Staining Acidic Phosphoproteins (Phosvitin) in Electrophoretic Gels

The principal phosphoglycoproteins of avian and amphibian egg yolk, known as phosvitins, have been examined extensively in developmental studies of estrogentic induction of protein synthesis (1) and, more recently, as substrates for protein kinases which phosphorylate the basic nuclear proteins (2,3). Phosvitin has few aromatic and basic amino acids (4) which can be detected by uv absorbance or by conventional anionic protein stains following electrophoresis. In addition, the high negative-charge density of clustered phosphorylserine residues (2) may prevent staining by charge repulsion of anionic dyes. Dilute solutions of cationic dyes like toluidine blue and acridine orange have been employed (5,6), but, in our experience, they penetrate gels slowly and also stain the residual negative charges of most electrophoretic matrices, including agarose, starch, and even polyacrylamide. The only specific method for localizing phosphoproteins in polyacrylamide gels requires several time-consuming steps to liberate orthophosphate by basic hydrolysis, to form an insoluble phosphomolybdate complex, and, finally, to stain this complex with methyl green dye (7). The exceptional affinity of contiguous phosphorylserines for trivalent metal ions (8) can, however, be exploited to detect phosvitin in gels. This strategem may also provide an alternative method for visualizing similar acidic phosphoproteins which have now been identified in brain, spermatozoa, and adrenal medulla (9,10).

Quantitative Detection of apo-Phosvitin by Coomassie blue Stain with Aluminum Mordant

Coomassie brilliant blue R250 in acid solution stains Al(III)-phosvitin but does not bind to the metal-free apo-protein (Fig. 1, A and B). Aluminum
salts added directly to the dye solution will promote formation of a metal-protein chelate in which Al(III) damps the strong negative charge and acts as a bridge between dye and phosphorylserine residue. As little as 40 ng of apo-phosvitin (containing about 0.13 nmol of phosphate) is easily detectable in our gel system (Fig. 2). Staining phosvitin with aluminum/Coomassie blue thus appears to be considerably more sensitive than the orthophosphate-entrapment method (7) which could detect only 1 nmol of phosphate. If the gels are not overloaded with protein, phosvitin stains as intensely as bovine serum albumin (Fig. 2).

**Qualitative Detection of Iron(III)-Phosvitin by Prussian blue Reaction**

The ferric-nitrilotriacetate chelate donates iron(III) specifically and stoichiometrically to apo-phosvitin (8). When fully saturated, iron(III)-phosvitin has a molar ratio of 1 Fe(III):2 phosphorylserine residues and has an electrophoretic mobility at alkaline pH that is not significantly different from the metal-free protein (Fig. 1, B and C). The iron-protein is easily visualized as “ferric ferricyanide” deposits (11) after soaking electrophoretic gels in any one of a variety of histochemical Prussian blue stains (Figure 1C). In our experience, intensity of staining is directly proportional to the amount of iron presented to the phosvitin...
sample prior to electrophoresis, providing the iron-binding capacity of the phosvitin has not been exceeded.

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