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# In Situ Detection Method for Glutaminyl Cyclase Activity in Polyacrylamide Gels

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that achieved by TLC with numerous buffers (1,2,5) or by HV-TLE protocols (2) when multiple samples are analyzed. We have found that when 1 µL of a dye solution (1% orange G, 1% phenol red in pH 3.5 buffer) was electrophoresed in parallel with the phosphoamino acid standards, orange G migrated slightly ahead of pSer and phenol red migrated with pTyr, providing useful visible markers for electrophoretic progression in our system.

When proteins are labeled by in vitro kinase reactions, the only radioactive compounds in the hydrolysates will be phosphopeptides, phosphoamino acids and phosphate. Under these conditions, a one-dimensional separation by TLE at pH 3.5 is considered adequate (2). When proteins are labeled in vivo and only partially purified, a high resolution two-dimensional HV-TLE/TLC separation system is required. These hydrolysates frequently contain labeled compounds that migrate with the phosphoamino acids (2). The procedure we describe is therefore limited to PAA of purified proteins and substrates labeled in vitro.

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## In Situ Detection Method for Glutaminyl Cyclase Activity in Polyacrylamide Gels

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Glutaminyl cyclase (QC; EC 2.3.2.5) is an enzyme associated with the posttranslational maturation of bioactive peptides in the neuroendocrine system. It acts on glutaminyl residues at the peptide amino terminus, cyclizing them to pyroglutamyl residues and producing ammonia in the process. The enzyme is widespread in mammalian tissues (2,5), and it has been suggested that multiple forms might be present. A method of detecting glutaminyl cyclase activity in polyacrylamide gels might, therefore, be useful in detecting tissue-specific variants.

Because QC produces ammonia in the course of catalysis, initial attempts were made to adapt detection methods published for other ammonia-producing enzymes, such as urease. However, indicator dyes, such as phenol red or the silver-staining method of de Llano et al. (3), proved unable to detect QC activity, probably because they depend on the rise in pH resulting from the production of ammonia, and the peptide substrates of QC might have buffered the system too much. However, an adaptation of our standard liquid-phase assay for QC activity (1), which links the production of ammonia to the conversion of nicotinamide-adenine dinucleotide [reduced] (NADH) to oxidized nicotinamide-adenine dinucleotide ($NAD^+$) with glutamate dehydrogenase to the filter paper overlay method of Nelson et al. (4), proved to be effective in detecting QC activity.

Bovine tissue samples were homogenized at a ratio of 0.3 g of tissue to 1 mL of 0.1 M Tris-HCl, pH 7.6. The extracts were subjected to two freeze/thaw cycles and clarified by centrifugation at 12 000× *g* for 15 min. They were then diluted 1:1 with a sample buffer (62.5 mM Tris-HCl, 20% glycerol, pH 6.8) that contained bromophenol blue as the running dye. Thirty microliters (ca. 90 µg) of each tissue sample were electrophoresed in the absence of detergent on a 10% NuPAGE™ gel (8 × 8 cm, 1-mm thick; Novex, San Diego, CA, USA) using the morpholino propanesulfonic acid (MOPS) buffer system (pH 7.7) at 20 V for 44 h at 4°C. Recombinant human QC (1.8 U) expressed as a fusion protein with glutathione *S*-transferase (GST) QC was treated in the same manner as a positive control (6). One unit of enzyme activity is defined as the amount of enzyme required to convert one nanomole of Gln-$NH_2$ (8 mM initial concentration) to pGlu-$NH_2$ in one minute (1).

Once electrophoresis was complete,

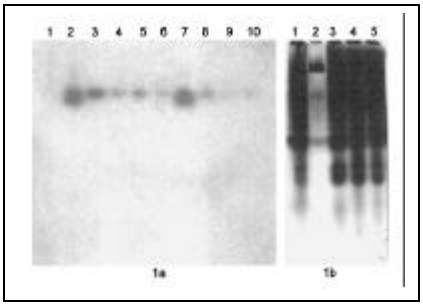


**Figure 1. Filter paper overlay of glutaminyl cyclase native gel viewed under UV light.** (a) Lane 1: mature bovine pituitary tissue extract; lane 2: recombinant human enzyme (positive control); lane 3: mature bovine hippocampus; lane 4: bovine calf hippocampus; lane 5: mature bovine striatum; lanes 6–10: duplicates of lanes 1–5. (b) Lanes are the same as 1–5 in Panel a except the filter paper has been removed and the gel stained for protein with Coomassie Blue.

the gel was washed (3× for 10 min) in 0.1 M Tris-HCl, pH 7.6. This washing of the gel appeared to be necessary for QC activity, possibly because of inhibitory components in the gel or electrophoresis buffer. Whatman No. 3 filter paper (Clifton, NJ, USA) cut to fit the gel was soaked in the following assay cocktail: 25 mg α-ketoglutarate, 5 mg arginine hydroxamate, 10 mg NADH, 30 mg Gln-$NH_2$ and 25 µL (30 U) glutamate dehydrogenase dissolved in 5 mL of the wash buffer. The saturated filter paper was placed on the gel, incubated at room temperature for 30 min and photographed under 330 nm UV light. A relatively long exposure time of 15 s with an F stop of 4.5 was necessary for optimum photography of the pale fluorescence. The results are shown in Figure 1a.

After photographing the filter paper overlay, lanes 1–5 of the gel were stained with Coomassie® Blue (Figure 1b) to ensure that the activity bands seen in the overlay did not indicate bulk protein. Control experiments showed a lack of reaction when substrate for glutaminyl cyclase was omitted from the assay cocktail. The order of reaction of various QC substrates on the overlay (Gln-Gln > Gln-$NH_2$ > Gln) also mirrored the reaction of recombinant QC with these compounds in solution (6). The expense of Gln-Gln ($430/g; Bachem, Torrence, CA, USA), however, favors the use of Gln-$NH_2$ ($70/g; Bachem) on a routine basis.

The method described here is capable of detecting glutaminyl cyclase activity in native polyacrylamide gels and should be useful in the study of QC variants in mammals as well as possibly in plants and bacteria.

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# Analysis of Cell-Cycle Profiles in Transfected Cells Using a Membrane-Targeted GFP

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At present, the most commonly used method for determining the cell-cycle profiles (G1, S and G2/M phases) in mammalian cells is to co-transfect the gene(s) of interest with a second plasmid expressing a cell-surface protein that can be identified by immunofluorescence-based cell staining and then analyze the fluorescently stained cells using flow cytometry (9,10). The cluster of differentiation (CD) proteins of T and B lymphocytes are often chosen as the cell-surface markers for this purpose because they are only expressed in T and B cells and, even when expressed at high levels, are not toxic to the common recipient cells used in transfection assays (9,10). However, detection of the cell-surface marker in these transfection assays requires a complicated protocol and reagents. The cells must be detached from tissue culture plates without trypsinization because the cell-surface marker can be destroyed by trypsin. Such treatment, however, makes it difficult to generate a single-cell suspension that is essential for flow cytometry analysis. After detachment from the plates, the cells have to be stained with a specific monoclonal antibody against the transfected cell-surface marker. To detect the signal in flow cytometry analysis, the monoclonal antibody used in the assay needs to be either directly conjugated with fluorescein isothiocyanate (FITC) or detected by FITC-conjugated anti-mouse