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Effects of Adenylic Acid on the Kinetics of Muscle Phosphorylase *a**

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It has long been known that muscle phosphorylase *b* has a strict requirement for adenylic acid (5′-AMP) (1). Phosphorylase *a*, on the other hand, is active without 5′-AMP (2) and although 5′-AMP may increase velocity somewhat, the increase according to most reports is not more than 50%. In connection with the use of phosphorylase and glycogen to measure low levels of inorganic orthophosphate, it was noticed that rates at 38° were very slow unless 5′-AMP was present. In exploring this further, it has been found that 5′-AMP can increase phosphorylase *a* activity 40 times or more when levels of glycogen, P_i, or glucose 1-phosphate are low. At 25° the 5′-AMP concentration necessary to produce half-maximal stimulation is very small, less than 1% of that required for phosphorylase *b* and only 10% of the concentration found by Cori, Cori, and Green (2) to produce half-maximal increments in phosphorylase *a* activity at high substrate levels.

An increase in temperature to 38° markedly increases the requirement for 5′-AMP and has a profound effect on the kinetic parameters of the enzyme even in the presence of 5′-AMP. With both glycogen and P_i at low levels, the temperature coefficient actually becomes negative above 25° with or without 5′-AMP present. The studies with low substrate levels have also shown an unusual degree of interdependence between the Michaelis constant for one substrate and the concentration of the other.

The fact that the rather striking effects of 5′-AMP on muscle phosphorylase *a* were not observed earlier is ascribed (*a*) to the fact that most measurements have been made at room temperature and (*b*) to the use of high substrate concentrations in most studies. By the use of highly sensitive fluorometric procedures, it was possible to make valid kinetic measurements at very low substrate levels.

EXPERIMENTAL PROCEDURE

Materials—Rabbit muscle phosphorylase *a* was obtained from Sigma Chemical Company as a twice crystallized product, and was recrystallized three or four times more in the presence of mercaptoethanol. It was practically free of amylo-1,6-glucosidase. Rabbit liver glycogen, used throughout, was obtained from Mann Research Laboratories, Inc. The glycogen contained originally 0.35 mmole of ADP and 0.17 mmole of AMP per kg, which was removed by dialysis at pH 4.8. (Dialysis against water or buffer at pH 8 was only partially effective.) The glycogen was finally analyzed enzymatically with pyruvate kinase, myokinase, and lactic dehydrogenase (3), and was found to contain less than 0.01 mmole of 5′-AMP per kg.

Measurement of Phosphorylase Activity—Activities in the direction of glycogen breakdown, with P_i as substrate, were followed directly in the fluorometer (1-ml volumes) in the presence of 2 mM magnesium acetate, 0.05 mM TPN^+, 1 to 3 μg per ml of crystalline muscle phosphoglucomutase, 0.7 to 2 μg per ml of yeast glucose-6-P dehydrogenase of about 30% purity, and usually 0.1 mg per ml of bovine plasma albumin. (Both enzymes were obtained from Boehringer and Sons through California Corporation for Biochemical Research.) The fluorometer sensitivity was adjusted ordinarily to give readings accurate to $\pm 1 \times 10^{-8}$ M, but was increased 3-fold for study of kinetics at low glycogen levels. This permitted reliable measurements with total product formation of not more than 10^{-7} M. When measurements were made at temperatures other than that of the room, the tubes were incubated in a water bath and removed from the bath at intervals to be read. Because of the negative temperature coefficient of TPNH fluorescence (about 1.5% per degree), the tubes were read within 6 or 8 seconds after removal from the bath. Appropriate temperature corrections were made before calculation of velocities. In one experiment the reactions were stopped with a slight excess of NaOH and read at room temperature. Phosphorylase was always preincubated in the final medium for 2 to 5 minutes; otherwise there is a slight lag in rate (2). The reaction was started with glycogen.

To measure velocities in the direction of glycogen synthesis, samples were incubated with glucose-1-P in the same medium used for rates in the opposite direction, except that phosphoglucomutase was omitted. After incubation for 5 to 20 minutes, the amount of unused glucose-1-P was measured fluorometrically. Aliquots were diluted in a fluorometer tube containing P-glucomutase plus additional TPN^+ and glucose-6-P dehydrogenase; the fluorescence of the resulting TPNH was read a few minutes later. Dilutions varied from 10- to 500-fold according to the level of glucose-1-P. Since both phosphorylase and P_i were diluted simultaneously, and final readings could be made quickly, there was no problem from back-reaction even though phosphorylase was not inactivated. Initial velocities were calculated from pseudo-first order rate constants assessed in the manner described by Cori *et al.* (2) (equilibrium concentrations of glucose-1-P were observed rather than calculated).

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RESULTS

In the presence of 1 mM P_i and 0.1 mM glycogen, calculated as glucosyl residue (16 mg per liter), 5′-AMP increases the activity of muscle phosphorylase *a* 10-fold at 12°, and 80-fold at 41° (Fig. 1). Under these conditions the temperature optimum is 23° in the presence of 5′-AMP and 18° in its absence. This unusual temperature phenomenon is greatly affected by the level of substrate, particularly that of glycogen (Table I). With low P_i (0.3 mM) and relatively high glycogen (1.8 mM), velocities were equal at 28° and 38°, whereas with 10 mM P_i and 1.8 mM glycogen, the rate was faster by 40% at 38° than at 28°. This agrees with Cori *et al.* (2), who found the temperature optimum in the reverse direction, with high levels of glycogen and glucose-1-P, to be 39°. However, under conditions of high substrate level, Madsen and Cori (4) found that 5′-AMP had a greater affect on phosphorylase *a* activity at low than at high temperature (velocity was increased 3-fold by 5′-AMP at 0°, but only 50% at 30°).

The negative temperature coefficient with low substrate is definitely not due to destruction of the enzyme. If, after in-

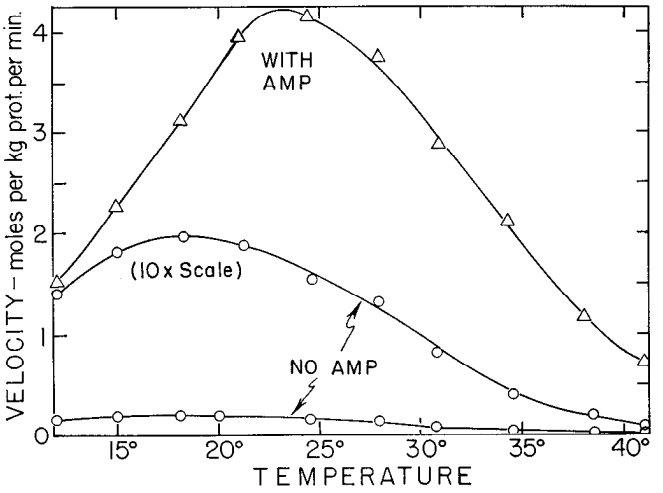

FIG. 1. Effect of temperature and 5′-AMP on phosphorylase *a* activity. P_i concentration was 1 mM, and glycogen concentration, 0.11 mM (calculated as total glucosyl residues). AMP when present was 0.1 mM.

TABLE I

Effect of temperature on phosphorylase a activity at different levels of P_i and glycogen

P_i	Glycogen	Velocity	
		28°	38°
mM	*mM*	*moles kg^{-1} min^{-1}*	
10.0	1.8	22.2	31.7
10.0	0.11	12.6	6.6
0.30	1.8	3.4	3.4
0.30	0.11	1.14	0.41
1.0	0.54	5.4*	6.1
1.0†	0.54	0.67*	0.12

* These are interpolated values from an experiment with other levels of glycogen.

† The 5′-AMP was omitted from these samples; all the rest contained 0.1 mM AMP.

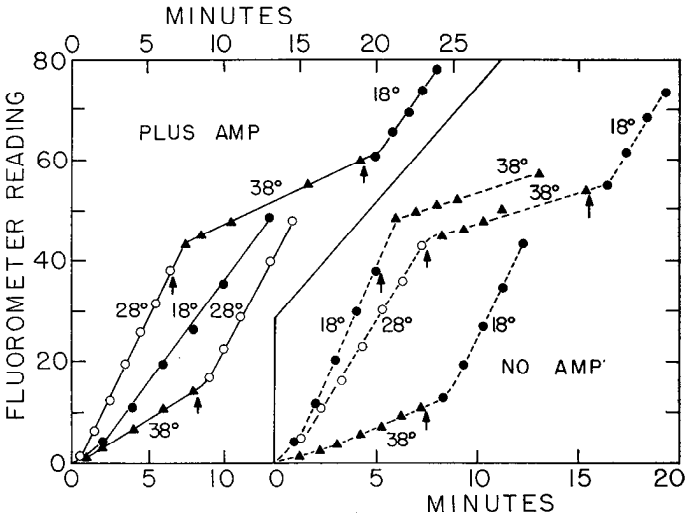

FIG. 2. Effect of temperature on velocity with low glycogen concentration. Samples (1 ml) were kept in a water bath at the indicated temperatures except for the few seconds required to measure the fluorescence. Fluorometric readings are equivalent to 7.7×10^{-8} M per division. The times of change to a different temperature are indicated by *arrows*. The fluorometer readings have been adjusted for the negative temperature coefficient of TPNH. The experiment with added 5′-AMP (0.11 mM) was conducted with 0.3 mM P_i, 0.11 mM glycogen (as glucosyl residue), and 0.36 μg of phosphorylase *a* per ml. For the experiment without 5′-AMP, P_i was increased to 1 mM and the amount of phosphorylase was increased 10-fold. Phosphorylase was added 5 minutes before the reaction was started (by adding glycogen). The rates at 18°, 28°, and 38° were, respectively, 0.87, 1.31, and 0.39 moles per kg of protein per minute with 5′-AMP, and 0.181, 0.134, and 0.035 mole per kg of protein per minute without 5′-AMP.

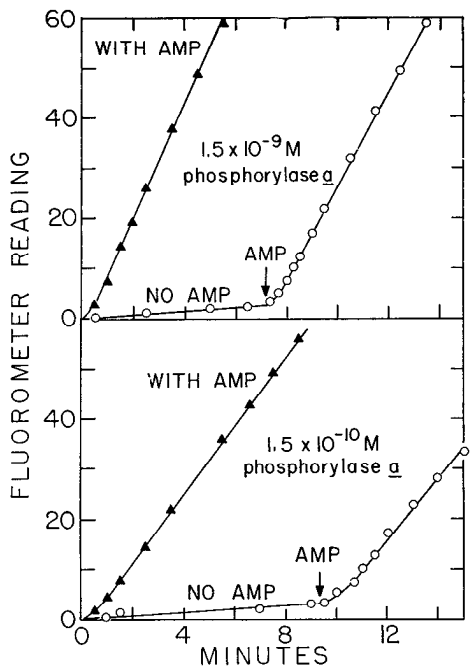

FIG. 3. Speed of action of 5′-AMP on phosphorylase *a*. Phosphorylase was preincubated for 5 minutes with either 0.3 mM P_i (*upper portion* of the figure) or 1.9 mM P_i (*lower portion*), and the reaction was started by adding glycogen to give a glucosyl concentration of 0.11 mM. AMP when present was 0.1 mM. The temperature was 28°. One fluorometer division is equivalent to 7.7×10^{-8} M TPNH.

cubation at 38°, the temperature was suddenly lowered, the rate immediately increased to the rate characteristic for the new temperature (Fig. 2). As seen, this is true both with and without 5′-AMP present.

The primary effect of 5′-AMP itself in these experiments is not one of stabilization; that is shown by experiments in which 5′-AMP was added either before phosphorylase or after phosphorylase had been allowed to act for 5 or 10 minutes (Fig. 3). Upon addition of the cofactor, the rate accelerated to approximately the control rate with a lag period entirely attributable to the auxiliary enzymes of the assay system. The fact that there was no demonstrable lag even with 1.5×10^{-10} M enzyme (0.077 μg of protein per ml) suggests that the phenomenon is intramolecular. (The facts presented do not rule out the possibility of a stabilizing affect of 5′-AMP during prolonged periods at elevated temperatures, and a few incidental observations would support this possibility.)

In the presence of 5′-AMP, the apparent Michaelis constant for P_i, K'_P, is a function of glycogen concentration (Fig. 4). Lines of the reciprocal plot intersect at almost the same extrapolated maximal velocities with the three glycogen levels shown. Values for K'_P range from 1.4 mM with 1.8 mM glycogen to 11 mM with 0.03 mM. Conversely, an increase in P_i concentration lowers the apparent Michaelis constant for glycogen, K'_G, which has the value of 0.04 mM with 10 mM P_i, and 0.25 mM with 0.1 mM P_i (Fig. 5). In this case, however, extrapolated maximal velocities clearly vary with P_i level.

In the absence of 5′-AMP, velocities are greatly reduced, as has already been seen, and yet extrapolated maximum velocities at infinite P_i concentrations are probably the same with or without 5′-AMP even at low glycogen concentration (Fig. 6). It will be noted that 0.034 mM glycogen (5.5 mg per liter) *with* 5′-AMP gives approximately the same rate curve as the 50-fold higher level of glycogen (1.8 mM) *without* AMP.

The effects of 5′-AMP on phosphorylase *a* are comparable for both glycogen synthesis and breakdown. Even with relatively high glycogen (5 mM), 5′-AMP greatly accelerates the rate of synthesis (Fig. 7); yet, as was found with P_i as substrate, extrapolated maximum velocities at infinite glucose-1-P concentration are the same. Consequently, with a low level of glucose-1-P, the addition of 5′-AMP results in a decrease in the apparent Michaelis constant for glycogen and an increase in the extrapolated velocities at infinite glycogen concentration (Fig. 8). The apparent Michaelis constant for glucose-1-P found by Cori *et al.* (2) was about 5 mM, *i.e.* 20 times that reported here. It is possible that the difference may be attributable to the fact that with the present fluorometric assay procedure, glucose-1-P levels

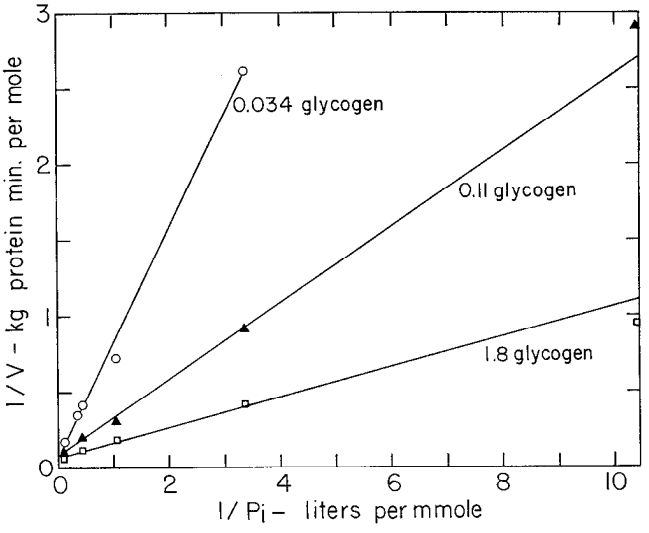

FIG. 4. Velocity of phosphorylase *a* activity as a function of P_i concentration at three levels of glycogen. The concentration of glycogen is given as millimoles of glucosyl residue per liter. AMP was present at a concentration of 0.1 mM. The temperature was 25°. The apparent Michaelis constants for P_i calculated from these curves are, respectively, 11.2, 3.8, and 1.5 mM in order from lowest to highest glycogen concentration.

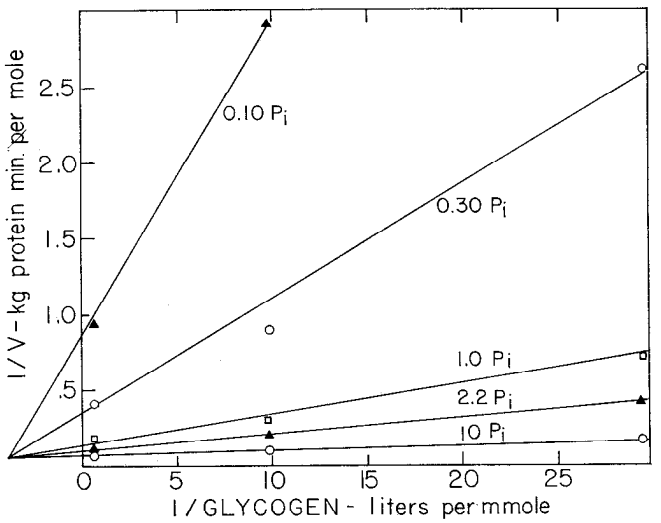

FIG. 5. Velocity of phosphorylase *a* activity as a function of glycogen concentration at five levels of P_i. Concentrations of P_i are millimolar. This figure is based on the same data as Fig. 4. The apparent Michaelis constants for glycogen calculated from these curves are 0.24, 0.21, 0.14, 0.12, and 0.04 mM (glucosyl residue) in order from the lowest to the highest P_i concentrations.

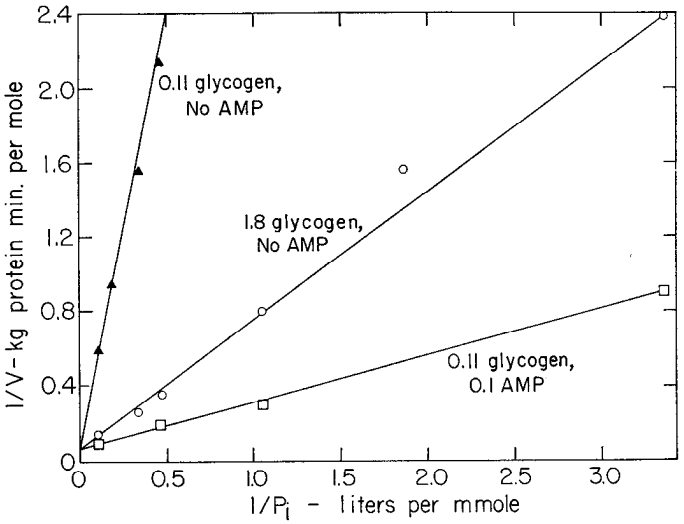

FIG. 6. Effect of 5′-AMP on phosphorylase *a* activity as a function of P_i and glycogen concentrations. AMP when present was at a concentration of 0.1 mM; glycogen levels are millimoles of glucosyl residue per liter. The temperature was 25°. The apparent Michaelis constants calculated from the curves for P_i with AMP absent are 70 and 10.5 mM, respectively, with 0.11 and 1.8 mM glycogen.

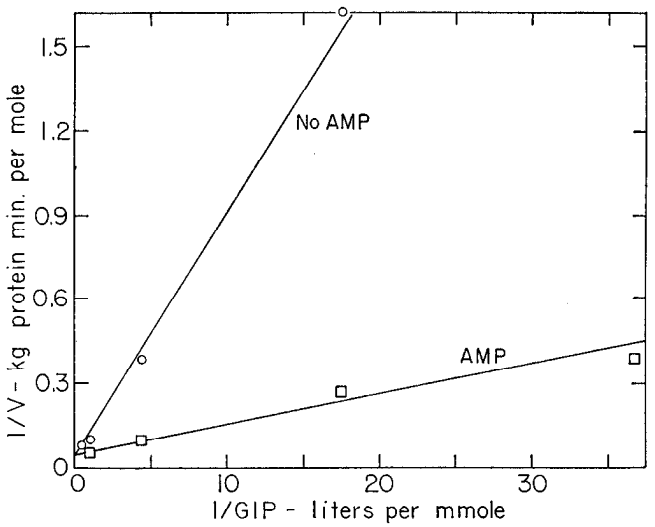

FIG. 7. Phosphorylase *a* activity as a function of glucose-1-P (*G1P*) concentration. Glycogen concentration was 5.4 mM. 5′-AMP when present was 0.01 mM. Temperature was about 26°. The apparent Michaelis constants for glucose-1-P calculated from these curves are 1.8 mM without AMP and 0.23 mM with AMP. Very similar constants were obtained with 0.5 mM glycogen.

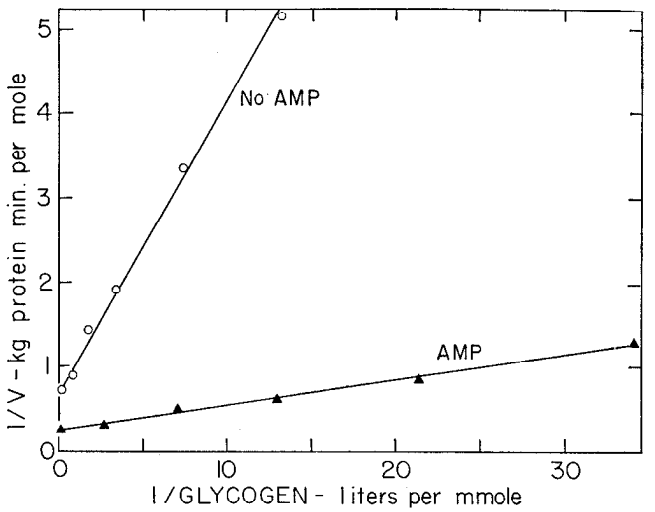

FIG. 8. Phosphorylase *a* activity in the direction of synthesis as a function of glycogen concentration. 5′-AMP when present was 0.01 mM. The reagent contained 0.055 mM glucose-1-P in 0.05 M imidazole buffer at pH 7. The temperature was about 26°. The apparent Michaelis constants for glycogen calculated from these curves are 0.5 mM without AMP and 0.12 mM with AMP.

as low as 0.025 mM could be readily studied, whereas with an assay procedure based on P_i formation, much higher substrate levels are ordinarily required.

The Michaelis constant for 5′-AMP with phosphorylase *a* is very low (Fig. 9) and is only slightly influenced by changing levels of substrates (Table II). This is in contrast to the findings of Helmreich and Cori (5) with phosphorylase *b*. On the other hand, K_{AMP} is increased 5-fold by raising the temperature to 38°, and if the ionic strength is brought to that of body tissues, K_{AMP} is increased to 4×10^{-6} M.

The effect of 5′-AMP seems to be quite specific. Tests were made with 5′-GMP, 5′-IMP, 5′-UMP, 5′-TMP, 5′-CMP, 3′-AMP, and 3′,5′-cyclic AMP. None was found with activity more than would be accounted for by a 0.2% contamination with 5′-AMP.

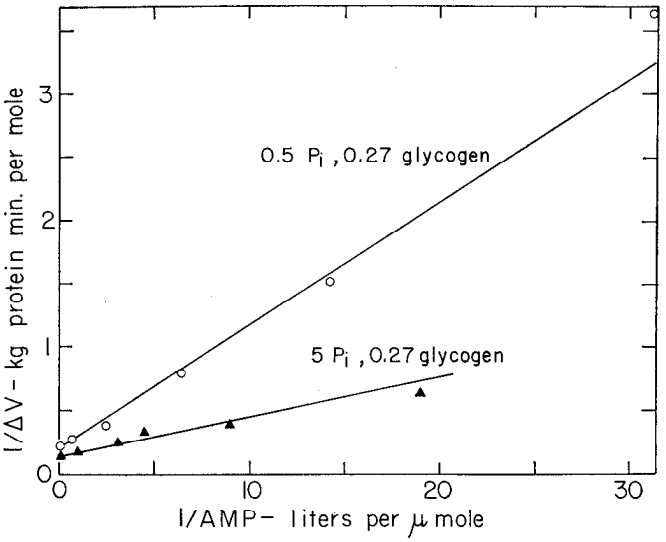

FIG. 9. Phosphorylase *a* activity as a function of 5′-AMP activity. The concentrations are millimolar. The apparent Michaelis constants for 5′-AMP calculated from these curves are 0.45 μM with 0.5 mM P_i, and 0.20 μM with 5 mM P_i.

TABLE II

Velocities with and without 5′-AMP at different P_i and glycogen concentrations

The Michaelis constants, K_{AMP}, are the concentrations of 5′-AMP which cause a half-maximal increase in velocity. Experiments A and B were made with 0.1 M imidazole-acetate buffer, Experiment C with 0.05 M imidazole-acetate buffer, and Experiment D with 0.04 M imidazole hydrochloride buffer in 0.125 M potassium acetate. All solutions were at pH 7.0.

Experiment	Temperature	P_i	Glycogen	Velocity		K_{AMP}
				−AMP	+AMP	
		mM	mM	moles kg^{-1} min^{-1}		μM
A	26°	0.5	0.27	0.28	5.1	0.45
	26	0.5	5.8	1.03	6.6	0.20
	26	5.2	0.27	2.32	11.9	0.20
	26	5.2	5.8	5.3	11.0	0.3
B	26	0.1	0.55	0.11	2.02	0.41
C	38	0.1	0.61	0.012	0.57	2.1
D	38	1.0	0.54	0.12	6.1	4.5

Analyses of Kinetic Data—The above findings can be fitted into the following arbitrary framework (*cf.* Alberty (6))

$$E + G \underset{}{\overset{K_G}{\rightleftharpoons}} EG \quad (1)$$

$$E + P \overset{K_P}{\rightleftharpoons} EP \quad (2)$$

$$EG + P \overset{K_P^G}{\rightleftharpoons} EXY \quad (3)$$

$$EP + G \overset{K_G^P}{\rightleftharpoons} EXY \quad (4)$$

$$EXY \rightarrow E + \text{products} \quad (5)$$

where G is glycogen, P is P_i, and rapid equilibrium is assumed except at the final step or steps. The velocity equation for this situation is

$$\frac{V}{v} = \frac{K_G K_P^G}{(G)(P)} + \frac{K_G^P}{(G)} + \frac{K_P^G}{(P)} + 1 \qquad (6)$$

An analogous equation would apply to the reverse situation. $K_G K_G^P$ is the term that Alberty combines into a single constant, K_{AB} (6), although he clearly indicates that it is numerically equal to the product of two constants as identified here.

By using the suggestions of Alberty (6) and of Frieden (7), it is possible to make estimates of the various dissociation constants from the kinetic data presented, together with similar data obtained at 38° (not shown). If the formulation is correct, at 25° with or without 5′-AMP (Table III) the presence of one substrate on the enzyme increases the affinity for the other as much as 20-fold. Estimates of K_P^G and of K_P are not made except as lower and upper limits, respectively, since the first is so low and the second is so high. From the data at 25°, K_P might be infinite, as demanded for strict compulsory order of addition; however, this seems unlikely since at 38° the kinetic values led to a real value for K_P. At 25°, 5′-AMP appears to decrease K_G to one-third, and both $K^G_{\text{glucose-1-P}}$ and K_P^G to one-seventh. Raising the temperature raises both of these constants, but in this case it is K_G which is most affected.

The apparent Michaelis constant for glycogen is considerably lower than that reported by Cori *et al.* (2), who found K'_G independent of 5′-AMP and equivalent to 1.3 mM (total glucosyl residues) with 16 mM glucose-1-P and no other buffer present. This may be compared with K'_G values of 0.12 and 0.5 mM, respectively, with and without 5′-AMP (Fig. 8). These values were obtained with low (0.05 mM) glucose-1-P. Fragmentary data at higher glucose-1-P levels do not indicate higher K'_G values. Conceivably part of the discrepancy may be attributed to the phenomenon of decreasing velocity with increasing chain length (2). In the present experiments the absolute changes in glycogen have been kept very small in both directions.

Madsen and Cori (8) made direct measurements of binding of phosphorylase *a* to glycogen and found a dissociation constant 10 times greater than the Michaelis constant (15 mM) found by Cori *et al.* (2). Furthermore, the binding was not affected by adding an equilibrium mixture of P_i and glucose-1-P. Because of these discrepancies between direct measurements and kinetic constants, an experiment was made with light scattering at 30- to 100-fold lower protein levels than those of Madsen and Cori. (The experiments are not intended to be definitive, but they are adequate to serve the present purpose.) The addition of glycogen resulted in a much greater increment in light scattering when phosphorylase was present than when it was absent. Linear plots were obtained between the reciprocal of this excess in light scatter and the reciprocal of glycogen concentration (Fig. 10). Glycogen scatters much more light than phosphorylase on a molar basis, and the values for excess light scatter extrapolated to infinite glycogen concentration were more than 10 times the light scatter of the phosphorylase by itself.[1] Therefore, the excess light scatter seems mainly attributable to an association of glycogen molecules rather than to the glycogen-phosphorylase association. Presumably 2 or 4 glycogen molecules combine with 1 molecule of phosphorylase. Regardless of the exact nature of the interaction, the concentration of glycogen which produces half-maximal excess of light scatter, K_G^S, may be closely related to the dissociation constant. In the presence of 5′-AMP, K_G^S was 0.65 and 0.28 mM, respectively, with 1.4×10^{-7} and 0.37×10^{-7} M phosphorylase, and in the absence of 5′-AMP a value of 1.25 mM for K_G^S was obtained (Fig. 10). In another experiment (not shown), in the presence of 5′-AMP and 1.1×10^{-7} M phosphorylase, the addition of 0.43 mM P_i and 0.17 mM glucose-1-P (*i.e.* an equilibrium mixture) lowered K_G^S from 0.59 to 0.18 mM.

Thus the results by light scattering agree surprisingly well with the kinetic results and confirm the effects of 5′-AMP, P_i, and glucose-1-P on K'_G. The results also suggest that higher

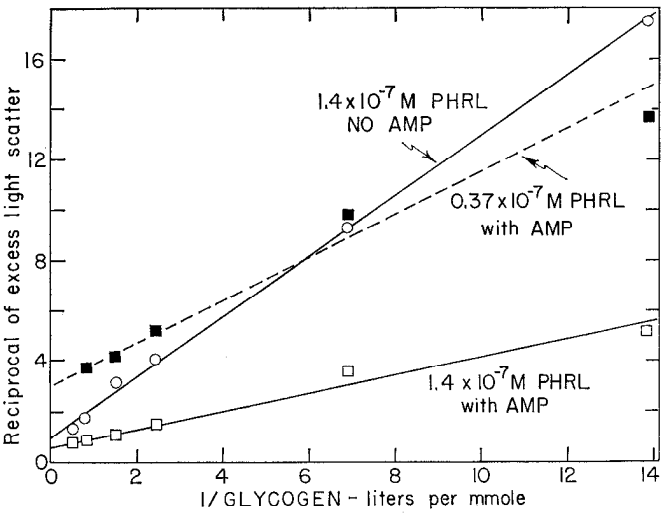

FIG. 10. Effect of glycogen concentration on light scatter in the presence of phosphorylase *a* (*PHRL*). Light scatter was measured at 350 mμ (90° angle) in an Aminco-Bowman spectrofluorometer. The experiments were performed in 0.1 M imidazole hydrochloride buffer at pH 7 in the presence of 0.01% bovine plasma albumin. The sum of the light scatter from glycogen and phosphorylase measured separately was deducted from the observed light scatter when both were present. It is the reciprocal of this difference (in arbitrary units) which has been plotted. Depending on the concentration, light scatter from the glycogen alone was 1.5 to 40 times greater than that from the higher of the two phosphorylase concentrations. AMP when present was 0.1 mM.

TABLE III

Dissociation constants for substrates of phosphorylase a

The glycogen constants are calculated in terms of total glucosyl residues.

Conditions	K_G	K_G^P	K_P	K_P^G	$K^G_{\text{glucose-1-P}}$
	mM	mM	mM	mM	mM
25°, no AMP	1.0	<0.2	>35	7	1.9
25°, 0.1 M AMP	0.26	<0.02	>20	1.1	0.23
38°, 0.1 M AMP	1.7	0.3	14	2.2	

[1] The crystalline phosphorylase when first diluted, although apparently in solution, scattered much more light than expected from its molecular weight. On standing at 25°, the light scatter diminished over a period of 30 to 60 minutes to a stable reading of about the expected value. This process, which is presumably one of disaggregation, could be greatly hastened by warming to 38° or by adding 0.1 mM 5′-AMP. This phenomenon seems to suggest that 5′-AMP can affect the configuration of phosphorylase.

values for K'_G may be obtained at high levels of phosphorylase. This is the expected result if a significant fraction of glycogen is tied up in phosphorylase combination. With a substrate that is larger than the enzyme, and which has many more available sites, it is difficult to calculate what proportion of the substrate should be considered free and what proportion bound at equilibrium. In the experiments of Madsen and Cori, with glycogen of 20,000,000 molecular weight, the molar ratio of phosphorylase to glycogen varied between 40:1 and 1,800:1. In the above light scattering experiments, assuming a molecular weight of 3,000,000 for the liver glycogen, the ratio varied between 1:1 and 40:1.

Reproducibility—The results reported above were obtained with phosphorylase recrystallized from two different lots of commercial enzyme. Although the results with the two preparations were indistinguishable, the experience of others has been that 5′-AMP enhancement may vary from one preparation to another (5). Therefore experiments were performed with four fresh phosphorylase *a* specimens. Two of these were made by adding ATP to crude rabbit muscle extracts which contained initially only phosphorylase *b*. In one case the reaction was stopped by dilution and the kinetic measurements were made at once. In the second case the phosphorylase *a* was partially purified but not crystallized. The other two specimens consisted of native phosphorylase *a* from rabbit and from rat. The animals were anesthetized, given 1 mg of epinephrine per kg, and 1 g of muscle was homogenized in a Waring Blendor with 100 ml of buffered solution containing cysteine, fluoride, and EDTA (8). Kinetic measurements were made on these samples with no purification whatsoever except that coarse particles were allowed to settle out. The total dilution was sufficient (1:50,000) to reduce native 5′-AMP to less than 5×10^{-8} M. Results with all four specimens of phosphorylase *a* were similar (Table IV) and agreed as far as they went with those obtained with the crystalline preparations.

TABLE IV

Effect of 5′-AMP on four phosphorylase a preparations

The analyses were carried out in 0.05 M imidazole buffer at pH 7.0. All the samples were obtained from skeletal muscle. The first two were made from phosphorylase *b* by adding ATP to crude preparations. The last two were native phosphorylase *a* specimens present in fresh homogenates of animals given epinephrine (see the text). Velocities are recorded as percentage of the activity of the sample with the highest substrate and 5′-AMP levels. Sample C apparently contained a little phosphorylase *b*.

Preparation	P_i	Glycogen	Velocity			K_{AMP}
			No AMP	2 μM AMP	220 μM AMP	
	mM	mM	%	%	%	μM
Rabbit A	0.5	0.5	0.7	17	23	0.5
	10	5	45	85	100	
Rabbit B	0.5	0.5	1.4	14	22	
	10	5	52	86	100	
Rabbit C	0.5	0.5	1.0	15	23	0.6
	10	5	35	67	100	
Rat	0.5	0.5	1.0	21	26	0.7
	10	5	53	83	100	

DISCUSSION

It is attractive to try to relate the effects of AMP on phosphorylase *a* to the anomalous temperature behavior. Possibly the most energetically stable configuration of the molecule provides easy accessibility of substrates to active sites, and this is reflected in low Michaelis constants. As temperature is increased, there occurs a transition to a configuration which offers poor accessibility of sites to substrates and hence higher Michaelis constants. AMP may be able to stabilize the more favorable configuration. As temperature increases, more AMP is required to maintain this configuration (*i.e.* a higher value for K_{AMP} is obtained), and as temperature is further increased, the favorable configuration is lost even with AMP present. With high levels of substrates, high activity is observed at relatively elevated temperatures, even in the absence of AMP. This would be expected if the rate-limiting step of the actual catalytic process were unaffected by the configuration.

The finding that phosphorylase *a*, like phosphorylase *b*, requires 5′-AMP for activity under certain circumstances raises the question of possible physiological significance. The first studies, made at room temperature and low ionic strength, gave such a low Michaelis constant for AMP that it seemed likely that phosphorylase *a* *in vivo* would always be fully active. The higher value of 4×10^{-6} M for K_{AMP} at physiological pH and ionic strength makes the possibility that phosphorylase *a* is not always fully active *in vivo* at least tenable.

Again, the fact that the AMP enhancement at room temperature is most evident with very low levels of substrates argued against physiological significance, but the experiments of Fig. 7 and Table I show a profound affect of AMP at 38° with 1 mM P_i (*i.e.* a concentration not far below the physiological range) when glycogen concentration was 0.1 to 0.5 mM. Although average concentrations of glycogen are much higher than this in tissues, because of the particulate nature of glycogen *in vivo* the concentration presented to phosphorylase might be quite low. Since, in addition, other factors such as glucose (9, 10) and ATP (11) might combine to further increase the AMP requirement, the possibility of a control function for 5′-AMP in relation to phosphorylase *a* activity cannot be dismissed. It has been shown that in brain, when the blood supply is cut off, even after phosphorylase *b* has been converted to phosphorylase *a*, glycogen does not begin to break down until AMP begins to increase (3). It might be suggested that the conversion of phosphorylase *b* to *a* renders the molecule more responsive to low levels of AMP and permits finer control of glycogenolysis, but that the same basic AMP control mechanism is inherent in both forms.

SUMMARY

1. In the presence of low levels of either glycogen, inorganic orthophosphate, or glucose 1-phosphate, skeletal muscle phosphorylase *a* is stimulated as much as 40-fold or more by very low levels of adenylic acid (5′-AMP).

2. With low substrate levels, the activity of phosphorylase *a* goes through a maximum at a temperature well below 38°. With 1 mM P_i and 0.1 mM glycogen (calculated as glucosyl residue), the temperature optimum was found to be 23° with 5′-AMP present and 18° in its absence. Taking the rate at 23° with 5′-AMP present as 100, the rates at 38° were 27° and 0.5, respectively, with and without 5′-AMP.

3. An increase in P_i concentration markedly lowers the apparent Michaelis constant for glycogen and vice versa. At

28°, the data are compatible with a mechanism that requires addition of glycogen before P_i. However, from measurements at 38°, it seems more likely that the enzyme can combine with either substrate alone, but that the presence of one substrate on the enzyme lowers the dissociation constant for the other by a factor of 10 or more.

4. The kinetic results with 5′-AMP are compatible with a 4-fold decrease by this nucleotide in the dissociation constant for glycogen and a 7-fold decrease in the dissociation constants for both P_i and glucose-1-P.

5. There appears to be a large temperature coefficient for the dissociation constant for P_i from phosphorylase *a*, and an even larger temperature coefficient for the glycogen dissociation constant.

6. Light scattering measurements have confirmed the conclusion from kinetic data that 5′-AMP, P_i, and glucose 1-phosphate lower the dissociation constant for glycogen.

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