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MODULATION OF PROTEIN FUNCTION

edited by
DANIEL E. ATKINSON
C. FRED FOX

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PREFACE

During the past two decades it has come to be recognized that biological functions of many, if not all, types are controlled to a very large extent through modulation of the functions of individual proteins or of multimolecular protein systems that result from interaction with metabolites or with specialized messenger compounds of low molecular weight. It was the aim of this symposium to bring together workers from several fields, all of which deal with such modulation of protein function. Discussion of representative metabolic control systems, ranging from single-enzyme responses to complex regulatory cascades, and the control of photosynthesis and of protein synthesis and enzyme inactivation dealt with the general topic at perhaps its most fundamental cellular level. Modulations and conformational changes in proteins that underlie higher-level interactions, such as those involved in cyclic nucleotide function, sensing and chemotactic response to foreign materials, and the complement system, were described. Two talks dealt with potential clinical relevance of phenomena of the types described by other participants. The common thread of functionally significant consequences of protein–small-molecule interaction led to extensive interaction among participants who work on widely diverse systems, and the editors hope that common thread will similarly unify this published record of the symposium.

We wish to thank the symposium speakers and poster session contributors for providing the basis of the program. We also wish to acknowledge the continuing support that the Life Sciences Division of ICN Pharmaceuticals, Inc., endows for the general support of this conference series, and, finally, we cite the generous contribution made by The National Foundation in partial support of the present meeting.

Daniel E. Atkinson

CONTENTS

III. PHOTOSYNTHESIS AND STORAGE POLYSACCHARIDES

IV. CASCADE SYSTEMS

V. PROTEIN PHOSPHORYLATION

MOLECULAR PROPERTIES OF PHOSPHOFRUCTOKINASE (PFK) RELEVANT TO MODULATION OF ITS FUNCTION[1]

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Studies during the past twenty years on the molecular properties of phosphofructokinase have contributed immensely to our understanding of its role as an important regulatory enzyme in glycolysis. Both covalent and non-covalent changes in enzyme structure have been reported. Evidence has been accumulating showing variation in phosphofructokinase activity in connection with different physiological conditions. In many cases the changes in enzyme activity is implied from indirect evidence and on the basis of what we already know of the properties of the enzyme. We wish to summarize briefly our current knowledge of some of the most important molecular properties of the enzyme. We will then report on some recent experiments on its allosteric sites and the nature of inhibition by vanadate. Finally, we will discuss briefly the relationship between these properties and the regulatory function of phosphofructokinase.

Molecular structure. Information based on data from our own laboratory on heart phosphofructokinase as well as laboratories of Lardy (1,2) and others indicates that the smallest fully active phosphofructokinase is a tetramer with a molecular weight of 360,000 and an $S_{20,w}$ value of 13. High enzyme concentration or the presence of fructose-1,6-P_2 or fructose-6-P favor the formation of high aggregates of the enzyme with an $S_{20,w}$ value as high as 54, while the presence of ATP or low enzyme concentration favor the low molecular form. The tetrameric form of the enzyme can be dissociated to dimers which are inactive. Enzyme protomers can be obtained in the presence of 4mM of SDS. Each protomer can be dissociated to 4 subunits with a molecular weight of 24,000 in the presence of 5M guanidine HCl.

Kinetics. Studies on the kinetics of phosphofructokinase have indicated that pH determines the nature of these kinetics (1). At pH 8.2, which is the optimal pH for enzyme activity, it exhibited Michaelis-Menten type of kinetics. At pH 6.9 typical allosteric kinetics are seen. The curve for ATP is hyperbolic until the activity

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TABLE 1

SOME OF THE IMPORTANT EFFECTORS OF PHOSPHOFRUCTOKINASE

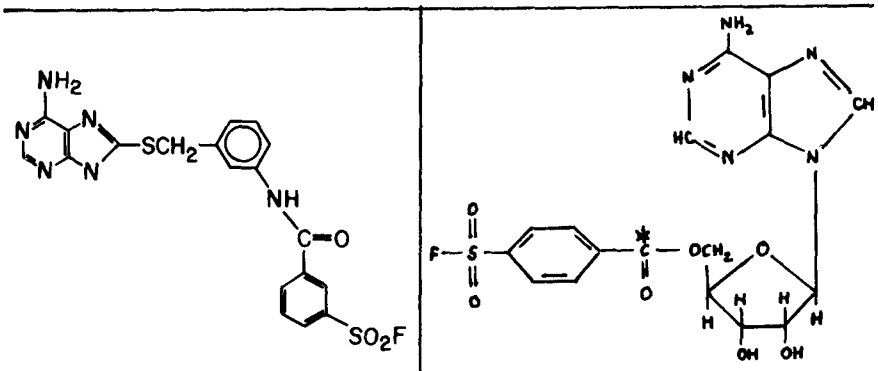
Inhibitors	Deinhibitors of ATP or Activators
ATP	3',5'-cyclic AMP
Citrate	5'-AMP
P-creatine	ADP
3-P-glycerate	Fructose 6-P
2-P-glycerate	Fructose-1,6-P_2
2,3-P_2-glycerate	Glucose-1,6-P_2
P-enolpyruvate	NH_4^+ , Pi

is maximal, followed by a steep inhibition curve as the ATP concentration is increased. In the presence of an activator the catalytic part of the curve is not changed while the inhibitory curve reaches almost a plateau after maximal activity. Thus activators exert their effect by relieving ATP inhibition, i.e. by "de-inhibition". The saturation curve for fructose-6-P at pH 6.9 is sigmoidal. Inhibitors will increase the sigmoidicity while activators will convert the sigmoidal kinetics to hyperbolic kinetics.

The list of allosteric effectors of mammalian phosphofructokinase (Table 1) is long and more agents are being added to it. Among the activators listed, AMP and Pi are of special interest since their levels are increased after anoxia. Furthermore, cyclic 3',5'-AMP, whose level is increased following administration of several hormones, also is one of the activators.

Nature of Allosteric Sites. Our approach to study the molecular properties of allosteric sites of PFK is largely through chemical modification and through identification of the structures that have been modified. Previously we have used photo-oxidation (3,4) and ethoxyformic anhydride (4) to modify sheep heart PFK. The modified enzyme became less sensitive to ATP inhibition in connection with the loss of ATP inhibitory binding sites; its sigmoidal kinetics for fructose-6-P was also abolished, while the catalytic effect was only slightly decreased. Reaction of ethoxyformic anhydride with PFK specifically modified four histidine residues per protomer; thus those histidine residues presumably served as cationic binding sites for inhibitory ATP.

The use of affinity label reagents is a more effective way of selectively binding to the site in the enzyme prior to its covalent reaction. Recently we have used two reagents that react covalently



8-[m-(m-FLUOROSULFONYLBENZAMIDO)BENZYLTHIO]ADENINE
(FSB-ADENINE)

5'-p-FLUOROSULFONYLBENZOYL ADENOSINE
(FSB-ADENOSINE)

FIGURE 1.

with sheep heart phosphofructokinase making it much less sensitive to inhibition by ATP (5,6). Modified enzyme becomes insensitive to activation by AMP, ADP and cyclic AMP.

Fig 1 shows the structure of these two compounds. The first compound used is 8-[m-(m-fluorosulfonylbenzamido)benzylthio] adenine which will be referred to as FSB-adenine (7). The other reagent is an adenosine derivative, 5'-p-fluorosulfonylbenzoyl adenosine which will be referred to as FSB-adenosine (8). Both affinity label reagents have been used to label several other enzymes. For example, FSB-adenine was first used by Graves to label the AMP site on glycogen phosphorylase (7). The adenosine reagent was used as an affinity label of the inhibitory DPNH site of bovine liver glutamate dehydrogenase by Colman (8). The catalytic sites of rabbit muscle pyruvate kinase (9) and mitochondrial ATPase (10) were also labelled with the same reagent following inactivation of the enzymes.

Our recent studies show that the adenosine reagent is a specific reagent for the AMP-ADP sites of phosphofructokinase and protects against ATP inhibition better than the adenine derivatives (Figure 2). This may be due to the presence of the ribose moiety which is important for the right orientation of the reagent molecule. Conditions were first established to abolish allosteric kinetics without affecting maximal enzyme activity at pH 8.2. This was achieved when the enzyme covalently binds approximately 1 mole of the reagent per protomer. The modified enzyme completely lost its sensitivity to inhibition by ATP at moderately low levels. Inhibition can only be produced at concentrations as high as 700μM.

The results summarized in Fig. 3 show the sensitivity of the enzyme to AMP activation when inhibited by ATP at a concentration that causes 60% inhibition. The results show that while the native enzyme is sensitive to activation by AMP at concentration as low as

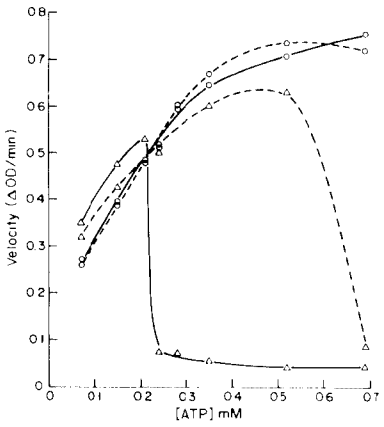


Fig. 2

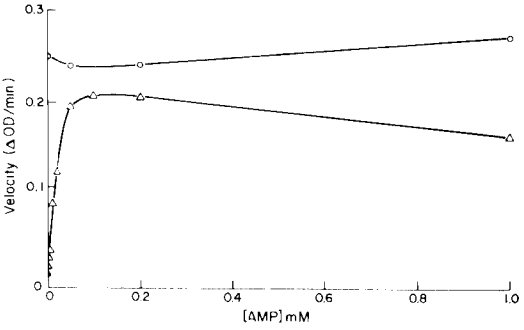


Fig. 3

Fig. 2. Sensitivity of native (▲) and modified (●) phosphofructokinase to inhibition by ATP. Enzyme modified by 5-$FSBO_2Bz$-adenosine and native enzyme were prepared as reported. Initial velocity of phosphofructokinase was measured at different ATP concentrations in the regular reaction mixture at pH 6.9 without AMP (straight line) or with 1 mM AMP (dashed lines). The concentration of fructose-6-P was fixed at 0.5 mM and the $MgCl_2$ at 1 mM.

Fig. 3. Sensitivity of native (▲) and modified (●) phosphofructokinase to activation by AMP. Enzyme activity was measured at pH 6.9 in the presence of 0.25 mM ATP, 0.5 mM fructose-6-P and 1 mM $MgCl_2$.

10μM, the modified enzyme is completely insensitive to AMP activation. Titration curves for the second substrate fructose 6-P showed no sigmoidal kinetics for the modified enzyme. Kinetics of the enzyme at pH 8.2, on the other hand, was not significantly influenced.

The effect of enzyme modification on nucleotide binding to PFK showed that the binding of AMP, cAMP and ADP was abolished following enzyme modification. In contrast, the modified and native enzyme did not differ significantly to the maximal amount of binding of the ATP imidoanalog, App(NH)p. Affinity of the modified enzyme to App(NH)p was reduced.

This reagent therefore appears to be affecting the allosteric sites specifically and does not appear to involve the catalytic sites of PFK. The fact that this reagent abolished the binding of the activators AMP, cAMP and ADP but only interfered with the affinity for the ATP analog App(NH)p indicates that occupation of the AMP site does not eliminate ATP binding. Thus, the desensitization to ATP inhibition by FSB-adenosine modification must result from a change in the interaction of PFK with ATP subsequent to ATP