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Regeneration of Enzymatic Activity after Sodium Dodecyl Sulfate/Polyacrylamide Gel Electrophoresis and Zinc Acetate Staining: The Example of Inositol 1,4,5-Trisphosphate 5-Phosphatase

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Regeneration of enzyme activity after sodium dodecyl sulfate–gel electrophoresis was investigated with a purified inositol 1,4,5-trisphosphate 5-phosphatase. In order to avoid silver or Coomassie blue staining, we have used zinc acetate. This staining procedure was sensitive, rapid, and reversible provided that zinc cations are chelated and activity is extracted after diffusion out of the gel. The method allows same gel lane staining and identification of the enzyme based on catalytic activity. © 1990 Academic Press, Inc.

Regeneration of enzymatic activity after SDS[1]/gel electrophoresis has now been described for several enzymes: phosphatidylkinase from bovine brain myelin (1), phosphatidylinositol-4-phosphatase kinase from human red blood cells (2), or adenylate cyclase from *Bordetella pertussis* (3). Ins(1,4,5)P_3 5-phosphatase activity has been reported in many tissues, isoforms of which are found in both the soluble and the particulate cell compartments (4). The enzymes catalyze the dephosphorylation of Ins(1,4,5)P_3 to Ins(1,4)P_2. Two soluble enzymes type I and type II have been purified from rat or bovine brain (5,6). Type I soluble enzyme could be identified as a 43-kDa protein by renaturation of enzyme activity following SDS/polyacrylamide gel electrophoresis (7). No activity was recovered in other parts of the SDS–gel. To do this, we had to stain one lane of the gel and renature enzymatic activity in another lane from the same gel. Attempts to renature Ins(1,4,5)P_3 5-phosphatase activity directly after silver or Coomassie blue staining were unsuccessful. In this report, we show that $InsP_3$ 5-phosphatase could be renatured after staining the gel in zinc acetate (8). This allows the direct localization of that particular enzyme after SDS–gel electrophoresis. The staining procedure is sensitive, rapid, and reversible, provided zinc cations are chelated.

[1] Abbreviations used: SDS, sodium dodecyl sulfate; $InsP_3$, inositol 1,4,5-trisphosphate.

MATERIALS AND METHODS

Materials

Materials for the purification and assay of $InsP_3$ 5-phosphatase were the same as those reported in Refs. (6,7). Type I $InsP_3$ 5-phosphatase was purified to a specific activity of 20–40 μmol/min/mg protein at 37°C and 30 μM $InsP_3$. Zinc acetate was from Merck. Rainbow protein molecular weight markers were from Amersham.

SDS–Gel Electrophoresis, Zinc Acetate Staining, and Extraction of the $InsP_3$ 5-Phosphatase Activity

Fractions of the purified enzyme were analyzed by SDS–10% polyacrylamide gels as described previously (7) with slight modifications. We used 12-cm separation gels and ran the electrophoresis at 250 V. Rainbow protein molecular weight markers were run in a parallel lane. For SDS–polyacrylamide gel electrophoresis, enzyme preparation was adjusted to 62 mM Tris–HCl, pH 6.8; 3% SDS; 5% 2-mercaptoethanol, 10% glycerol. The mixture was run without boiling on SDS–10% polyacrylamide gel (10). After electrophoresis at 4°C, the gel was silver stained (9). Alternatively, the gel was stained with zinc acetate as follows: the gel was washed with 200 ml of water for 3 min. After removal of the water, the gel was soaked in 200 ml of 0.3 M zinc acetate for 45 s until protein bands appeared: proteins were visualized by contrast on a black sheet. The bands appeared transparent

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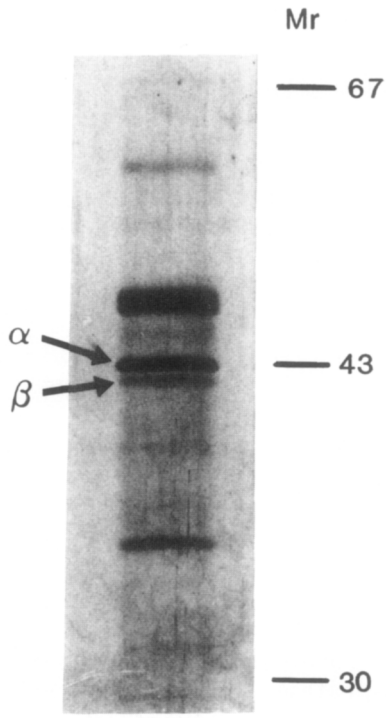

FIG. 1. Silver-stained gel of purified $InsP_3$ 5-phosphatase. A sample containing approx 1.5 nmol/min, activity determined at 1 μM $InsP_3$ (i.e., about 0.5 μg), of purified enzyme was subjected to SDS/10% polyacrylamide gel electrophoresis. Positions of M_r standards are shown on the right.

as a result of "gel background staining" (8). The gel was cut into 2-mm slices and each was washed six times with 3 ml of 250 mM Tris–EDTA, pH 9.0, at 0°C to chelate the Zn cations. The bands were then homogenized in 0.4 ml 50 mM Hepes, pH 7.5; 25% sucrose; 0.1% Triton X-100, 47 mM 2-mercaptoethanol. After overnight incubation at 4°C, polyacrylamide gel fragments were removed by centrifugation and activity was determined in the presence of 1% Triton X-100 after 30–60 min of incubation at 37°C. Recovery of $InsP_3$ 5-phosphatase activity after electrophoresis was between 10 and 40%.

RESULTS AND DISCUSSION

Although we had previously reported that Ins(1,4,5)P_3 5-phosphatase could be renatured after SDS/gel electrophoresis and activity detected in the 43-kDa region (7), reversible staining of the gel followed by extraction of the protein was not possible by classical Coomassie blue or silver staining. No activity was recovered using these methods (not shown). Alternatively, polypeptides separated by SDS/gel electrophoresis could be stained by heavy metal salts without a fixation step (8,11). In our experiments, we have used zinc acetate which allows the detection of proteins up to 0.15 μg of protein loaded onto the gel. Using longer separation gels (i.e., 12 cm) as reported previously (7), we have now identified two bands "α" and "β" in the 43-kDa region (Fig. 1). The position of α and β bands could be easily followed by comigration with ovalbumin present in the rainbow protein molecular weight markers run in an adjacent lane. These two polypeptides which were very close to each other could be visualized by silver (Fig. 1), Coomassie blue, or zinc acetate staining (Fig. 2), the last procedure being the most rapid. In contrast to silver, zinc acetate staining is reversible provided Zn cations are chelated. Moreover, $InsP_3$ 5-phosphatase could be extracted after enzyme diffusion out of the gel: enzymatic activity was specifically associated with the lower molecular band β, whereas α had little activity (Table 1). In general, α had 8% of the total activity extracted and presumably resulted from a contaminating polypeptide.

In conclusion, this procedure allows the identification of the protein/enzyme after SDS–gel electrophoresis as localized by enzymatic assay. It is particularly rapid and does not require electrotransfer to nitrocellulose as previously used for phosphatidylinositol-4-phosphate ki-

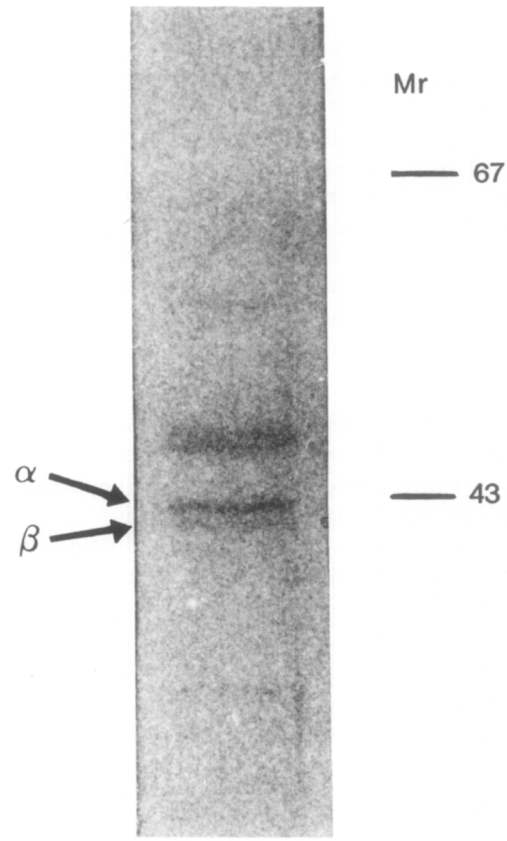

FIG. 2. Zinc-stained gel of purified $InsP_3$ 5-phosphatase. A sample containing approx 0.78 nmol/min as determined at 1 μM $InsP_3$ was applied to the gel. The gel was stained by zinc acetate as described under Materials Methods. The position of "α" and "β" bands was localized by comigration with ovalbumin. $InsP_3$ 5-phosphatase was assayed in α and β bands after chelating Zn^{2+} ions and renaturation of enzyme activity. Activities recovered in α and β bands were 10.0 and 83.0 pmol/min, respectively (i.e., about 1 and 11% from the initial activity applied to the gel).

TABLE 1

Comparison of $InsP_3$ 5-Phosphatase Activity of "α" and "β" Extracted Slice after SDS/Polyacrylamide Gel Electrophoresis

Experiment	$InsP_3$ 5-phosphatase (pmol/min)	
	α	β
1	0.2	5.3
2	5.3	24.3
3	7.0	123
4	7.2	300
5	10.0	83.0

Note. The α and β bands were visualized by zinc acetate staining (Fig. 2), excised, and homogenized as described under Materials and Methods. $InsP_3$ 5-phosphatase was determined at 1 μM $InsP_3$ after 60 min of incubation at 37°C. The data are from five different experiments made with samples of independent enzymatic preparations (i.e., of different total activity) of purified $InsP_3$ 5-phosphatase.

nase (2). Although our procedure may be specific for a particular group of enzymes which include the $InsP_3$ 5-phosphatase, we have used the same method for regeneration of $InsP_3$ 3-kinase after SDS/gel electrophoresis.

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