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HUMAN PLATELET PHOSPHORYLASE*

SIMON KARPATKIN AND RICHARD M LANGER

Department of Medicine, New York University Medical Center, 550 First Avenue, New York, N Y 10016 (U S A)

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SUMMARY

Human platelets contain phosphorylase activity similar in some respects to that of skeletal muscle Sucrose gradient analysis of partially purified phosphorylase revealed a phosphorylase *b* dimer with apparent molecular weight of 177 000, $s_{20,w}$ = 8 9 S, a phosphorylase *a* dimer with apparent molecular weight 177 000, $s_{20,w}$ = 8 9 S, and a phosphorylase *a* tetramer with apparent molecular weight of 326 000, $s_{20,w}$ = 13 5 S AMP-independent phosphorylase activity (phosphorylase *a*) *in vitro* could be varied from 2 3% to 90% of total activity by suitable incubation or dialysis with MgATP, NaF or EDTA Of interest is the observation of an active phosphorylase *a* dimer (AMP-independent) Phosphorylase *a* dimer was the predominant form of phosphorylase *a* with the experimental condition employed

A kinetic analysis of phosphorylase *b* revealed a sigmoidal AMP-dependence curve with $^1/_2v_{max}$ = 6 10^{-5} M

Phosphorylase *a* had an apparent K_m for glycogen and orthophosphate of 0 2 and 0 7 mM, respectively ATP and ADP served as noncompetitive inhibitors with respect to glycogen and competitive inhibitors with respect to orthophosphate The apparent K_i for ATP and ADP with respect to orthophosphate was 0 82 and 2 9 mM, respectively The apparent K_i for ATP and ADP with respect to glycogen was 2 5 and 3 5 mM, respectively

INTRODUCTION

Human platelets play a dual role in the maintenance of hemostasis (1) They form a platelet plug which is essential for the repair of injury to blood vessels (2) They provide a phospholipid surface that is required for optimum coagulant interaction[1,2] Human platelets are a unique tissue in that the performance of their physiologic function is associated with their physical disintegration Consequently, the requirement for a return to steady-state levels of metabolic intermediates is no longer ap-

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plicable Thus, we are dealing with a tissue which performs its physiologic biochemical activity with a terminal release of energy[3]

Platelets have properties similar to those of skeletal muscle in the possession of a contractile protein ATPase, thrombosthenin, similar to actomyosin[4] They also contain similar concentrations of adenine nucleotide and of glycogen[3], which when depleted result in impaired platelet function[5–8] They have a predominantly aerobic glycolytic metabolism[9,10] with a glycolytic rate which is 14 times that of the red blood cell[11] and 4 times that of primate skeletal muscle[12] Since glycogenolysis represents 45% of total lactate production[3,10], a study of one of the enzymes regulating this major metabolic energy source was instituted[13]

Human platelet phosphorylase was partially purified and assayed in both the direction of glycogen synthesis and of glycogen degradation for total phosphorylase activity and AMP dependence Sucrose gradient estimations of phosphorylase molecular weights were obtained for AMP-dependent and AMP-independent phosphorylase activity Kinetic measurements of phosphorylase interaction with substrates as well as with AMP, ATP and ADP were also obtained

METHODS

Preparation of platelets

All procedures were performed at 0° unless otherwise noted. Human platelet-rich plasma, collected in acid–citrate–dextrose solution, was obtained 1–2 h after phlebotomy Platelets were separated from plasma by differential centrifugation and were washed in a modified human Ringer solution (pH 7 1) as described previously[3,10]

Extraction of platelet phosphorylase

Platelet phosphorylase was extracted at a low temperature (−196°) in an extractant similar to that employed by DANFORTH *et al* [14] The frozen pellet of above was ground to a fine powder as described and was thoroughly mixed with 3 vol /g wet weight of 40 mM β-glycerol phosphate buffer (pH 6 8), 10 mM mercaptoethanol, 5 mM EDTA, 20 mM NaF and 60% glycerol Control experiments revealed that 5 mM EDTA and 20 mM NaF were optimum concentrations for fixation and extraction of phosphorylase *b* and phosphorylase *a*, respectively The mortar was then transferred to an ice bucket where it was kept until thawing took place. The thawed slurry was reground and transferred to a centrifuge tube This was sedimented at 27 000 × *g* for 10 min The supernatant was then treated with charcoal (neutralized norite A), 40 mg/ml, to remove AMP Recentrifugation of this material provided the phosphorylase enzyme mixture for enzymatic assay Repetition of the charcoal procedure did not result in any appreciable decrease of phosphorylase activity or percent phosphorylase *a* This extract represented a 7-fold purification*

* The difficulty in obtaining large quantities of fresh human platelets (1 g wet weight per 500 ml whole blood) precluded attempts at further purification of human platelet phosphorylase Nevertheless, the prime role played by this tissue in hemostasis and its significant dependence on glycogenolysis, was considered significant justification for obtaining the data to be presented The AMP dependence of phosphorylase *b* (Fig 1) and the kinetic data of phosphorylase *a* (to be discussed below) did provide reasonable and useful information with regard to the regulation of this enzyme in human platelet glycogenolysis The same can be said for sucrose gradient analysis

Phosphorylase activity

This was measured in both directions, *i e* towards glycogen synthesis with the liberation of P_i and towards glycogen degradation with the liberation of Glc-1-*P*

1 Glycogen synthesis 0 2 ml of the tissue extract was added to 0 8 ml of the incubation mixture (tissue dilution of 1 20) at 30° The tissue AMP added to the assay cuvette was equivalent to $1\ 3 \cdot 10^{-5}$ M The incubation mixture contained 7 5 mM β-glycerolphosphate buffer (pH 6 8), 7 5 mM Glc-1-*P*, 5 mM mercaptoethanol, 10 mM NaF, 0 5 mM EDTA and 7 2 mM glycogen (glucose units) with or without 0 5 mM AMP Aliquots (0 2 ml) were removed at 0, 10, 20 and 40 min and were added to 8 ml of 0 125 M sodium acetate buffer (pH 4) P_i liberated was assayed in duplicate by the method of LOWRY AND LOPEZ[15] Measurements were linear with time in the presence or absence of 0 5 mM AMP Nonlinear measurements, when present, were discarded The assay gave zero readings in the absence of Glc-1-*P* or tissue extract and was linear with tissue extract

2 Glycogen degradation Tissue extract, 20 μl, (tissue dilution 1 100) was added to a cuvette containing a final volume of 0 5 ml and 20 mM β-glycerolphosphate buffer (pH 6 8), 16 3 mM glycogen (glucose units), 2 mM potassium phosphate, 3 mM $MgCl_2$, 0 46 mM NADP, 2 μg glucose-6-phosphate dehydrogenase and 10 μg phosphoglucomutase with or without 1 mM AMP at 30° The tissue AMP contribution to the assay cuvette was equivalent to $2\ 5\ 10^{-6}$ M following charcoal extraction and tissue dilution Rates were zero in the absence of P_i or glycogen Rates were linear with time as well as with tissue extract

Sucrose gradient analysis

Sucrose gradients were employed in a manner similar to that reported by MARTIN AND AMES[16] Continuous gradients of 5–20% sucrose in 20 mM β-glycerol phosphate buffer (pH 6 8), 5 mM mercaptoethanol with or without 20 mM NaF, 5 mM $MgCl_2$, 1 mM ATP or 5 mM EDTA were run in a Beckman L2 ultracentrifuge employing a SW-39 head with total volume of 250 μl carefully layered onto the gradient Catalase was assumed to have a molecular weight of 250 000, $s_{20,w} = 11\ 3$ S (ref 17) Gradients were centrifuged at 37 000 rev /min for 10–13 h at 4° The gradient was tapped, and 4-drop aliquots were collected This was diluted by addition of 300 μl of 20 mM β-glycerol phosphate buffer (pH 6 8), 20 mM NaF, 5 mM EDTA and 5 mM mercaptoethanol, and alternate tubes were assayed for enzyme activity in the presence and absence of 1 mM AMP Marker protein, catalase, was assayed at 406 mμ Molecular weights were calculated by employing the treatment of MARTIN AND AMES[16], *e g* $s_1/s_2 = (M_1/M_2)^{2/3}$, where s_1 and s_2 are sedimentation constants of two different proteins and M_1 and M_2 are their respective molecular weights The assumption was made that $s_1/s_2 = R_1/R_2$, where R_1 and R_2 represent the distance traveled from the meniscus of the sucrose gradient

Materials

Distilled deionized water was used at all times All chemicals were reagent grade Glucose-6-phosphate dehydrogenase, type X, EDTA, $NADP^+$, ATP, ADP and AMP were obtained from the Sigma Chemical Co (St Louis, Mo) Norite A was obtained from Fisher Scientific (New York, N Y), and shell fish glycogen was obtained from Mann Research Co (New York, N Y) It was dissolved in distilled water and treated

twice with charcoal, 60 mg/g glycogen The glycogen in the absorbed solution was then precipitated twice with absolute alcohol, was dissolved in β-glycerol phosphate buffer and was assayed for glycogen content

RESULTS

Phosphorylase activity

When measured in the direction of glycogen synthesis, total phosphorylase activity was 67 7 $\pm$ 9 7 μmoles P_i per g per h (10 experiments, $\pm$ S E) Under basal conditions (directly extracted from 0° washed platelets in the presence of EDTA and NaF), platelet phosphorylase *a* (AMP-independent activity) represented 49 $\pm$ 8% of total activity (10 experiments)

When phosphorylase was measured in the direction of glycogen breakdown, total phosphorylase activity was 17 5 $\pm$ 0 7 μmoles Glc-1-*P* per g per h (7 experiments, $\pm$ S E) Percent phosphorylase *a* represented 56 $\pm$ 7 4% (8 experiments, $\pm$ S E) (Phosphorylase was assayed in the direction of glycogen breakdown for the remainder of the investigation)

Phosphorylase b

When the tissue extract of above was dialyzed in the extractant media, *minus* the glycerol (*i e* contained 20 mM NaF and 5 mM EDTA), 1 ml of tissue extract per liter of dialysis fluid, 12 hours at 4°, the % of phosphorylase *a* decreased to 37 $\pm$ 9% (5 experiments, $\pm$ S E , $P < 0\ 05$)* Addition of glycerol to the assay mixture had no effect on the 37% phosphorylase *a* obtained following dialysis The small decrease in percent phosphorylase *a* following dialysis could be further enhanced by redialysis to remove NaF (*i e* same dialysis fluid, *minus* NaF, conditions favoring phosphorylase phosphatase) followed by incubation at 37° for 1–2 h in the presence of 5 mM EDTA Under these conditions, phosphorylase *a* declined to 15 $\pm$ 6% (6 experiments) When phosphorylase was extracted from platelets with the above extraction media, *minus* NaF, the percent phosphorylase *a* was again 56% before dialysis However, following dialysis against the same extractant, *minus* glycerol, the percent phosphorylase *a* decreased to 9% and incubation at 37° was unnecessary The lowest phosphorylase *a* obtained with the above type manipulations was 2 3%

When low percent phosphorylase *a* preparations were assayed in the presence of increasing AMP concentrations, sigmoidal curves were obtained (Fig 1) similar to the AMP dependence of skeletal muscle phosphorylase *b* The AMP concentration at which velocity was $^1/_2 v_{max}$ was $6 \cdot 10^{-5}$ M It is thus apparent that concentrations of AMP of $2\ 5 \cdot 10^{-6}$–1 3 10^{-5} M (see MATERIALS AND METHODS) could not have been responsible for the relatively high percentage of phosphorylase *a* obtained prior to dialysis.

Sucrose gradient analysis of phosphorylase b

A phosphorylase extract containing 20 mM β-glycerolphosphate buffer (pH 6 8), 5 mM mercaptoethanol and 5 mM EDTA was incubated at 37° for 1 h This resulted in

* Similar results were obtained when the enzyme was assayed in the direction of glycogen synthesis Tissue extract obtained with minimal manipulation, *i e* directly out of plasma, revealed 56% phosphorylase *a* prior to dialysis and 33% following dialysis (average of 4 experiments)

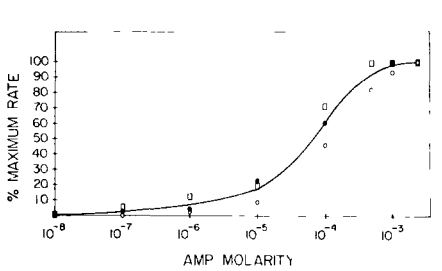

Fig 1 AMP saturation curve for phosphorylase *b* activation, plotted semi-logarithmically Low percentage of phosphorylase *a* prepared as described in MATERIALS AND METHODS was assayed in the direction of glycogen breakdown with increasing concentration of AMP Experimental data from three different preparations are plotted AMP concentration at one-half maximal velocity was 6 10^{-5} M

Fig 2 Sucrose gradient analysis of phosphorylase containing 98% phosphorylase *b* This preparation was obtained as described in MATERIALS AND METHODS A 5-ml continuous gradient of 5–20% sucrose containing 20 mM β-glycerol phosphate buffer, 5 mM mercaptoethanol and 5 mM EDTA was layered with 200 μl of tissue extract and 2 mg catalase marker It was centrifuged at 37 000 rev /min at 4° for 11 h in a Beckman L2 ultracentrifuge employing a SW-39 head Alternate tubes were assayed for phosphorylase activity in the absence (○—○) and presence (●—●) of 1 mM AMP Catalase protein was determined from the absorbance at 406 mμ, (×— — —×)

a preparation containing 2 3% phosphorylase *a* When this preparation was centrifuged in a sucrose gradient containing 20 mM β-glycerolphosphate buffer (pH 6 8) 5 mM EDTA and 5 mM mercaptoethanol, one enzyme peak was obtained (Fig 2) This peak contained phosphorylase activity in the presence of 1 mM AMP but absence of enzyme activity in the absence of 1 mM AMP The apparent molecular weight for this preparation was 151 000, $s_{20,w} = 8$ 1 S and probably represents phosphorylase *b* (refs 18–20)

Phosphorylase a

When the phosphorylase extract was dialyzed overnight at 4° against 20 mM β-glycerolphosphate buffer (pH 6 8) *plus* 5 mM mercaptoethanol, 5 mM $MgCl_2$ and 20 mM NaF, no significant increase in phosphorylase *a* was obtained Neither was an increase obtained when this mixture was incubated at 37° for 1–2 h However, if 1 mM ATP was added to this mixture which was then incubated at 37° for 1 h, (conditions favoring phosphorylase *b* kinase) phosphorylase *a* increased to 88 ± 1 3% (6 experiments) It was also soon apparent that incubation at 37° was unnecessary, since dialysis of the above mixture at 4° for 12 h in the presence of 1 mM ATP was also effective, resulting in conversion to 86% phosphorylase *a*

Kinetic analysis of regulators of phosphorylase a

A phosphorylase preparation was prepared by extracting tissue extract as described in methods but with the absence of 5 mM EDTA This tissue extract was then dialyzed at 4° overnight against the same extractant, *minus* 60% glycerol, *plus* 5 mM $MgCl_2$ and 1 mM ATP This preparation did not lose activity during dialysis and was now refractory to AMP activation (0–10% activation)

The K_m for glycogen for this phosphorylase *a* extract was approx 0 2 mM (Figs 3A and 3B) Both ATP and ADP served as noncompetitive inhibitors of phos-

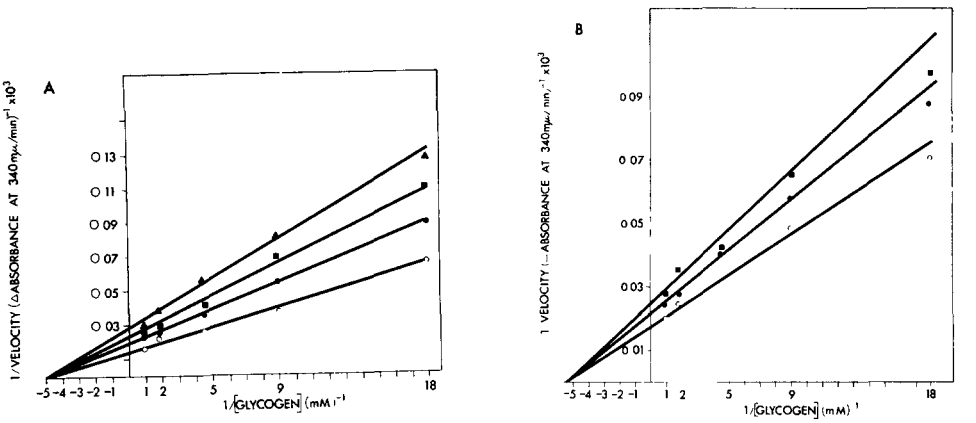

Fig 3 A Lineweaver–Burk plot of phosphorylase *a* for interaction of glycogen and ATP Three levels of ATP are given ○, 0 mM, ●, 1 mM, ■, 2 mM, ▲, 3 mM Glycogen K_m for this preparation was 0 212 mM (glucose units) Respective v_{max} values for these ATP inhibitor concentrations were 71 4, 52 6, 43 5 and 35 1 The K_i for ATP was 2 5 mM B Lineweaver–Burk plot of phosphorylase *a* for interaction of glycogen and ADP Two levels of ADP are given ○, 0 mM, ●, 0 75 mM, ■, 1 5 mM Glycogen K_m for this preparation was 0 197 mM Respective v_{max} values for these ADP inhibitor concentrations were 58 8, 47 6 and 40 8 The K_i for ADP was 3 5 mM Assay for A and B was in the direction of glycogen breakdown at 30° The assay cuvette contained 20 mM β-glycerol phosphate buffer, pH 6 8, 2 mM potassium phosphate, variable glycogen, 3 mM $MgCl_2$, 20 mM NaF, 0 46 mM $NADP^+$, 2 μg glucose-6-Phosphate dehydrogenase, 10 μg phosphoglucomutase and 20 μl of phosphorylase *a* tissue extract (1 100 tissue dilution)

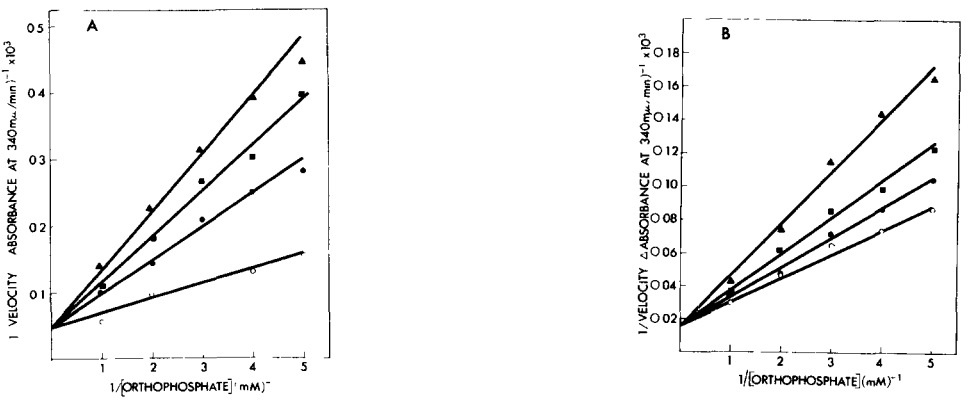

Fig 4 A Lineweaver–Burk plot of phosphorylase *a* for interaction of orthophosphate and ATP Three levels of ATP are given ○, 0 mM, ●, 1 mM, ■, 1 5 mM, ▲, 2 5 mM Respective K_m values for this phosphorylase preparation and these ATP inhibitor concentrations were 0 528, 1 18, 1 61 and 1 99 mM The K_i for ATP was 0 82 mM B Lineweaver–Burk plot of phosphorylase *a* for interaction of orthophosphate and ADP Three levels of ADP are given ○, 0 mM, ●, 0 75 mM, ■, 1 5 mM, ▲, 3 0 mM Respective K_m values for this phosphorylase preparation and these ADP inhibitor concentrations were 0 938, 1 25, 1 40 and 1 88 mM The K_i for ADP was 2 9 mM Assay for A and B was in the direction of glycogen breakdown at 30° The assay cuvette contained the same as in Fig 1 except for glycogen which was held constant at 5 6 mM (glucose units) and orthophosphate which was varied

phorylase *a* when glycogen was varied The K_i for ATP and ADP were 2 5 and 3 5 mM, respectively (Figs 3A and 3B)

The K_m for orthophosphate of phosphorylase *a* varied between 0 53 and 0 94 mM (Figs 4A and 4B) Both ATP and ADP served as competitive inhibitors of phosphorylase *a* when orthophosphate was varied ATP appeared to be a more potent inhibitor of orthophosphate than was ADP Thus the K_i for ADP was 3 5-fold greater than the K_i for ATP, *i e* 2 9 and 0 82 mM, respectively (Figs 4A and 4B)

Sucrose gradient analysis of phosphorylase a

When dialyzed phosphorylase extract was treated with 20 mM β-glycerol-phosphate buffer (pH 6 8), 5 mM mercaptoethanol, 20 mM NaF, 5 mM $MgCl_2$ and 1 mM ATP at 37° for 100 min, a preparation was obtained containing 85% phosphorylase *a* When this was centrifuged on a sucrose gradient (same as above except for ATP), again one enzyme peak was obtained (5 experiments) with apparent molecular weight of 179 00, $s_{20,w} = 9$ 0 S (Fig 5) The enzyme activity was present in the absence, as well as in the presence, of 1 mM AMP and is probably a phosphorylase *a* dimer[21-24]

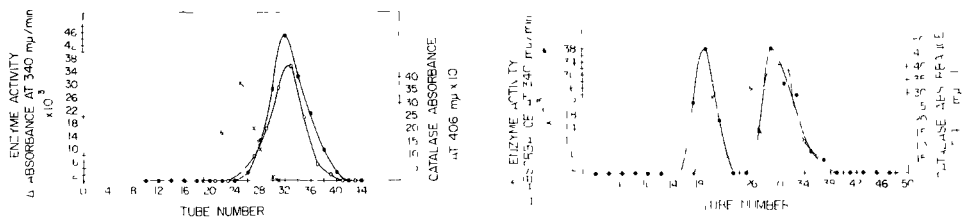

Fig 5 Sucrose gradient analysis of phosphorylase containing 85% phosphorylase *a* This preparation was obtained by dialysis at 4° for 12 h in the presence of 20 mM β-glycerol phosphate buffer, pH 6 8, 20 mM NaF, 5 mM $MgCl_2$, 1 mM ATP This was next incubated at 37° for 200 min and then layered on a 5–20% sucrose gradient containing all of the above, except for 1 mM ATP The gradient was centrifuged at 37 000 rev /min for 13 h at 4° Alternate tubes were assayed for phosphorylase activity in the absence (○—○) and presence (●—●) of 1 mM AMP Catalase protein was determined from the absorbance at 406 mμ, (×— — —×)

Fig 6 Sucrose gradient analysis of phosphorylase containing 84% phosphorylase *a* This preparation was obtained and treated indentically as in Fig 5 except for the omission of the 37° incubation step

When an 85% phosphorylase *a* preparation was obtained in the absence of heating, *i e* by employing a similar mixture as above, but was dialyzed overnight at 4°, two enzyme peaks were obtained (Fig 6) The higher molecular weight peak, apparent molecular weight 306 000, $s_{20,w} = 12$ 9 S, had an identical enzyme activity in the absence and presence of 1 mM AMP and probably represents a phosphorylase *a* tetramer The lower molecular weight peak, apparent molecular weight 173 000, $s_{20,w} =$ 8 9 S, had slightly greater enzyme activity in the presence of 1 mM AMP than in its absence This peak probably represents a phosphorylase *a* dimer with a small admixture of a phosphorylase *b* dimer Attempts at finding tetramer were not always successful and may be related to the relatively low enzyme concentration employed, inherent in a partially purified enzyme system Thus, only 4 out of 7 enzyme preparations dialyzed at 4° under conditions leading to varying percentage phosphorylase *a* resulted

in tetramer formation In 3 out of these 4 cases, the tetramer peak was minor The one example of major tetramer peak is shown in Fig 6 Further attempts at consistently inducing tetramer formation proved unsuccessful (see DISCUSSION)

Assay of 17 dimer preparations had a molecular weight of 177 000 ± 2800 (S E), $s_{20,w} = 8\ 9$ S No significant differences were noted in molecular weights of phosphorylase *a* or *b* dimers The tetramers averaged an apparent molecular weight of 326 000 ± 12 500 (4 experiments), $s_{20,w} = 13\ 5$ S

DISCUSSION

Human platelets are glycolytically and glycogenolytically active Glycogenolysis contributes approximately one-half of glycolytic flux through the Embden–Meyerhof pathway, the principal metabolic pathway[3,9,10,25] The regulation of glycogenolysis is mediated by a platelet phosphorylase which has been found to be similar in some respects to skeletal muscle phosphorylase The presence of a phosphorylase in human platelets, which is activated by AMP, has been previously reported from this laboratory[13,26] as well as from other laboratories[27,28] However, the molecular weights as well as the kinetic interaction of phosphorylase with substrates, glycogen and orthophosphate, and modifiers, AMP, ATP, ADP, have not been previously reported Human platelet phosphorylase exists as 2 functional forms with respect to enzyme activity as modified by AMP a sensitive phosphorylase *b* form, which is inactive in the absence of AMP, (apparent molecular weight 177 000, $s_{20,w} = 8\ 9$ S) and an insensitive phosphorylase *a* form which is active in the absence of AMP (apparent molecular weight 326 000, $s_{20,w} = 13\ 5$ S) (or active dimer, apparent molecular weight 177 000, $s_{20,w} = 8\ 9$ S) Although these molecular weights are less than that reported originally by KELLER AND CORI[18], they are in reasonable agreement with more recent reevaluations of rabbit skeletal muscle phosphorylase[29,30], In rabbit skeletal muscle, phosphorylase *b* and phosphorylase *a* have been shown to consist of 2 or 4 subunits which make up a dimer and tetramer species, respectively[31,18,19] Interconversion of phosphorylase *b* $\rightleftharpoons$ phosphorylase *a* is mediated by the enzymes, phosphorylase phosphatase[32] and phosphorylase *b* kinase[33] with the additional requirement of MgATP for kinase activity This has been found to result in the incorporation of 4 phosphoryl groups per mole of tetramer, phosphorylase *a*[34] Similar subunits appear to be operative in human platelets as well as the enzyme apparatus for the interconversion of the two phosphorylase species Platelet phosphorylase also contains an active phosphorylase *a* dimer, *i e* an enzyme that is active in the absence of AMP with a molecular weight similar to that of phosphorylase *b* dimer In this respect, WANG AND GRAVES[21] have reported interconversion of a phosphorylase *a* tetramer to an inactive phosphorylase *a* dimer by increasing temperature, increasing salt concentration, decreasing pH and protein dilution In another communication, these authors[22] presented suggestive evidence for the presence of an active phosphorylase *a* dimer In human platelets, temperature and protein dilution also appear to enhance the formation of an active phosphorylase *a* dimer Recently, HELMREICH *et al* [24] have reported the presence of an inactive phosphorylase *a* dimer obtained from the phosphorylase *a* tetramer by the addition of glucose In an earlier report, METZGER *et al* [35] presented light-scattering evidence for an active phosphorylase *a* dimer WANG AND GRAVES[21] have also noted stabilization of the phosphorylase *a* tetramer by the presence of AMP or alkaline pH

We have been unable to duplicate these observations with human platelet active phosphorylase *a* dimer We were unable to enhance phosphorylase *a* tetramer formation. It is conceivable that the experimental conditions employed resulted in too low an enzyme concentration In this respect, these data are consistent with the suggestion that active phosphorylase *a in vivo* may exist as the phosphorylated dimer rather than the tetramer Similar conclusions were drawn by METZGER *et al* [35]

Of further interest, with respect to platelet phosphorylase, is the recent report of KARPATKIN AND LANGER[13] wherein inactive phosphorylase dimer as well as monomer could be converted to phosphorylase *b* and phosphorylase *a* in the presence of MgATP

Of interest was the observation of strong ATP and ADP competitive inhibition of human platelet phosphorylase *a* for orthophosphate In rabbit skeletal muscle, phosphorylase *b* is more strongly inhibited by ATP than is phosphorylase *a*[36]

Although human platelet total phosphorylase activity represents approx 2 4% that reported for frog skeletal muscle (per g wet weight, 5 5% per mg protein*)[14] the rate of glycogenolysis of human platelets[3,10] is still considerably greater than that reported for frog skeletal muscle[14,38] This rate might conceivably be attributed to the high percentage of "resting" human platelet phosphorylase *a*, 45% (ref 26), when compared to resting skeletal muscle phosphorylase of 3% (ref 14) As reported by KARPATKIN AND LANGER[26], activation of glycogenolysis by physiologic agents known to initiate platelet plug formation (agglutination and contraction of platelets) did not result in the further conversion of phosphorylase *b* to phosphorylase *a* as is the case with skeletal muscle contraction[14] Observations of a similar nature were noted in rat heart muscle[36,40,41], as well as in an I strain of mice without phorylase *b* kinase[42] Changes in platelet glycogen and orthophosphate, as well as in ATP, ADP, AMP, Glc-6-*P* (modifiers of phosphorylase[41,43]) following platelet aggregation by physiologic agents, have also been reported by KARPATKIN AND LANGER[3] The present kinetic data on human platelet phosphorylase along with the intracellular changes of substrates and modifiers of platelet phosphorylase mentioned above might conceivably explain the activation of glycogenolysis during simulated platelet plug formation[3,10] in the absence of changes in percent phosphorylase *a*

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* Frog skeletal muscle contains 267 mg protein per g wet weight[37] Human platelets contain 114 mg protein per g wet weight [39]

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