BBA 66138

STUDIES ON THE ALLOSTERIC ACTIVATION OF GLYCOGEN PHOSPHORYLASE *b* BY NUCLEOTIDES

II. NUCLEOTIDE STRUCTURE IN RELATION TO MECHANISM OF ACTIVATION*

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(Received March 16th, 1970)

SUMMARY

The effect of thirty-two nucleotides and related compounds on the activity of glycogen phosphorylase *b* has been examined to further understand the mechanism of nucleotide activation of this enzyme. All nucleoside 5′-monophosphates, 2′-deoxyadenosine 5′-phosphate and adenosine 5′-phosphoramidate activated this enzyme with the maximum activation ranging from 10 to 60% that of AMP activation. The mechanism of the activation by these nucleotides was examined kinetically, since AMP and IMP activation of phosphorylase *b* were previously found to exhibit different kinetic properties[1]. All the other nucleotide activators enhanced the v_{max} of the enzyme without changing the enzyme affinity toward the substrate, glucose-1-*P*. When these nucleotides were used as activators, the substrate saturation curve was sigmoidal suggesting strong homotropic cooperativity. Thus, the kinetic mechanism of the enzyme activation by these nucleotides resembled that of IMP activation. Furthermore, while AMP caused partial association of phosphorylase *b*, IMP and the other activators induced enzyme association only in the presence of glucose-1-*P*. The nucleotide activation of phosphorylase *b* could be enhanced by NaF and certain polycations: spermine and protamine. Although the nucleotide specificity for phosphorylase *b*′, a tryptic derivative of phosphorylase, was essentially the same as that for phosphorylase *b*, the activation of the enzyme derivative was not sensitive to polycations. F^-, however, still showed stimulation of nucleotide activation.

INTRODUCTION

In the first paper of this series, the effect of IMP on the catalytic and structural properties of muscle glycogen phosphorylase *b* (α-1,4-glucan:orthophosphate glucosyl-

* Part of the work was presented at the Canadian Federation Meetings, Kingston, 1968.

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transferase, EC 2.4.1.1) was examined[1]. The results of this study indicated that the allosteric mechanism of phosphorylase *b* activation by IMP was significantly different from that of AMP activation. This difference suggested that the allosteric transitions could be best explained by a direct application of the "induced-fit" theory of KOSHLAND[2], a suggestion which had been made previously for the allosteric transitions of glycogen phosphorylase *a* (ref. 3). In the present study, the effects of various nucleotides on the structure and activity of phosphorylase *b* have been examined in an attempt to further elucidate the relationship between the allosteric activation mechanism of the enzyme and the structure of the nucleotides. The results show that each nucleoside 5'-phosphate may activate phosphorylase *b* to a different extent. The kinetic mechanism of activation by these nucleotides resembles that of IMP activation. In contrast to AMP, the various activators cause partial association of phosphorylase *b* only when glucose-1-*P* is present. The extent of both activation and association of the enzyme by nucleotides depends on the nucleotide used. These results can readily be explained on the basis of the induced-fit theory.

During the course of this investigation, two independent laboratories have presented evidence for the functional groups on the nucleotides that appear to be important for the activation of and binding to phosphorylase *b* (refs. 4, 5). Similar conclusions are reached in the present paper.

METHODS

Glycogen phosphorylase *b* was prepared by the method of FISCHER AND KREBS[6] and was recrystallized 4 or 5 times. The crystallized enzyme was treated with Norit A before use to free it from nucleotides. Phosphorylase *b'* was prepared by controlled tryptic hydrolysis of phosphorylase *a* or *b* (refs. 7, 8). The hydrolysis was stopped by addition of trypsin inhibitor and the enzyme was used without prior removal of trypsin and trypsin inhibitor.

Glucose-1-*P*, shellfish glycogen, nucleotides and their analogues, with the exception of GMP and dGMP, were purchased from Sigma Chemical Company and were the highest grade obtainable. GMP and dGMP were purchased from Calbiochem Company. Glycogen was purified with Norit A according to the procedure of SUTHERLAND AND WOSILAIT[9]. Reagent grade NaF was purchased from Baker Chemical Company. Spermine tetrahydrochloride was obtained from Mann Research Laboratories. Protamine sulphate, bovine pancreatic trypsin and egg white trypsin inhibitor were obtained from Calbiochem Company. Contamination of the structural analogues by AMP and other nucleotides was tested by ascending paper chromatography as described previously[1]. Solvent systems were water-saturated $(NH_4)_2SO_4$–isopropanol–0.1 M sodium phosphate buffer (pH 7.2) (79:2:19, by vol.) for most of the nucleotides, and isopropanol–acetic acid–1% aqueous $(NH_4)_2SO_4$ (45:35:20, by vol.) for dAMP. No contamination of nucleotides could be detected. Initial velocity of phosphorylase was measured at 30° in the direction of glycogen synthesis by a method similar to the procedure of ILLINGWORTH AND CORI[10]. Kinetic data were processed, wherever applicable, using an Olivetti Programma 101 desk computer according to a program, modified slightly, provided by HENSON AND CLELAND[11], which provides values for K_m, v_{max}, their standard errors of deviation, slopes and intercepts.

Maximal activation (A_{max}) of each nucleotide activator was expressed as the

TABLE I

EFFECTS OF STRUCTURAL ANALOGUES OF AMP ON PHOSPHORYLASE *b* ACTIVITY

Compound (17.5 mM)	A_{max}*	
	16 mM Glucose-1-P (%)	*100 mM Glucose-1-P (%)*
Control		
AMP	100	100
Modifications in 5'-phosphate		
Adenosine	0	0
2'-AMP	0	0
3'-AMP	0	0
Cyclic-2',3'-AMP	0	0
Cyclic-3',5'-AMP	0	0
Adenosine 5'-phosphoramidate	30	57
Adenosine 5'-nicotinate	0	0
Diadenosine 5'-pyrophosphate	0	0
Modifications in adenine ring		
CMP	18	36
GMP	6	46
IMP	20	64
UMP	23	45
XMP	13	16
Modifications in ribose and base		
Ribose	0	0
Ribose-5-*P*	0	0
Deoxyribose	0	0
Pyrimidine, purine	0	0
Purine riboside	0	0
Adenine	0	0
Cytidine, guanosine, inosine	0	0
Thymidine, uridine, xanthosine	0	0
TMP	5	10
2'-dAMP	13	55
2'-dCMP	0	0
2'-dGMP	0	0
2'-dUMP	0	0
2'-dXMP	0	0

* Percentage of maximal activation (see METHODS).

percentage of AMP activation. Since double reciprocal plots of nucleotide activation were nonlinear, a rough estimation of the apparent K_a was calculated at 100 mM glucose-1-*P* (saturating) from a Hill plot[12]. The apparent K_a was obtained as the concentration of the activator giving half-maximal activation where $\log v/(v_{max} - v)$ equals 1.0. In the presence of 0.3 M NaF, double reciprocal plots of $(1/v)$ against $(1/A)$ were linear enabling direct calculation of the K_a's.

Ultracentrifugal runs were made on a Spinco Model E analytical ultracentrifuge equipped with Schlieren optics. All experiments were carried out at a temperature of 20 ± 1° and a rotor speed of 60 000 rev./min. Protein concentrations ranging from 3 to 6 mg/ml were routinely used. Sedimentation coefficients were determined with

the aid of a Nikon Model 6C microcomparator and were corrected for viscosity and for the density of the buffer to water at 20°.

RESULTS

The effect of thirty-two nucleotides and related compounds on the activity of phosphorylase *b* was studied in an attempt to delineate the structural requirement of the nucleotide for enzyme activation. Since the v_{max} of IMP activation can be greatly enhanced at high concentrations of glucose-1-*P* (ref. 1), the effect of these nucleotides and related compounds on the activity of phosphorylase *b* was investigated at both 16 and 100 mM glucose-1-*P*. The data in Table I represents the mean values of five surveys, reproducible to within ± 5%, carried out with phosphorylase *b* in the presence of these effectors at concentrations of 17.5 mM. Activation was expressed as the percent activation relative to AMP (A_{max}). Values of 5% or less were considered to be negligible. For simplicity in analysing their activation effects, the structural analogues of AMP are classified into three main groups in which modifications occur (a) in the 5′-phosphate moiety, (b) in the adenine ring, and (c) in the ribose and base moieties. All nucleoside 5′-monophosphates, 2′-deoxyadenosine 5′-phosphate (dAMP), and TMP activated the enzyme at values ranging from 10 to 60% of AMP activation. Other 2′-deoxynucleotides, 2′-AMP, 3′-AMP, cyclic-2′,3′-AMP, cyclic-3′,5′-AMP, bases or other analogues did not cause enzyme activation. These results suggested that both the 5′-phosphate and the 2′-hydroxyl groups on the ribose moiety are important in the nucleotide activation of phosphorylase *b*.

All the nucleotide activators and most of the nonactivating compounds listed in Table I probably interact with phosphorylase *b* at the AMP binding site. Kinetic studies indicate that they are linear competitive inhibitors with respect to AMP. The inhibition constants, K_i's, are listed in Table II. The relative magnitude of the K_i values should reflect the binding strength of the inhibitors to phosphorylase *b*. Among the weak activators, the K_i values increased in the following order: dAMP, CMP, IMP, GMP, TMP, XMP, and UMP. The increase in the K_a values follow the same order with the exception of CMP (Table III). Among the compounds which did not activate, adenine, 3′-AMP, and 2′-AMP bound the strongest to the enzyme, whereas the cyclic AMP's were weak inhibitors. dCMP was a weaker inhibitor than was CMP. Among the free bases, only adenine and inosine inhibited enzyme activity to a significant extent.

When IMP instead of AMP was used as an activator, phosphorylase *b* showed a sigmoidal response toward glucose-1-*P* (ref. 1). Similar kinetic behavior was observed with the activation by other nucleotide activators listed in Table I. In Fig. 1, the initial velocity is plotted against glucose-1-*P* concentration for three nucleotide activators: dAMP, CMP, and UMP. These kinetic plots are sigmoidal even at high concentrations of activator. In addition, IMP only effects v_{max} of phosphorylase *b* without changing the enzyme affinity toward glucose-1-*P*. The activation of phosphorylase *b* by the other nucleotides again resembles IMP activation in this aspect. The double reciprocal plots with respect to glucose-1-*P* for dAMP, CMP and UMP activation were parabolic making it difficult to determine K_m for glucose-1-*P*. The substrate concentration required for half-maximum activation, however, could be estimated from the double reciprocal plots. For all these activators the value of this concentration ranged from 25 to 35 mM.

TABLE II

INHIBITION CONSTANTS OF AMP ANALOGUES FOR PHOSPHORYLASE *b*
Initial velocity was measured with 16 mM glucose-1-*P*, 1% glycogen

Compound	K_i *(0.1 mM)*
Control	
AMP	0.5*
Modifications in 5'-phosphate	
2'-AMP	14.0
3'-AMP	10.5
Cyclic-2',3'-AMP	41.0
Cyclic-3',5'-AMP	21.0
Modifications in adenine ring	
CMP	9.0
GMP	11.0
IMP	9.0
UMP	78.0
XMP	41.0
Modifications in ribose and base	
Ribose	**
Ribose-5-*P*	**
Deoxyribose	**
Pyrimidine	**
Purine	22.0
Purine riboside	32.0
Adenine	10.8
Cytidine	150.0
Inosine	21.5
Thymidine	55.0
Uridine	**
Xanthosine	**
TMP	11.5
2'-dAMP	5.0
2'-dCMP	29.0
2'-dXMP	23.0

* The K_a value of AMP.
** No inhibition at 4.16 mM concentration of analogue.

In the presence of ATP, an inhibitor for glycogen phosphorylase *b*, the initial velocity against glucose-1-*P* concentration plot is sigmoidal[13]. Similar kinetic plots with respect to glucose-1-*P* were observed with phosphorylase *b* in the presence of the nucleotide inhibitors listed in Table II. In Fig. 2, the kinetics of interaction between nucleotides and glucose-1-*P* are depicted for two nucleotide inhibitors: 3'-AMP and cyclic-3',5'-AMP. The glucose-1-*P* saturation curves are pronouncedly sigmoidal in the presence of these nucleotide inhibitors.

A number of ions: F^-, protamine and polyamines have been found to effect the interaction between phosphorylase *b* and AMP (refs. 8, 14, 15). The effect of these ionic species on the activation of the enzyme by many of the compounds listed in Table I was investigated. The data in Table IV represents the mean of three surveys, reproducible to within $\pm$ 9%, carried out with phosphorylase *b* (16 mM glucose-1-*P*) in the presence of various activators at concentrations of 5 mM. The A_{max} values of

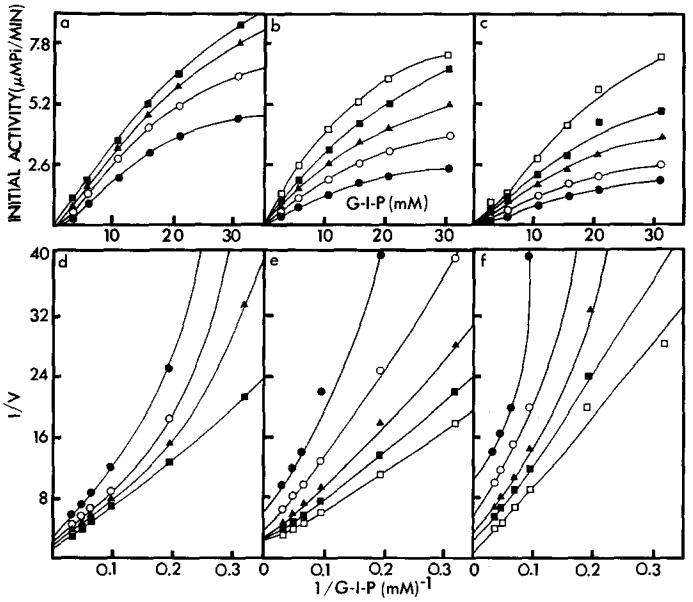

Fig. 1. The kinetics of phosphorylase *b* activation by several structural analogues of AMP. Upper curves: the initial velocity of phosphorylase *b* as a function of glucose-1-*P* concentration at the following levels of activators: □, 0.313 mM; ■, 0.625 mM; ▲, 1.25 mM; ○, 2.08 mM; ●, 4.16 mM. The activators are: (a) dAMP; (b) CMP; (c) UMP. Different enzyme preparations were used for the three analogues. Lower curves: d, e, and f are double reciprocal plots of the data from a, b, and c, respectively.

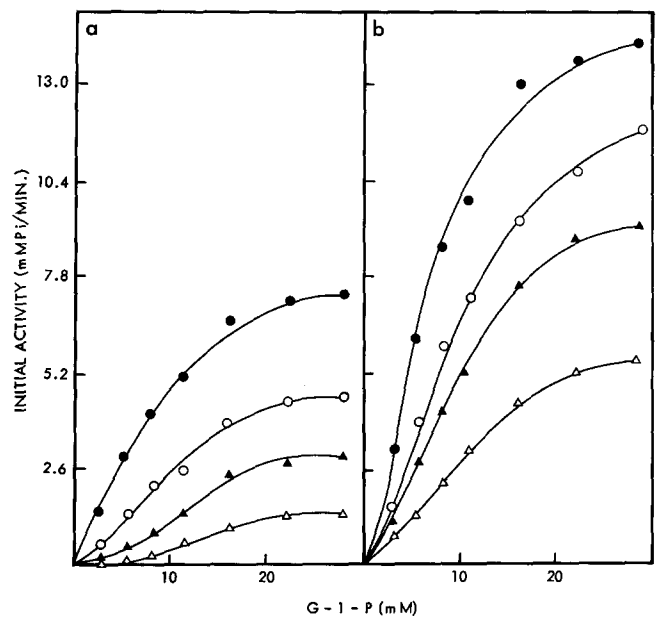

Fig. 2. The initial velocity of phosphorylase *b* as a function of glucose-1-*P* concentration at 83.3 μM AMP in the presence of: (a) 3′-AMP; (b) cyclic-3′,5′-AMP. Analogue concentrations were: ●, 0; ○, 0.52 mM; ▲, 1.04 mM; △, 2.08 mM. Different enzyme preparations were used for the two analogues.

TABLE III

ACTIVATION CONSTANTS AND MAXIMUM VELOCITY OF AMP ANALOGUES FOR PHOSPHORYLASE *b*

Apparent K_a calculated at 100 mM glucose-1-*P* (see METHODS). Average of three determinations. v_{max} is expressed as moles P_i released/min per mg of protein. Activator concentrations ranged from 0.208 to 8.33 mM except that AMP concentrations were 10-fold lower (10 μM). A change in activator concentration from 6.3 to 8.33 mM (10-fold lower for AMP) did not increase the v_{max} by more than 12%. Thus, the v_{max} values could be determined visually from plots of initial velocity *versus* activator concentration

Compound	K_a *(0.1 mM)*	v_{max}
AMP	0.5	122
Adenosine 5′-phosphoramidate	33.0	74
2′-dAMP	10.0	56
CMP	30.0	49
GMP	15.0	47
IMP	12.5	81
TMP	39.5	24
UMP	39.0	52
XMP	35.0	24

the activators were increased over the values for the native enzyme by a factor of 2–3 in the presence of NaF, protamine, and spermine. The only exception is XMP, whose activation of phosphorylase *b* was not enhanced in the presence of polycations.

The kinetics of activation of all activators tested were normalized in the presence of NaF suggesting that the cooperative interactions have been "desensitized" by the F^-. The linear Lineweaver–Burk plots enabled direct calculation of the K_a values for the activators from replots of the slope against activator concentration. These values, together with the apparent K_a values for the activation of the enzyme in the absence of NaF, are listed in Table V. NaF decreased the K_a values for all nucleoside 5′-monophosphates by approx. 5-fold.

FISCHER *et al.*[16] have reported that limited tryptic hydrolysis of phosphorylase *a* resulted in the release of a specific phosphohexapeptide and concomitant formation of phosphorylase *b*′. Since GRAVES *et al.*[18] have recently shown that the allosteric

TABLE IV

EFFECTS OF NaF AND POLYAMINES ON REACTION OF AMP ANALOGUES WITH PHOSPHORYLASE *b*

Compound (5 mM)	A_{max} (%)*			
	No addition	*NaF (0.3 M)*	*Protamine (80 mg/ml)*	*Spermine (5 mM)*
AMP	100	100	100	100
2′-dAMP	21	68	59	62
CMP	17	55	39	34
GMP	14	71	46	53
IMP	35	86	75	87
TMP	9	26	18	22
UMP	18	62	58	53
XMP	7	17	9	7

* Percentage of maximal activation (see METHODS).

TABLE V

EFFECT OF NaF ON THE ACTIVATION CONSTANTS OF AMP ANALOGUES FOR PHOSPHORYLASE *b*

Compound	K_a *(mM)*	
	No addition	*0.3 M NaF*
AMP	0.05	0.005
Adenosine 5′-phosphoramidate	3.30	0.880
2′-dAMP	1.00	0.200
CMP	3.00	0.173
GMP	1.50	0.530
IMP	1.25	0.240
TMP	3.95	0.950
UMP	2.90	0.323
XMP	3.50	0.830

behavior of phosphorylase *b′* differed from that of phosphorylase *b*, the effects of several structural analogues of AMP on the activity of phosphorylase *b′* were investigated. Table VI represents the mean values of three surveys, reproducible to within

TABLE VI

EFFECTS OF STRUCTURAL ANALOGUES OF AMP ON PHOSPHORYLASE *b′* ACTIVITY

Compound (17.5 mM)	A_{max} *(%)**	
	16 mM glucose-1-P	*100 mM glucose-1-P*
Control		
AMP	100	100
Modifications in 5′-phosphate		
Adenosine	0	0
2′-AMP	0	0
3′-AMP	0	0
Cyclic-2′,3′-AMP	0	0
Cyclic-3′,5′-AMP	0	0
Modifications in adenine ring		
CMP	22	60
GMP	4	31
IMP	18	36
UMP	19	24
XMP	8	36
Modifications in ribose and base		
Ribose, ribose-5-*P*	0	0
Pyrimidine, purine	0	0
Purine riboside	0	0
Adenine	0	0
Cytidine, inosine	0	0
Thymidine, uridine	0	0
TMP	5	17
2′-dAMP	12	31
2′-dCMP, 2′-dGMP, 2′-dUMP	0	0

* Percentage of maximal activation (see METHODS).

TABLE VII

EFFECTS OF NaF AND POLYAMINES ON REACTION OF AMP ANALOGUES WITH PHOSPHORYLASE *b'*

Compound (5 mM)	A_{max} (%)*			
	No addition	*0.3 M NaF*	*Protamine (80 mg/ml)*	*Spermine (5 mM)*
AMP	100	100	100	100
2'-AMP	0	0	0	0
3'-AMP	0	0	0	0
Cyclic-2',3'-AMP	0	0	0	0
Cyclic-3',5'-AMP	0	0	0	0
2'-dAMP	20	44	18	27
2'-dCMP	0	0	0	0
CMP	19	48	19	23
GMP	12	15	7	9
IMP	26	65	29	27
TMP	6	28	15	18
UMP	18	61	31	22
XMP	9	29	9	7

* Percentage of maximal activation (see METHODS).

$\pm$ 7%, showing the effect of AMP analogues on phosphorylase *b'* activity at two different concentrations of glucose-1-*P*. There was no apparent difference between the activation properties of phosphorylase *b'* and *b*, only the nucleoside 5'-monophosphates and dAMP activated these two enzyme forms. The activation of phosphorylase *b'* by these compounds was still dependent upon the concentration of glucose-1-*P*.

Since the kinetics of AMP activation of phosphorylase *b'* were not affected by NaF, spermine or protamine[8,14,15], surveys were carried out to examine the effect of these ionic species on the activation by AMP analogues. The data in Table VII are mean values of three surveys carried out with different samples of phosphorylase *b'*. The standard deviations were less than $\pm$ 8%. As observed in the case of the activation of phosphorylase *b'* by IMP (refs. 8, 14), the activation by the majority of the other activators was only slightly affected by addition of protamine or spermine. However, F^- stimulated the nucleotide activation of phosphorylase *b'*. This stimulation resembled that obtained by increasing the level of glucose-1-*P* (Table VI).

In order to see whether the difference between nucleotide activation may be correlated with differences in enzyme structure induced by these nucleotides, the physical properties of the enzyme were examined. Incubation of phosphorylase *b* with AMP results in the partial association of the dimeric form of the enzyme into a tetramer[17]. IMP can cause association of the enzyme into a tetrameric species only in the presence of glucose-1-*P* (ref. 1). Evidence similar to that obtained for the association of phosphorylase *b* in the presence of IMP was obtained in the present study for the other activators of the enzyme (Fig. 3). At concentrations of 10 mM, the activators adenosine 5'-phosphoramidate, dAMP, CMP, TMP, UMP, GMP, XMP, and the nonactivating dUMP caused varying degrees of phosphorylase *b* association in the presence of 0.1 M glucose-1-*P*. The weakest activators TMP and XMP showed only slight association of phosphorylase *b*. In contrast, phosphorylase *b* sedimented

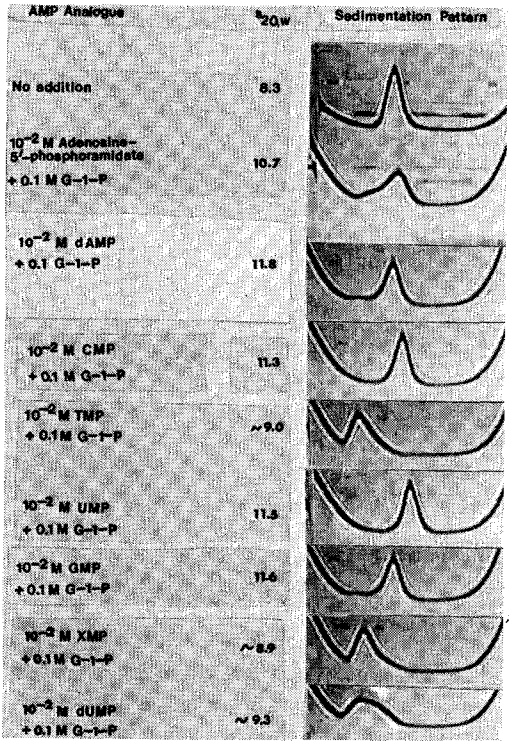

Fig. 3. Effects of structural analogues of AMP on the association of phosphorylase *b*. Ultracentrifugation was at 20° in 0.03 M cysteine–0.04 M glycerophosphate (pH 6.8). Protein concentration varied from 4.5 to 6.0 mg/ml. Time of sedimentation was 32 min at 59 780 rev./min. Direction of sedimentation was from left to right.

as a homogeneous protein with an $s_{20,w}$ value of approx. 8.3 S in the presence of both 0.1 M glucose-1-*P* and 10 mM concentrations of 2′-AMP, 3′-AMP, cyclic-3′,5′-AMP, dCMP, or dGMP. The fact that the majority of the nonactivating nucleotides did not cause association of phosphorylase *b* in the presence or absence of glucose-1-*P* suggests that enzyme association is probably related to activation.

DISCUSSION

The functional groups in AMP that are important for binding or activation of glycogen phosphorylase *b* have recently been investigated in two laboratories[4,5]. Results in the present study support the conclusions made by these investigators. All the ribonucleoside 5′-phosphates tested in our study activated phosphorylase *b*. In addition, dAMP and adenosine 5′-phosphoramidate were also found to be activators. Among all activators used, AMP was most effective both in terms of maximum activation and enzyme affinity.

The kinetics of IMP activation differs from that of AMP activation[1]. It was suggested that this difference in kinetic behavior stems from the difference in conformations of enzyme which are induced by these two nucleotides. The present study showed that the kinetic mechanism of activation by other nucleotides resembles that

of IMP activation. Only the v_{max} of the enzyme reaction was dependent on the nucleotide concentration whereas AMP enhanced both the v_{max} and the enzyme affinity toward glucose-1-*P*. In addition, while AMP can cause partial association of phosphorylase *b*, the other nucleotides, like IMP, induce enzyme association only in the presence of glucose-1-*P*. These results further support the previous suggestion regarding the relationship between the kinetic behavior and conformation of the enzyme.

The nucleotide activators used in the present study represent AMP analogues modified at a variety of positions. They, however, activate the enzyme with the same kinetic mechanism, which is distinctive from that of AMP activation. Thus, the unique kinetic behavior of AMP activation cannot be attributed to any one of these specific groups on this molecule. In the first paper of this series[1], the induced-fit theory of KOSHLAND[2] has been used to explain the kinetic property of glycogen phosphorylase *b*. Results in the present study can also be rationalized on the basis of this theory. One important prediction of the induced-fit theory is that the final conformational state of an enzyme is a result of complementary interactions between the enzyme and its effectors. Therefore, different effectors may induce a continuum of conformational states. The observation that different nucleotides can cause a different extent of activation as well as association of phosphorylase *b* is in agreement with this prediction. Since AMP, among these activators, possesses the highest enzyme affinity, it may be assumed to contain the maximum number of interacting groups. Thus, this nucleotide may induce conformational change in phosphorylase *b* to a greater extent than any of the other nucleotide activators. This effect of AMP on the enzyme conformation, therefore, distinguishes it from other nucleotides both in kinetic behavior of activation as well as in its effect on enzyme association.

A single specific serine residue per monomeric unit of phosphorylase is phosphorylated during the phosphorylase *b* to *a* conversion. The formation of phosphorylase *b'* from controlled tryptic digestion of phosphorylase *b* involves the release of a hexapeptide which contains this specific serine residue[8,16]. This peptide probably does not participate directly in the binding of nucleotide activators, since nucleotide specificity of phosphorylase *b* and *b'* is essentially the same. In view of the preponderance of positive charge in the primary structure around this serine residue, it has been proposed that this region of the enzyme is involved in intraelectrostatic interactions[16]. This is supported by the observation that polyamines stimulate AMP activation of the phosphorylase *b* form of the enzyme selectively. The present study shows that the activation of phosphorylase *b*, but not *b'* by any of the nucleotide activators tested can be enhanced by spermine. The stimulation of phosphorylase *b* by F^- has also been considered as due to the existence of this hexapeptide region of the enzyme[15,17]. Although AMP activation of phosphorylase *b'* is not stimulated by F^- (ref. 17), this anion has been shown in the present study to stimulate the activation of phosphorylase *b'* by certain other nucleotide activators. Thus, the stimulation of nucleotide activation of phosphorylase *b* by F^- would appear to be due to structural entities of the enzyme other than or in addition to this region.

ACKNOWLEDGMENTS

The work is supported by a grant from Medical Research Council of Canada, MA 2381. We are indebted to Mr. Erwin Wirch for his technical assistance.

J.H.W. is a Medical Research Council Scholar.

REFERENCES

1 W. J. BLACK AND J. H. WANG, *J. Biol. Chem.*, 243 (1968) 5892.
2 D. E. KOSHLAND, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 98.
3 J. H. WANG AND W. J. BLACK, *J. Biol. Chem.*, 243 (1968) 4641.
4 D. M. MOTT AND A. L. BEIBER, *Biochem. Biophys. Res. Commun.*, 30 (1968) 363.
5 T. OKAZAKI, T. A. NAKAZAWA AND O. HAYAISKI, *J. Biol. Chem.*, 243 (1968) 5266.
6 E. H. FISCHER AND E. G. KREBS, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 5, Academic Press, New York, 1962, p. 369.
7 G. T. CORI AND C. F. CORI, *J. Biol. Chem.*, 158 (1956) 459.
8 J. H. WANG, P. M. HUMNISKI AND W. J. BLACK, *Biochemistry*, 7 (1968) 2037.
9 E. W. SUTHERLAND AND W. D. WOSILAIT, *J. Biol. Chem.*, 218 (1956) 459.
10 B. ILLINGWORTH AND G. T. CORI, *Biochem. Prep.*, 3 (1953) 1.
11 C. P. HENSON AND W. W. CLELAND, *Biochemistry*, 3 (1964) 339.
12 W. E. L. BROWN AND A. W. HILL, *Proc. Roy. Soc. London, Ser. B*, 94 (1922–1923) 297.
13 N. B. MADSEN, *Biochem. Biophys. Res. Commun.*, 15 (1964) 390.
14 E. G. KREBS, *Biochim. Biophys. Acta*, 15 (1954) 508.
15 R. W. SEALOCK AND D. J. GRAVES, *Biochemistry*, 6 (1967) 201.
16 E. H. FISCHER, D. J. GRAVES, E. R. S. CRITTENDEN AND E. G. KREBS, *J. Biol. Chem.*, 234 (1959) 1698.
17 M. M. APPELMAN, Ph.D. Thesis, University of Washington, 1962.
18 D. J. GRAVES, S. MANN, G. PHILIP AND R. J. OLIVEIRA, *J. Biol. Chem.*, 243 (1968) 6090.