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PLASMA MEMBRANE PHOSPHORYLATION BY ENDOGENOUS PHOSPHATE DONORS IN HUMAN BLOOD PLATELETS

SELECTIVITY OF THE ACTION OF DIBUTYRYL CYCLIC AMP

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SUMMARY

Incubation of platelet-rich plasma with $^{32}P_i$ leads to cellular uptake of the isotope and covalent incorporation into several cell constituents. Plasma membranes isolated from intact labelled platelets, delipidated and solubilized in sodium dodecyl sulfate, show, upon gel electrophoretic analysis, three main peaks of radioactivity: two in the molecular weight range 100 000–30 000 and an additional very slow migrating component strongly positive by the periodic acid-Schiff reaction. Treatment of the cells with dibutyryl cyclic AMP under conditions just sufficient to completely inhibit platelet aggregation leads to an increased isotope incorporation. Electrophoretic analysis of membranes isolated from dibutyryl cyclic AMP-treated cell reveals: (a) no change in the general pattern of distribution of the isotope, (b) no difference in the isotope incorporation to the two components of lower mol. wt. and (c) a marked increase (> 100 %) in isotope incorporation in the slow migrating material as compared to membranes isolated from control cells. This material can be extracted from platelet plasma membranes after treatment of the membranes for 5 h with Triton X-100, at a detergent-to-protein ratio of 7.5. When the membrane material extracted with Triton X-100 is subjected to gel chromatography in Agarose (Biogel A-15m), the phosphorylated material that corresponds to the slow migrating band in polyacrylanide gel electrophoresis is eluted with or very close to the void volume of the column. Isoelectric focussing of this fraction, shows a single radioactive peak corresponding to an isolectric point of 3.78. The isolated component is pronase-sensitive, contains 52 % of carbohydrate and 15 % sialic acid. Analysis of the stability of the bound phosphate suggests that about 43 % of it is bound as acylphosphate. The results reported, obtained through an approach that closely resembles physiological conditions are compatible with the participation of this membrane phosphoglycoprotein in the phenomena of platelet aggregation.

Abbreviations: cyclic AMP, adenosine 3′ : 5′-cyclic monophosphate; dibutyryl cyclic AMP, N^6,$O^{2'}$-Dibutyryl adenosine 3′ : 5′-cyclic monophosphate; $PheMeSO_2F$, phenylmethyl-sulfonyl-fluoride; $TosPheCH_2Cl$, L-1-tosylamide-2-phenylethyl-chloromethyl ketone; $TosylCH_2Cl$, N-α-*p*-tosyl-L-lysine chloromethyl ketone.

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INTRODUCTION

An important aspect of the process of haemostasis is the mechanism by which blood platelets are transformed following injury so that they adhere to each other, initiating a process that will culminate with the formation of thrombi [1–3].

Adhesion and aggregation are direct consequences of membrane properties. In addition many of the events associated with the platelet reaction, such as the release reaction and contractile activity either involve the plasma membrane or are a consequence of its stimulation.

Several compounds have been shown to be inhibitors of platelet aggregation [3]. Of the many compounds that inhibit platelet aggregation, prostaglandin E_1 is the most potent inhibitor described so far [4, 5] and the role of cyclic AMP in mediating the action of prostaglandin E_1 has been well established [6–8]. Cyclic AMP itself is a weak inhibitor of platelet aggregation while its substituted derivative, dibutyryl cyclic AMP, has been shown to be a potent inhibitor [9–11]. Recently, a well documented paper dealing with the endogeneous phosphorylation of isolated platelet membranes by ATP and the characteristics of a membrane-bound protein kinase has been published [12].

The experiments to be described in the present paper were designed in order to study the phosphorylation of the platelet plasma membranes under experimental conditions as close as possible to the physiological ones (intact platelets in their own plasma and $^{32}P_i$ as the source of phosphate). Using this approach it was found that dibutyryl cyclic AMP preferentially increases the degree of phosphorylation of a glycosilated protein not previously described in platelet plasma membranes.

MATERIALS AND METHODS

Platelet-rich plasma. Human blood was obtained from healthy donors at the Blood Bank of the Hospital Universitario, Caracas. Blood (450 ml) was collected in plastic bags (Fenwal JF-15-ES), containing 63 ml of acid citrate dextrose (17 mM citric acid 90 mM tri-sodium citrate 16 mM NaH_2PO_4 and 140 mM D-glucose). The bags were centrifuged in a PR 2 International Centrifuge at 190 $\times$ *g* for 15 min at 18 °C and the supernatant platelet-rich plasma was carefully collected in a transfer bag without anticoagulant (Fenwal TA-3-M). In all cases, the platelet-rich plasma was freed of residual erythrocytes by repeated centrifugation at 1000 $\times$ *g* for 55 s. Although this treatment will remove part of the large-heavy fraction of platelets, it will also yield a cleaner platelet preparation (contamination with red cells and leukocytes never exceeded one per 20 000 and one per 30 000 platelets, respectively). The platelet-rich plasma was always kept at room temperature (23–25 °C) and used within 2.5 h. Platelet-poor plasma was obtained by centrifugation of platelet-rich plasma at 3000 $\times$ *g* for 10 min followed by 30 min centrifugation of the supernatant plasma at 30 000 $\times$ *g*.

Platelet aggregation. A Gilford 240 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio, USA), modified to include a magnetic stirrer under the cell compartment, was used to measure the extent of platelet aggregation [13, 14].

Platelet-rich plasma (1 ml) was incubated in the cuvette for 3 min at 37 °C. After this incubation, the cuvette was transferred to the spectrophotometer and stirring was initiated. Aggregation was triggered after 15 s by addition of 50 μl of an ADP solution in 0.15 M NaCl, pH 7.2, to obtain the desired final ADP concentration. The slope of the plot of absorbance versus time during the first 10–15 s was used to obtain saturation curves. In experiments designed to study the effect of length of incubation with dibutyryl cyclic AMP on the degree of inhibition of the aggregation, a saturation curve for ADP was always established before, to select the concentration of ADP giving the maximum and half-maximum effects.

^{32}P incorporation in intact platelets suspended in plasma. Platelet-rich plasma was incubated for the desired time with 100 μCi/ml of $^{32}P_i$ made 0.15 M in NaCl, at 37 °C. Gentle stirring of the incubation mixture was done at 15 to 20-min intervals. For the experiments designed to study the effect of dibutyryl cyclic AMP on the incorporation of ^{32}Pi by platelets, the platelet-rich plasma, after addition of the isotope was divided into two equal aliquots and dibutyryl cyclic AMP was added to one of them. At the end of incubation, control and dibutyryl cyclic AMP-treated cells were centrifuged down at $4000 \times g$ for 15 min, washed twice with 10 volumes of 100 mM phosphate 0.15 M NaCl (pH 7.2), and solubilized in 2 % sodium dodecyl sulfate. After protein determination aliquots were taken for radioactivity counting.

Iodination of intact platelets. Platelets from platelet-rich plasma were washed as described by Baenziger and Majerus (method I) and suspended in 10 ml of the resuspension buffer [15]. Iodination was accomplished as described by Czech and Lynn [16].

Platelet plasma membranes were obtained through a combination of the glycerol-lysis technique described by Barber and Jamieson [17] and a method described by Brunette et al. [18] that makes use of an aqueous two-phase polymer system developed by Albertsson [19]. Platelet-rich plasma, 20 ml per tube, was carefully layered on 30 ml of a glycerol gradient (0–40 %) and centrifuged initially at 1000 $\times$ g for 30 min and subsequently for 15 min at 5000 $\times$ g. The supernatant was completely removed by aspiration. The platelet pellet was disrupted with a stirring disc and lysis accomplished by adding 15 ml of 1 mM $ZnCl_2$. Stirring was continued until the suspension appeared homogenous (approx. 10 min). The suspensions were then transferred to a pre-cooled all-glass homogenizer, type B, (Kontes Glass Co., Vineland, N.J., U.S.A.), and subjected to 25 up-and-down strokes. The homogenate was centrifuged at 20 000 $\times$ g for 15 min. The pellets were suspended by means of a Vibromixer in 5 ml of top phase (polyethylenglycol) and then, 5 ml of bottom phase (Dextran) were added. The two phases were mixed and centrifuged at 11 000 $\times$ g for 10 min. The membranes were collected at the interface of the two-phase system. The interface together with the top and most of the bottom phase were carefully transferred to another tube, remixed, and the centrifugation carried out again. This step was repeated twice to remove any trapped contaminants. After the last centrifugation, material at the interface was carefully collected and diluted with five volumes of Tris/sucrose pH 7.5. The membranes were then sedimented from the suspension by centrifugation for 10 min at 35 000 $\times$ g and the pellet washed twice with the same solution. The membranes prepared as described, were immediately used or otherwise stored in 30 % glycerol at −25 °C. Electron microscopy of material obtained as described before, was done after fixation for 1 h in 5 % cacodylate-buffered glutaral-

dehyde. The activity of several marker enzymes were also determined in order to have another criteria with regard to the purity of the preparation: $(Na+K^+)$-ATPase (EC 3.6.1.3) was measured at 37 °C in 100 mM Tris (pH 7.5), containing 2 mM Mg^{2+}, 60 mM Na^+ and 5 mM K^+ in a final volume of 1.0 ml. The $(Na^+ + K^+)$-ATPase activity was obtained from the difference observed when 100 μM ouabain was included in the reaction mixture. *p*-Nitrophenylphosphatase (EC 2.1.1.2) was measured at 37 °C in 50 mM acetate (pH 4.6), containing 5.5 mM *p*-nitrophenylphosphate and 0.1 % Triton X-100 in a final volume of 1.0 ml. After stopping the reaction with 4.0 ml of 0.1 N NaOH, the absorbance was read at 405 nm ($\varepsilon_M = 1.73 \cdot 10^4\ M^{-1} \cdot cm^{-1}$). Acid β-glycerophosphatase (EC 3.1.3.2) was measured at 37 °C in 25 mM acetate (pH 4.6), containing 25 mM β-glycerophosphate and 0.05 % Triton X-100. Glucose-6-phosphatase (EC 3.1.3.9) was determined as described by Kaulen et al. (20). NADH: cytochrome *c*-oxidoreductase (EC 1.6.99.3) was determined in 20 mM Tris · HCl (pH 7.4), containing 0.12 mM NADH and 66 μM potassium ferrocyanide as acceptor. The absorbance was read at 340 nm ($\varepsilon_M = 6.2 \cdot 10^3\ M^{-1} \cdot cm^{-1}$). All activities were expressed as μmol of substrate consumed or product released per min per mg of membrane protein.

Polyacrylamide gel electrophoresis. The membranes, suspended in an appropriate volume of 1 mM each $TosPheCH_2Cl$ and $TosLysCH_2Cl$ or 1 mM $PheMeSO_2F$ were solubilized by addition of 10 % sodium dodecyl sulfate to a final concentration of 2.5 %, followed by 2-mercaptoethanol at a final concentration of 1 %. Solubilization of the membrane was usually aided by heating the sample in boiling water for 10 min. Use of 6 M urea or heating overnight at 50 °C did not change the results.

Once solubilized, the membranes were subjected to a lipid extraction with 10 volumes of cold chloroform/methanol (60 : 40) overnight. The protein precipitate was centrifuged at 4000 × *g* for 10 min and washed twice with fresh chloroform/methanol. The precipitate from the last washing was solubilized in 2.5 % sodium dodecyl sulfate/ 1 % mercaptoethanol in 0.125 M Tris, pH 6.8, and used for polyacrylamide gel electrophoresis. The stacking gel was 3 % and the running gel was 6 % acrylamide respectively. The buffer for the electrophoresis was 25 mM Tris (hydroxymethyl) amino methane (Trizma base), 192 mM glycine, 1 % sodium dodecyl sulfate and 10 % glycerol, pH 8.3. The fixing and staining of the gels with Coomassie blue as well as the staining for carbohydrates using the periodic acid-Schiff procedure was done as described by Fairbanks et al. [21]. Slicing of the gels for radioactivity counting was accomplished in unfixed gels, frozen with dry-ice. The slices were incubated overnight in scintillation counting vials with 0.5 ml of 0.5 M NaOH at 50 °C. After cooling, 5 ml of Insta-gel (Packard Instrument Co., Ill., U.S.A.) was added to each vial and cooling continued in the dark for 2 h before starting the counting procedure.

Extraction of membrane proteins with Triton X-100. Membranes were extracted at room temperature with continuous stirring for 4.5 h, at a detergent-to-membrane protein ratio of 7.5 and a final protein concentration of 1mg/ml. It was found that under these conditions, 80 % of the ^{32}P radioactivity was consistently extracted from the membranes. Any insolubilized material was removed by centrifugation at 25 000 × *g* for 20 min.

Isoelectric focussing was carried out in an LKB 8100 electrofocussing equipment (LKB-Produkter AB, Stockholm-Bromma, Sweden). The sample, in 0.75 %

Triton X-100 was made 2 % with the corresponding ampholine (pH 3.5–10.0 or pH 2.5–6.0) and was used as the light solution for the sucrose gradient.

pH stability of the phosphate bond. The phosphorylated component obtained in the electro-focussing experiments was extensively dialyzed against 1 % sodium dodecyl sulfate or 1 % Triton X-100 and aliquots (approx. 2000 cpm) were incubated for 30 min at pHs ranging from 2.0 to 13.0. At the end of the incubation, the sample was filtered through a Sephadex G-200 column (1.0 × 30 cm) equilibrated with the same detergent at pH 7.5. The percentage of hydrolysis was calculated from the counts remaining in the void volume fraction. All buffers were used at 0.2 M concentration. Bound phosphate released by treatment with hydroxylamine was measured as above after incubation of the sample with 3 M neutral hydroxylamine at 30 °C. The control sample included NaCl instead of hydroxylamine.

Extraction and identification of platelets' nucleotides was done as described by Murakami et al. [22].

Total hexose content was determined by the anthrone method as described by Scott et al. [23]. Samples from isoelectric focussing were extensively dialyzed against 1 % sodium dodecyl sulfate in order to remove as much as possible of Triton X-100, which was found to interfere severely with the anthrone method.

Sialic acid was determined as described by Warren [24].

Protein was determined by the method of Lowry, as modified by Wang and Smith [25].

Materials. Dibutyryl cyclic AMP, ADP, *p*-nitrophenylphosphate, *β*-glycerophosphate, lactoperoxydase, pronase, *n*-butyric acid, sodium dodecyl sulfate and Triton X-100 were all the best grade reagents obtained from Sigma Chemical Co., St. Louis, Missouri, USA. ^{32}P, as orthophosphate and $Na^{131}I$, both carrier free, were obtained from New England Nuclear, Boston, Mass., U.S.A. All other reagents were the best grade commercially available.

RESULTS AND DISCUSSION

Incubation of platelet-rich plasma with $^{32}P_i$ at 37 °C for 1h indicates that platelets are capable of incorporating $^{32}P_i$ and that the level of incorporation is increased 2–3 times by the addition of dibutyryl cyclic AMP. Extensive delipidation of the samples, before counting, produces a 30 % decrease in the number of counts in both the controls and the dibutyryl cyclic AMP-treated cells, suggesting that the difference in labelling observed is not due to a difference in incorporation of the isottope into lipidic molecules. When isolated plasma membranes were incubated with $^{32}P_i$ under the same conditions used with platelet-rich plasma, no detectable labelling was found. Sodium butyrate (100 μM) was without effect on the incorporation of $^{32}P_i$ by intact platelets suspended in its own plasma.

Extraction and identification of the labelled nucleotides, as described in Methods reveals that under our experimental conditions, $^{32}P_i$ can be incorporated into the metabolic pool of nucleotides in intact platelets [26].

Inhibition of the aggregation phenomena by dibutyryl cyclic AMP

Salzman et al. [11] have shown that the action of dibutyryl cyclic AMP requires its entry into the platelet, where it mimics the functions of intracellular cyclic

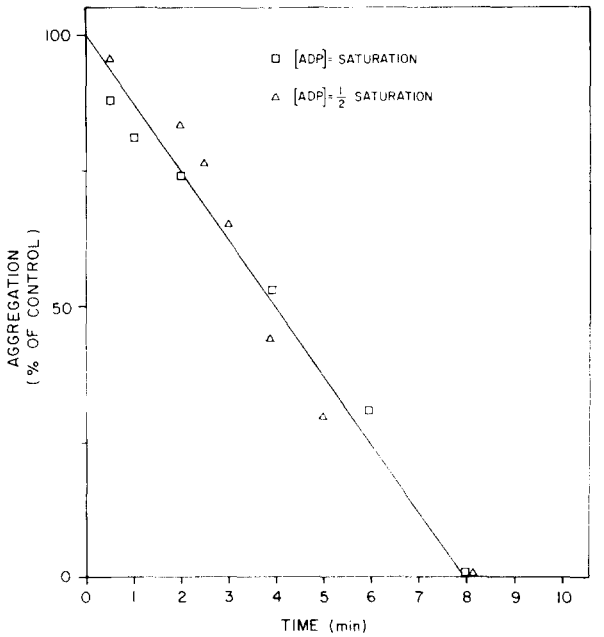

Fig. 1. Time dependence for the inhibition of platelet aggregation induced by incubation with 1 mM dibutyryl cyclic AMP. Each point represents the mean value obtained from three different batches of platelets. Temperature, 37 °C.

AMP, and that the platelet uptake of the cyclic nucleotide is a time-dependent process. As shown in Fig. 1, there is an inverse lineal relationship between time of incubation with dibutyryl cyclic AMP and the degree of inhibition of platelet aggregation. This relationship is not influenced by varying the concentration of the aggregating stimuli (ADP), and complete inhibition is obtained after approx. 7 min of incubation, irrespective of whether the final concentration of ADP was saturating or below the saturating range.

Phosphorylation pattern of plasma membranes isolated from labelled intact platelets

Plasma membranes, obtained from control platelets incubated with $^{32}P_i$ and subjected to polyacrylamide gel electrophoresis in sodium dodecyl sulfate, show a pattern of isotope distribution qualitatively similar to that obtained from membranes derived from dibutyryl cyclic AMP-treated cells (Fig. 2). However, quantitatively both preparations show a significant difference in the degree of labelling of a membrane material that does not migrate into the gel, even at gel concentrations of 1.7 % acrylamide. Similar results are obtained in the presence of 1 mM aminophylline, although this compound is not as effective as dibutyryl cyclic AMP. Staining of the gels with Coomasie blue did not reveal any stainable material on top of the gels, or at most, only a very faint coloration could be observed. However, treatment with periodic acid followed by Schiff reagent gives a very intense purple band at the top, in addition to the other three glycoprotein bands described also by other investigators [27–30]. No significant difference, however, can be observed between controls and

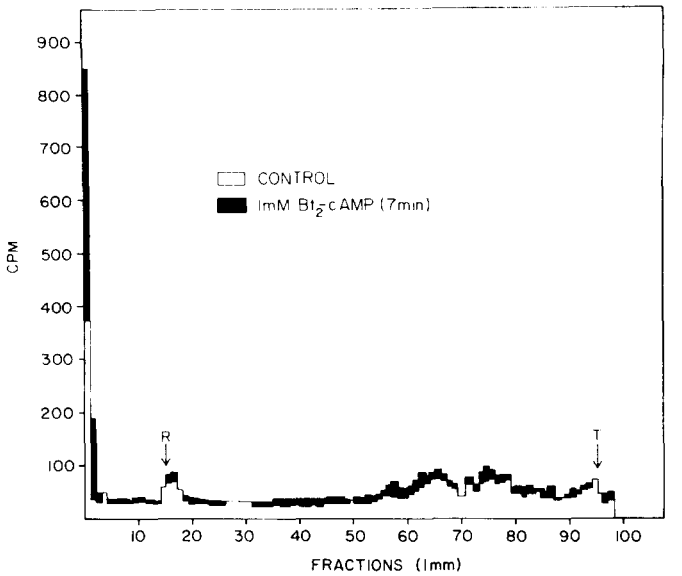

Fig. 2. ^{32}P-labelling of human platelet membrane. Isotopic pattern of 150 μg of delipidated membrane protein, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. R, beginning of running gel (6 % acrylamide); T, position of tracking dye at the end of the run; □, control; ■, 1 mM dibutyryl cyclic AMP (7 min).

cells treated with dibutyryl cyclic AMP.

Although, the increase in the ^{32}P incorporation of the slow-migrating material could simply reflect the general increase of incorporation of the isotope, a preferential labelling of this material, modulated by dibutyryl cyclic AMP, can not be excluded. In this regard, it is interesting to note that ^{32}P incorporation in the other two phosphorylated components does not appreciably change under the same conditions.

Our results are somewhat different from those obtained by Steiner [12]. Although the procedure for membrane solubilization is quite the same in both cases, Steiner does not observe any degree of ^{32}P label remaining on top of the gels, even at 5 % gel concentration and the general pattern of isotope distribution in the lower molecular weight components, although very similar, does in our case show only minor quantitative differences between controls and dibutyryl cyclic AMP-treated cells (the ratio varying from 1.2 to 1.4). On the contrary, this ratio increases between 2 and 3 times for the material at the top of the gel. These differences might be explained by the different experimental approach used (intact platelets and inorganic ^{32}P as the source of phosphate vs. isolated platelet membranes and ATP acting as the phosphate donor used in ref. 12).

Artifacts introduced by contamination of the membranes with other subcellular constituents seem unlikely. The procedure used here for obtaining platelet plasma membranes yields a membrane preparation which morphologically and in terms of enzyme markers (Table I) seems clean from mitochondria, lysozomes or microsomes. Furthermore, it is simpler and less time-consuming than other methods previously described for the same purpose [17, 31].

Gel filtration of plasma membranes solubilized in sodium dodecyl sulfate

TABLE I

ENZYMATIC CHARACTERIZATION OF PLATELET PLASMA MEMBRANES ISOLATED AS DESCRIBED IN METHODS

Marker enzyme		μmol/mg per min	
		Barber & Jamieson [18]	Kaulen & Gross [33]
Total ATPase	0.050	0.011	n. r.*
$(Na^+ + K^+)$-ATPase (ouabaine sensitive)	0.010	0.005	0.004
pNPPase	0.180	0.410	0.770
βGPase	n.d.**	n.r.	0.009
Glucose-6 phosphatase	n.d.	n.r.	0.002
NADH: cytochrome *c* reductase	n.d.	n.r.	n.r.

* Not reported.
** Not detectable.

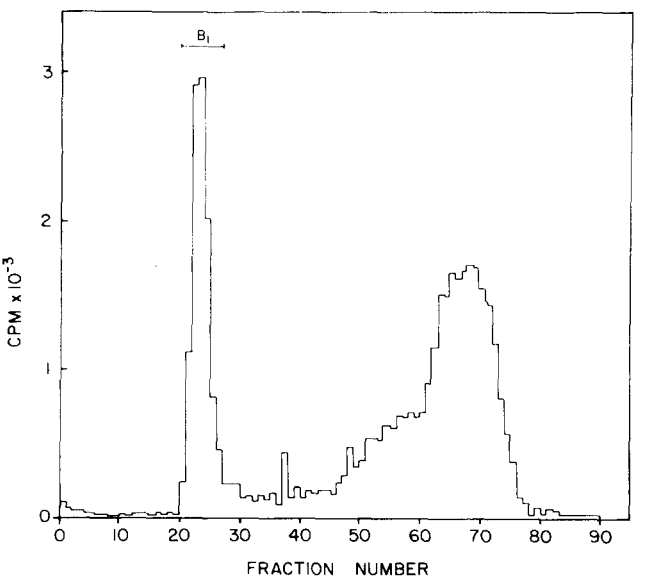

Fig. 3. Gel filtration of ^{32}P-labelled platelet plasma membranes solubilized in 1 % sodium dodecyl sulfate. Column (1.6×38 cm) of Bio-Rad A-15m eluted with 1 % sodium dodecyl sulfate. Fraction volumes of 0.7 ml were collected.

(1 %), through a column of Bio-Rad A-15m equilibrated with 1 % sodium dodecyl sulfate, yields the elution pattern of ^{32}P-labelled components shown in Fig. 3. Although a better resolution was obtained with Bio-Rad A-15m, filtration through Sepharose 4B gave the same general pattern. When the fractions, corresponding to the radioactive peak eluted with the void volume of the column (B_1) were pooled, concentrated and subjected to polyacrylamide gel electrophoresis in sodium dodecyl

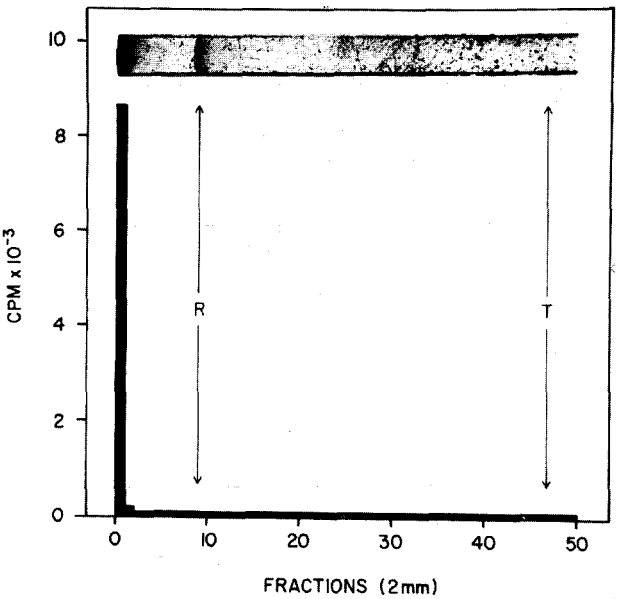

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel analysis of fraction B_1, obtained as indicated in Fig. 3 (80 μg of protein). Bottom, ^{32}P distribution. Top, periodic acid-Schiff staining. R, and T, as in Fig. 4.

sulfate, all of the radioactivity detected was located on top of the gel and it coincided with a strong periodic acid-Schiff positive reaction developed at the same position of the gel (Fig. 4).

Extraction of platelet membrane proteins with Triton X-100. Triton X-100 used as described in Methods (at a detergent: protein ratio of 7.5 (w/w) and a membrane protein concentration of 1 mg/ml) extracts 70–80 % of the radioactivity that had been incorporated in platelet plasma membranes as ^{32}P after 4–5 h of constant stirring at room temperature. Under these conditions, the solubilized protein represents 72 % of the total protein. Increasing the extraction time to 12 h does not appreciable increase the amount of radioactivity solubilized. Extraction times less than 2.5 h failed to solubilize any material eluting with the void volume of the columns, suggesting that solubilization of such material requires partial disorganization of the membrane structure. Fractionation of the solubilized membranes, through Bio-Gel A-15m or Sepharose 4B equilibrated with 0.75 % Triton X-100, yields a pattern of elution absolutely comparable to the one obtained after sodium dodecyl sulfate solubilization. Radioactive fractions corresponding with the void volume were pooled and subjected to isoelectric focussing.

Isoelectric focussing of the fractions eluting with the void volume. A preliminary isoelectric focussing, between pH 3.0 and 10.0 yielded a single radioactive fraction at pH approx. 4. Refinement of the focussing, between pH 2.5 and 6.0 resulted in the pattern shown in Fig. 5. The pI of the phosphorylated component corresponds to 3.78.

A total hexose content of 52 % (based on protein) for this component, is in

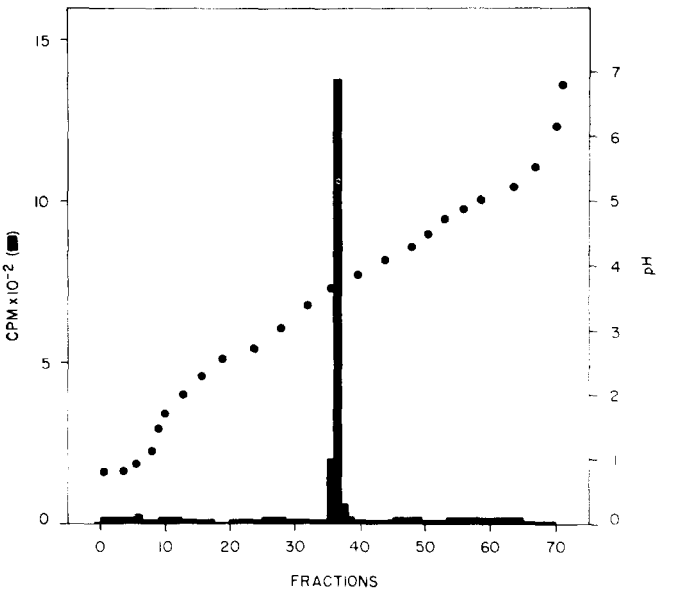

Fig. 5. Isoelectric focussing analysis of fraction B_1 obtained as indicated in Fig. 3. 300 μg of protein was distributed in the sucrose gradient.

agreement with the strong periodic acid-Schiff positive reaction observed at the top of the polyacrylamide gels and supports the idea of the glycoproteic nature of the phosphrylated component. The sialic acid content was found to be 15 %. Pronase treatment of the phosphorylated component, followed by gel filtration through Sepharose 4B, completely abolish the peak of radioactivity merging from the column with the fractions corresponding to the void volume.

Although various types of glycopeptides from platelet plasma membranes, with sialic acid contents ranging from 14 to 28 %, have been characterized [27–30], it is interesting to note that none of them behave electrophoretically in polyacrylamide gel electrophoresis as the glycoprotein described in this paper, and up to date no relationship with phosphorylation events mediated by cyclic nucleotides have been described for such glycopeptides.

The character of the phosphate bond was determined as described in Methods by studying the stability of the bound phosphate towards pH variation. As seen in Fig. 6, hydrolysis, as measured by the release of $^{32}P_i$, is most rapid at the extremes of pH, a fact which imposes a U shape on the stability curve. Curves of such shape are a general property of acylphosphates [32–36]. Reaction of the phosphorylated component with 3 M neutral hydroxylamine at room temperature produces the release of 43 % of the label. These results suggest that part of the bound phosphate exists as acylphosphate. The functional significance of this finding is not clear. Smith et al. [37] have reported on two nuclear histone kinases which catalyse the formation of acid-labile histone phosphates. It is not clear however if they belong to the acyl-phosphate type.

The data so far described suggest that we are dealing with a phosphoglyco-

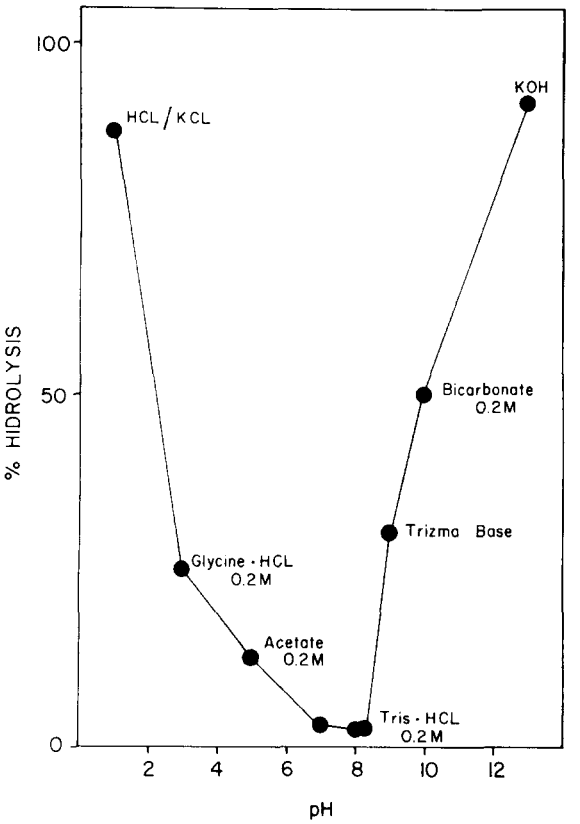

Fig. 6. Effect of pH on the decomposition of phosphorylated component B_1, isolated by isoelectric focussing.

protein of high apparent molecular weight whose phosphorylation may be, at least in part, regulated by the availability of cyclic nucleotides. The position of this component in the membrane can not yet be precisely defined. The glycoprotein nature of it and its relative difficulty of extraction with Triton X-100 might suggest the possibility of a transmembrane location [38, 39]. However, more experimental evidence is needed in order to clarify this question.

Our results are suggestive of a functional role for this phosphoglycoprotein in the triggering of the release reaction and subsequent platelet aggregation. In this regard, a likely function might be the regulation of the concentration of intracellular ionic calcium, modulated through the action of internal or external stimuli on this membrane component. Although direct experimental support is not available so far, current work in our laboratory is being done in this direction.

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