intermediates .....	87
A. Standard Procedure for Detection of Imine Intermediates Using Sodium Borohydride .....	88
B. Borohydride Reduction of D-Erythrulose-1-Phosphate/Aldolase Complexes .....	88
C. pH Dependence of the Borohydride Reduction of Glycolaldehyde Phosphate and Aldolase .....	89
D. Potassium Ferricyanide Oxidation of Enamine .	90
 RESULTS	 91
I. Synthesis and Characterization of Substrates and Substrate Analogs .....	91
II. $K_m$ and $V_{max}$ of D-Fructose 1-Phosphate and L-Erythrulose 1-Phosphate .....	98
III. Determination of Dissociation Constants of Organophosphate Esters by Fluorescence Quenching	98
IV. Binding of D-Erythrulose 1-Phosphate to Aldolase	125
V. Detection of Imine Intermediates .....	127
VI. Detection of Enamine Intermediates .....	139
 DISCUSSION	 143
I. Specificity of Aldolase for the Keto Form of Fructose 1-Phosphate .....	143
II. The $pK_a$ of the Imino-Hydrogen of Glycolaldehyde Phosphate and Aldolase .....	143
III. Binding of D-Erythrulose 1-Phosphate to Aldolase: Identification of the Imine and Enamine Intermediates as Members of the E-D <sup>*</sup> Complex and Interaction of the 3-Hydroxyl Group with the Enzyme .....	146
IV. Binding Constants and Maximum Quenching of Phosphate Esters .....	149
A. Percentage Maximum Quenching of Aldolase Intrinsic Fluorescence .....	149
B. Binding of Monophosphates that Lack a Carbonyl	

Group .....	151
C. Binding of Monophosphates Containing a Carbonyl Group .....	151
1. Noncovalent and Covalent Complex Formation .....	151
2. The Effect of Electron-Withdrawing Groups on Binding .....	156
3. Formation of Carbinolamine .....	159
4. Rate Determining Step of the D-Erythrulose 1-Phosphate Binding Reaction .....	168
5. Construction of Free Energy Diagrams for the Binding Reactions of Dihydroxyacetone Phosphate and D-Erythrulose 1-Phosphate	170
 Conclusion	 171
 BIBLIOGRAPHY	 174
 APPENDIX 1: Derivation of an Equation Describing Binding of Alkyl Phosphates to Aldolase in the Presence of Inorganic Phosphate .....	 A-1
APPENDIX 2: Relationship of $K_m$ to the Dissociation Constant of Substrates of Aldolase .....	A-4