Separation and Identification of Type 1 and Type 2 Protein Phosphatases from Rabbit Reticulocyte Lysates*

(Received for publication, June 11, 1982)

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Protein phosphatase type 1 and type 2 activities (designated PP-1 and PP-2, respectively) from rabbit reticulocyte lysates have been identified and characterized based on criteria previously established for similar activities in rabbit skeletal muscle and rabbit liver. These include (a) chromatographic separation on DEAE-cellulose, (b) substrate specificity toward glycogen phosphorylase a and the a- and b-subunits of phosphorylase kinase, (c) differential sensitivity to the heat-stable protein phosphatase inhibitors-1 and -2, and (d) sensitivity to MgATP. When total lysate phosphatases are assayed in the presence of 1 mM MnCl₂, protein phosphatase type 1, which is designated PP-1, is the predominant phosphatase activity. However, when phosphatase assays are carried out with MgATP concentrations similar to those in the lysate, type 2 activity is diminished, and the levels of type 1 (41%) and type 2 (59%) phosphatase activities are comparable. A small proportion (6%) of total lysate phosphatase is tightly bound to the ribosomes, where type 1 phosphatase predominates.

At least five species of protein phosphatases can be identified in lysates. These constitute two forms of protein phosphatase type 1, one of which (designated F₁) is dependent on MgATP and a lysate activator protein F₂, and F₁ and F₂ have been identified previously in skeletal muscle. Three species of protein phosphatase type 2 have been identified and designated PP-2A₁, PP-2A₂, and PP-2B based on criteria recently established for rabbit skeletal muscle and rabbit liver phosphatases, which display similar phosphatase profiles. Lysate protein phosphatases types 1, F₁, F₂, and F₃ can all act on phosphorylase a and the a- (type 2) or b- (type 1) subunit of phosphorylase kinase. PP-2B, a Ca²⁺/calmodulin-dependent phosphatase, specifically dephosphorylates the a-subunit of phosphorylase kinase, but does not act on phosphorylase a. The heat-stable protein phosphatase inhibitor-2 from skeletal muscle completely blocks the activity of the two type 1 phosphatases (PP-1, F₁), but has no effect on the three species of type 2 protein phosphatase. A preliminary assay of the two heat-stable phosphatase inhibitors in lysates indicates significant levels of inhibitor-2, but little or no detectable inhibitor-1.

Several proteins associated with the protein-synthesizing machinery of reticulocyte lysates have been shown to be phosphorylated in vitro by various protein kinases. These include the initiation factors eIF-4B, eIF-5, the a- and b-subunits of eIF-2, several subunits of eIF-3, and one or more ribosomal proteins (1–6). The roles of some initiation factors are now partly understood. However, the functions of the phosphorylated components remain unknown, with the single exception of phosphorylated eIF-2a. The evidence now indicates that the inhibition of lysate protein synthesis induced by heme deficiency, or by low concentrations of double-stranded RNA, is due primarily to the phosphorylation of eIF-2a by specific protein kinases which are activated in lysates by the appropriate stimuli (6–12). Several of the protein kinases involved in the phosphorylation events cited above have been isolated from reticulocytes. These include the cAMP-dependent protein kinases (types I and II) (13, 14), the heme-regulated eIF-2a kinase (6–10), the double-stranded RNA-dependent eIF-2a kinase (6, 11, 12) and several other cAMP-independent protein kinase activities (2, 10, 15). By contrast, little is known about reticulocyte protein phosphatases. Recent studies with several other tissues have demonstrated that protein phosphatases may be classified into two groups, designated type 1 (PP-1) and type 2 (PP-2) (16, 17). This classification is based on criteria which include (i) chromatographic separation on DEAE-cellulose (17); (ii) substrate specificity towards glycogen phosphorylase and the a- and b-subunits of phosphorylase kinase (16, 17); (iii) sensitivity to MgATP (16, 17); and (iv) sensitivity to the heat-stable phosphatase inhibitors-1 and -2, which inhibit type 1 phosphatases specifically (16–20).

Two forms of type 1 protein phosphatase have been identified. One of these, which is simply designated PP-1, is in the
active state at all stages of purification, while the second (F2) is dependent on MgATP as well as an activator protein (F6) for its activity (21-23). The properties of these enzymes are indistinguishable by a number of criteria (23).

Type 2 protein phosphatases include three enzymes designated 2A, 2A, and 2B. PP-2A consists of three subunits (35,000, 59,000, and 69,000 daltons) while PP-2A contains just two of these subunits (35,000 and 69,000 daltons). Both of these enzymes are active on a variety of substrates, including phosphorylase a and the a-subunit of phosphorylase kinase (17). The 35,000-dalton components of PP-2A and PP-2A appear to be the same polypeptide and represent the catalytic subunit (17, 24). PP-2B, a Ca2+/calmodulin-dependent enzyme, is active against the a-subunit of phosphorylase kinase but does not significantly dephosphorylate phosphorylase a (17, 25).

A number of studies on reticulocyte phosphatase activities have been reported. Mambiy and Traugh (26-28) have partially purified several protein phosphatase activities from reticulocyte lysates. These were characterized in part, based on their chromatographic properties, substrate specificities including eIF-2, and cation sensitivity, but the relationship of these activities to the type 1 and type 2 phosphatases is not clear. Grankowski et al. (29) have partially purified a fraction from lysates which displays phosphatase activity toward two of the subunits of phosphorylated eIF-2, as well as a 100,000-dalton phosphorylated polypeptide associated with eIF-2a kinase activity. Crouch and Safer (30) have isolated a highly purified protein phosphatase from lysates which acts as an eIF-2a phosphatase and displays the properties of a type 2A phosphatase as demonstrated by Cohen et al. (17) and Stewart et al. (31). However, these same investigators have also shown that rabbit skeletal muscle PP-1 and reticulocyte PP-2A dephosphorylate eIF-2a at a similar relative rate in vitro (31).

Based on these observations, the specificity of protein phosphatases toward eIF-2a both in vitro and in situ remains to be clarified.

The present study was undertaken to determine the distribution and relative levels of protein phosphatases type 1 and type 2 in the lysate as a first step towards assessing the roles of these enzymes in the regulation of protein synthesis. The results demonstrate that type 1, type 2A, and type 2B protein phosphatases are present in the reticulocyte lysate, and that under the appropriate assay conditions (in the presence of 1 mM MnCl2), PP-2A represents the major phosphorylase phosphatase activity in the lysate. Interestingly, when phosphorylase activities are determined in the presence of 1 mM MgCl2/0.5 mM ATP, which approximates normal lysate concentrations, the relative activity levels of PP-1 and PP-2A are similar. In this regard a related study on the sensitivity of endogenous eIF-2a phosphatase to inhibitor-2 in protein synthesizing systems has shown that (i) PP-1 activity is reduced, (ii) eIF-2a is phosphorylated, and (iii) protein synthesis is inhibited (32).

EXPERIMENTAL PROCEDURES

Protein and Enzyme Preparations—Phosphatase activator protein F6 (33), phosphorylase b (34), phosphorylase kinase (35), inhibitor-1 (36), inhibitor-2 (36), and the catalytic subunit of the cyclic AMP-dependent protein kinase (19, 30) were purified from rabbit skeletal muscle. 

\[ ^{32} \text{P-labeled phosphorylase a (5 x 10}^8 \text{cpm/mmol)} \]

was prepared from phosphorylase b using purified phosphorylase kinase and [\(^{32} \text{P}\)]ATP (23, 37). Protein phosphatase 1 was purified 2000-fold from rabbit skeletal muscle (23). 

\[ ^{32} \text{P-labeled phosphorylase kinase (3 x 10}^8 \text{cpm/mmol)} \]

was prepared by using purified cyclic AMP-dependent protein kinase and [\(^{32} \text{P}\)]ATP (23, 57); the labeled product contained approximately 1 phosphate per each a- and b-subunit. Protein concentrations were determined by the method of Bradford (38).

Protein Phosphatase Activities in Reticulocyte Lysates—Reticulocytes and reticulocyte lysates were prepared as described previously (39, 40). Reticulocyte lysates were diluted 5-fold in 50 mM Tris-HCl (pH 7 at 25°C), 1 mM EDTA, 30 mM 2-mercaptoethanol, and then gel-filtered through Sephadex G-50 in the same buffer to remove low molecular weight inhibitors (19, 20, 41). Protein phosphatase assays were routinely carried out at a final lysate dilution of 1:3000. Dilutions of this magnitude were necessary to obtain the maximum specific activity for both PP-1 and PP-2A in the lysate.

Phosphorylase Phosphatase—Activity was assayed in the presence and absence of inhibitor-2 (100 units assay) utilizing [\(^{32} \text{P}\)]phosphorylase a (10 μM, 5 x 10^6 cpm/mmol), bovine serum albumin (0.33 mg/ml), and lysate at a final dilution of 1:3000 or DE-52 fractions at a final dilution of 1:150. Where indicated in the legends, assays were carried out under three different conditions: (i) in the presence of 1 mM MnCl2; (ii) in the presence of 1 mM EDTA; or (iii) in the presence of 1 mM MgCl2/0.5 mM ATP (MgATP).

Inubations were at 30°C and under these conditions reactions were linear for 30 min or longer. Phosphatase activity was measured by the increase in acid-soluble radioactivity (20, 37). One unit of phosphorylase phosphatase activity is defined as that amount of enzyme which releases 1 nmol of phosphate from phosphorylase a/min in the standard assay (20, 37). One unit of inhibitor-2 activity is defined as the amount of protein which produces 50% inhibition of phosphorylase phosphatase in the standard assay (20).

Phosphorylase Kinase Phosphatase—Activity was assayed under conditions identical to those described above for phosphorylase phosphatase, except that all assays were carried out in the presence of 1 mM MnCl2 and with [\(^{32} \text{P}\)]phosphorylase kinase (3 μM, 3 x 10^6 cpm/mmol) labeled in the a or b subunits, as substrate. Protein phosphatase activity values reported in these experiments were also determined by the molybdate-extraction method as described (43). This procedure distinguishes between acid-soluble label ([\(^{32} \text{P}\)]P-) released by phosphatase or proteinase activity. In all cases, the data obtained by this method confirmed the value for protein phosphatase obtained by the standard assay (20).

Separation of Lysate PP-1 and PP-2 by Chromatography on DEAE-cellulose (DE-52)—Lysates were diluted 1:3 in 5 mM Tris-HCl (pH 7), 0.1 mM EDTA, 30 mM 2-mercaptoethanol (buffer A), and then applied to a DE-52 column. The column was washed with buffer A supplemented with 50 mM NaCl until the effluent protein concentration was less than 20 μg/ml. The phosphatase activities were then eluted with a linear salt gradient of 50-400 mM NaCl in buffer A.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out as described previously (47).

Procedures to Detect Inhibitor-1 and Inhibitor-2 in Reticulocyte Lysates—Reticulocyte lysates (1.0 ml) were diluted 5-fold in 10 mM Tris-HCl (pH 7.4 at 25°C), 1.0 mM EDTA and then heated at 90-95°C for 5 min. Precipitated protein was removed by centrifugation (12,000 x g, 15 min), and the supernatant collected. The pellet was washed in 5 ml of 10 mM Tris-HCl (pH 7.0, 25°C) and collected by centrifugation. The two supernatants were combined and then lyophilized. The lyophilized protein was reconstituted in H2O, at a protein concentration of 0.3-0.5 mg/ml. The protein solution was made 10% with trichloroacetic acid and the protein allowed to precipitate at room temperature for 5 h. The precipitate was collected by centrifugation (15,000 x g, 10 min) and resuspended in 50 mM Tris-HCl (pH 7.0, 25°C); the pH was adjusted to 8.0 and the solution was dialyzed extensively against 50 mM Tris-HCl, 0.1 mM EDTA. Assays for inhibitors-1 and -2 were carried out as previously described (19, 20), and were based on the zivity of the inhibitor preparation to inhibit skeletal muscle PP-1 both (i) prior to and after phosphorylation by the cyclic AMP-dependent protein kinase, and (ii) after treatment with PP-1 (in the presence of Mn2+) to dephosphorylate any phosphorylated inhibitor-1 present in the isolated fraction. The ability of the inhibitor preparation to inhibit PP-2A, was also examined.

RESULTS AND DISCUSSION

PP-1 and PP-2 Activity Levels in Unfractionated Reticulocyte Lysates—Phosphorylase phosphatase activity of the lysate was measured under a variety of assay conditions as shown in Table I. In the presence of 1 mM MnCl2 which elicits maximal activity for PP-2A, and utilizing 32P-phosphorylase a as substrate, a total phosphorylase phosphatase activity of
**Reticulocyte Protein Phosphatases**

**Phosphorylase phosphatase activity in reticulocyte lysates**

Lysate phosphatase activity was assayed with \(^{32}P\)phosphorylase a as substrate. Activity conditions are described under “Experimental Procedures.” As shown, lysate phosphatase activity was assayed in the presence of 1 mM MnCl\(_2\), 1 mM EDTA, or 1 mM MgCl\(_2\)/0.5 mM ATP. In one series of assays, phosphatase activities were determined in ribosomes obtained from lysates by sedimentation as described in the text. Where indicated, assays contained 100 units of inhibitor-2. PP-1 is measured as the difference in phosphorylase phosphatase activity determined in the absence and presence of inhibitor-2. PP-2 activity is defined as inhibitor-2-insensitive activity. Values for total phosphorylase phosphatase and for per cent inhibition by inhibitor-2 are expressed as the mean ± the standard error of the mean.

| Assay conditions | Phosphorylase phosphatase | Inhibition by inhibitor-2 | Protein phosphatase-1 | Protein phosphatase-2 |
|------------------|--------------------------|--------------------------|-----------------------|-----------------------|
| Lysate           |                          |                          |                       |                       |
| + 1 mM MnCl\(_2\) (15) | 14.2 ± 1.10              | 15.8 ± 0.85              | 2.20                  | 12.00                 |
| + 1 mM EDTA (12)  | 9.2 ± 0.08               | 25 ± 1.20               | 2.30                  | 6.90                  |
| + 1 mM MgCl\(_2\) | 4.6 ± 0.00               | 40 ± 2.40               | 1.85                  | 2.80                  |
| + 0.5 mM ATP      |                          |                          |                       |                       |
| Ribosomes         | 0.84 ± 0.07              | 58 ± 2.80               | 0.49                  | 0.35                  |

* Number of preparations assayed is given in parentheses.

**Units/ml** = concentration in undiluted lysate.

14.2 units/ml of lysate was observed. In the presence of 1 mM MnCl\(_2\) and 100 units of inhibitor-2, to specifically inhibit PP-1 (16, 17, 41, 42, 44), there was a 16% inhibition of the total phosphorylase phosphatase activity (Table I). Therefore, under these assay conditions, 84% of the total phosphorylase phosphatase activity was presumed to be PP-2A (12 units/ml). When the assays were carried out in the presence of 1 mM EDTA which has been shown to depress PP-2A but not PP-1 activity in other tissue extracts (16, 17), lysate PP-2A was decreased 50% whereas PP-1 activity displayed a slight increase of 5-10%, as observed previously for muscle PP-1 (23).

Phosphorylase phosphatase assays were also carried out in the presence of 1 mM MgCl\(_2\)/0.5 mM ATP (MgATP), concentrations which are comparable to those found in the lysate. Under these conditions, the activity of PP-2A, but not that of PP-1, was markedly decreased (16, 17), which resulted in comparable levels of activity for the two enzymes. PP-2A activity was 23% (2.8 units/ml of lysate) and PP-1 activity was 84% (1.85 units/ml of lysate) even when assayed in the presence of 1 mM MnCl\(_2\) (Table I). Hence, relative to the ratios of PP-1 and PP-2A in the lysate, there is an evident enrichment of PP-1 in the ribosome-associated preparation.

**Level of Inhibitor-1 and Inhibitor-2 in Reticulocyte Lysates**—The heat-treated reticulocyte lysate fraction under

![Fig. 1. Chromatography of reticulocyte protein phosphatases. A. separation of lysate phosphorylase phosphatase activities on DEAE-cellulose. The phosphatase activity profiles were derived from the chromatography of 9 ml of lysate on a DEAE-cellulose column (1.5 × 10 cm) using a linear gradient (360 ml) of 50-400 mM NaCl in 5 mM Tris-HCl (pH 7.0) with 0.1 mM EDTA, 30 mM β-mercaptoethanol. Fractions (5 ml) were collected and assayed at a final dilution of 1:150 in the presence of 1 mM MnCl\(_2\) except where MgATP was added. The substrate in all assays was \(^{32}P\)phosphorylase a as described under “Experimental Procedures.” ● — ●, PP-1 activity which is inhibited by 100 units of inhibitor-2 (the difference in phosphorylase phosphatase activities observed in the absence and presence of inhibitor-2). ▲ — ▲, F\(_2\)/MgATP-dependent PP-2A activity (F\(_2\)) which is inhibited by 100 units of inhibitor-2. ◆ — ◆, PP-2 activities which are insensitive to 100 units of inhibitor-2. Data are plotted as a percentage of the \(^{32}P\) counts/min released in the phosphatase assay of the peak fraction of PP-2A. PP-1, protein phosphatase type 1; PP2A, protein phosphatase type 2A; PP2B, protein phosphatase type 2B. Other protein phosphatase designations are as in A.**
“Experimental Procedures” contained PP-1 inhibitor activity comparable to that observed in similar preparations from rabbit skeletal muscle (data not shown). This activity was not changed significantly on pretreatment with either the cyclic AMP-dependent protein kinase (to phosphorylate inhibitor-1) or by an excess of skeletal muscle protein phosphatase-1 in the presence of Mn$^{2+}$ (to dephosphorylate any phosphorylated inhibitor-1). These preliminary data suggest that there is little or no inhibitor-1 in the lysate, and the major protein phosphatase-1 inhibitor activity could be attributed to inhibitor-2 or a similar inhibitor. However, saturating levels of a partially purified heat-stable inhibitor-2 fraction from lysates, which inhibited PP-1 over 95%, also inhibited PP-2A about 30%. Whether the inhibition of PP-2A is due to the presence of specific inhibitor of this phosphatase remains to be determined.

Separation of Lysate PP-1 and PP-2 on DEAE-cellulose—In order to separate the type 1 and type 2 protein phosphatase activities, lysates were chromatographed on DEAE-cellulose (DE52) as described under “Experimental Procedures.” A typical elution profile is shown in Fig. 1. Initially, all fractions were monitored for phosphorylase phosphatase activity in the presence of 1 mM MnCl$_2$. Under these conditions, three peaks of activity were observed eluting at 150 mM, 210 mM, and 300 mM NaCl, respectively. This resembles similar protein phosphatase profiles reported for rabbit liver and rabbit skeletal muscle, except for differences in the ratios of the type 1 and type 2 activities in these three tissues (16, 17, 42, 44). In order of elution, these activities have been designated PP-1, PP-2A, and PP-2A$_2$ (Fig. 1A) (16, 17). The total recovery of phosphorylase phosphatase activity from the column represented over 80% of the lysate phosphatase activity originally loaded. Moreover, the relative levels of PP-1 and PP-2 activities recovered were in good agreement with the values in the unfractionated lysates based on the data obtained using inhibitor-2 (see Table I). Inhibitor-2 was found to inhibit the first peak selectively (PP-1), which indicated that the second and third peaks represented type 2A phosphatases. These data tend to confirm the specificity of inhibitor-2 and provide further support in favor of its use in determining the levels of type 1 and type 2A protein phosphatase activities, since assays were carried out in the absence of MnCl$_2$, but in the presence of MgATP plus a protein activator fraction previously designated F$_a$ (17, 21-23, 33). There was over a 3-fold increase in the phosphorylase phosphatase activity in the region of PP-1 (Fig. 1). This MgATP-induced activity, which is therefore the sum of F$_a$-dependent and F$_a$-independent protein phosphatase activities, is completely sensitive to inhibition by inhibitor-2. The F$_a$-dependent PP-1 activity has been previously designated F$_c$ phosphatase (21-23) (Fig. 1); these data confirm lysate F$_c$ as a type 1 phosphatase. The F$_a$ fraction used in these assays was isolated from skeletal muscle (33). However, F$_c$ was also found in lysates. Using the MgATP-dependent F$_c$ phosphatase isolated from the lysate profile in Fig. 1, the column fractions were assayed for F$_c$ activity. As shown for skeletal muscle (21, 22, 33), all of the F$_c$ activity eluted in the 50 mM NaCl-buffer A wash (F$_c$ not shown). Under these conditions of assay, we estimate that there is sufficient endogenous lysate F$_c$ to permit maximal activation of endogenous lysate F$_c$ within 10 min. In addition, assuming efficient recovery of all lysate activities in the chromatographic profiles (see Fig. 1), we estimate that the PP-1 plus F$_c$ activities in the lysate represent 40% of the total phosphorylase phosphatase activity. It should also be noted that in the presence of MgATP, the PP-2A activities which elute from the DEAE-cellulose column are over 90% inhibited, and similar results are observed in other tissues (16, 17).

One further point of interest in the profile in Fig. 1 is that different lysates displayed variable activity ratios for PP-2A$_1$ and PP-2A$_2$. In one lysate, for example, the two peaks were of nearly equal magnitude (data not shown). The reason for this is not entirely clear, but it has been observed that in the rechromatography of PP-2A$_1$ on DEAE-cellulose, a high proportion (50-80%) of the activity elutes in the position of PP-2A$_2$, of PP-2A$_1$. At present, PP-2A$_1$ is thought to be composed of three distinct subunits of 35,000, 59,000, and 69,000 daltons, whereas PP-2A$_2$ contains only two of these subunits, 35,000 and 69,000 daltons (17, 44). This suggests the possibility that PP-2A$_1$ is generated from PP-2A$_2$ by the chromatographic procedure. This result is of interest due to the finding that the endoplasmic reticulum phosphatase isolated by Crouch and Safer (30) corresponds to PP-2A$_2$ (17, 31, 44). In addition, the possibility cannot be excluded that the observed variability in PP-2A$_1$ and PP-2A$_2$ activity levels is due to a processing step prior to chromatography on DEAE-cellulose.

The lysate fractions from the DEAE-cellulose chromatography were also assayed for phosphorylase kinase phosphatase using [$^{32}$P]phosphorylase kinase labeled in the α- and β-subunits as substrates (Fig. 1B). In the phosphatase profile, in addition to PP-1, PP-2A, and PP-2A$_2$, a new but small peak of phosphatase activity was detected which eluted just ahead of PP-1 (Fig. 1B). This activity corresponds to similar peaks described previously for rabbit skeletal muscle and liver which have been designated PP-2B (17). Lysate PP-2B is insensitive to inhibitor-2 and contains no phosphorylase phosphatase activity as measured by its inability to dephosphorylate [$^{32}$P]phosphorylase a. PP-2B from rabbit skeletal muscle is a Ca$^{2+}$-dependent enzyme that is stimulated 10-fold by calmodulin (25). The antipsychotic drug trifluoperazine has been used extensively to probe for calmodulin-dependent reactions (45). Recent studies show that trifluoperazine inhibits PP-2B specifically, and therefore can be used to establish the presence of this enzyme (46). When the various lysate phosphatases separated on DEAE-cellulose were assayed using [$^{32}$P]phosphorylase kinase as substrate, only the phosphatase activity eluting in the region corresponding to PP-2B was inhibited by 100 μM trifluoperazine (data not shown). It should be noted that no Ca$^{2+}$ or calmodulin was added in the PP-2B activity assays with phosphorylase kinase as substrate, since calmodulin is present in the substrate (subunit δ) (47); and Mn$^{2+}$ effectively substitutes for the Ca$^{2+}$ requirement (25). When unfractionated lysates were assayed for phosphorylase kinase phosphatase activity in the presence of inhibitor-2 to block PP-1 activity, the addition of 100 μM trifluoperazine inhibited 10-15% of the total PP-2 activity in the lysate, which is in good agreement with the levels of PP-2B activity measured in the DEAE-cellulose elution profile (Fig. 1B) for all five protein phosphatase species, over 95% of the acid-soluble [$^{32}$P]phosphate as determined by the molybdate-extraction procedure (43).

Specificity of Reticulocyte PP-1, PP-2A, and PP-2B Toward the α- and β-Subunits of [$^{32}$P]Phosphorylase Kinase—Phosphorylase kinase is a 16-subunit enzyme (α,β,γ,δ) (47). Both the α- and β-subunits are phosphorylated by the cAMP-dependent protein kinase (35, 37). An additional criterion in distinguishing type 1 and type 2 protein phosphatase activities is that PP-1 preferentially dephosphorylates the β-subunit, whereas PP-2 preferentially dephosphorylates the α-subunit of phosphorylase kinase (16, 17, 44). The peak DEAE-cellulose fractions of PP-2B, PP-1, and PP-2A$_2$ were suitably diluted.

1 J. G. Foulkes, unpublished observations.
and assayed for phosphorylase kinase phosphate activity with $[^{32}P]$phosphorylase kinase as substrate (Fig. 2). As predicted, based on the properties of these enzymes established in other tissues (16, 17, 31, 44), reticulocyte PP-2B dephosphorylated the $\beta$-subunit at least 20-fold more rapidly than the $\alpha$-subunit; and PP-2A dephosphorylated the $\alpha$-subunit 4- to 6-fold more rapidly than the $\beta$-subunit (Fig. 2).

The data presented here show that at least five species of protein phosphatase can be identified in reticulocyte lysates. These constitute two forms of PP-1, one of which (F criticize dependent) on MgATP and an activator protein, Fm. In addition, three species of PP-2 have been identified (PP-2B, PP-2A, and PP-2A). In a preliminary estimation of inhibitor-2 in -2 in lysates, we find little or no detectable inhibitor-1. However, inhibitor-2 is present in heat-treated lysates at a concentration which corresponds to at least 50% of the specific activity of inhibitor-2 in a similar preparation from rabbit skeletal muscle tissue (20). One of the purposes in originating this study was to examine the role of phosphatases in the regulation of protein synthesis. This will ultimately require the purification of the lysate protein phosphatases in order to investigate their substrate specificities toward the various phosphorylated initiation factors and other phosphorylated components involved in protein chain initiation.

Acknowledgments—We would like to thank Dr. P. Cohen (Dundee, Scotland), Dr. L. S. Jefferson (Hershey, PA), and Dr. M. London (Cambridge, MA) for the use of their laboratories during the time much of this work was carried out. We would also like to thank Drs. P. Cohen and I. M. London for reading the manuscript and for their comments. We thank Dr. B. Hemmings, B. Caudwell, and Dr. A. Stewart (all of the University of Dundee) for the skeletal muscle preparations of Fm (B. H.), phosphorylase $b$ (B. C.), phosphorylase kinase (B. C.), and protein phosphatase-1 (A. S.). We also thank Dr. M. Clemens (St. George’s, London) for reticulocyte lysate samples.