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Measurement of specific activity of radioactive proteins in starch gels

The problems of measuring concentration and soft β-radioactivity of protein fractions resolved by starch gel electrophoresis have been largely overcome by new procedures incorporating a modification of the method of LOWRY *et al.*[1] and the use of a scintillator cocktail compatible with high concentrations of starch, gel buffer and urea. The techniques have been applied specifically to the analysis of mixtures of S-carboxymethylated proteins from wool roots[2,3] but would be equally useful for the estimation of concentration and specific activity of other proteins fractionated by electrophoresis in starch or other neutral media.

Experimental

Wool root protein, dissolved in 8 *M* urea, 0.001 *M* EDTA, and 0.01 *M* Tris–HCl, pH 9.4, was applied on paper (0.5 mg in 50 μl; Whatman No. 3MM, 3 cm × 0.7 cm) to a starch gel[4] (16.2 cm × 5.3 cm × 0.7 cm) and run at 10 V/cm overnight until the distance between paper and front was 12 cm. The gel was removed from the perspex frame and sliced with a wire cutter into 40 pieces 3 mm long. The pieces were placed in glass scintillator vials containing 2 ml of water, broken up with a nylon rod, and the suspension sonicated at 1.5 A on an MSE ultrasonicator for several seconds until cleared.

1 ml was withdrawn by pipette for protein determination. To the remainder (1.8 ml) was added 15 ml of a scintillator cocktail consisting of two parts of the mixture xylol–dioxan–ethanol (5:5:3), containing 80 g/l naphthalene, 5 g/l 2,5-diphenyl-oxazole (PPO) and 0.25 g/l 1,4-bis[2-(5-phenyl-oxazolyl)]-benzene (POPOP)[5], with one part of Triton X100. The sample and cocktail were thoroughly shaken and counted in a Beckman LS200 spectrometer.

To the 1 ml of sonicated material kept for protein determination was added 1 ml of double strength solution C of LOWRY *et al.*[1] (alkaline copper solution). The mixture was shaken, left for 10 min, and 0.2 ml of solution E of LOWRY *et al.*[1] (FOLIN'S phenol reagent) added. The mixture was immediately shaken and left for 30 min for colour development.

To the reacted mixture was added 3 ml of 0.5% cetyl trimethylammonium bromide (CTAB) in water-saturated *n*-butanol. This was then shaken vigorously, transferred to 15-ml glass tubes and centrifuged on a bench centrifuge for a few minutes to separate the phases. The top organic phase was removed by pipette and its absorption measured at 750 nm and 420 nm in a Beckman DBG spectrophotometer. The results were compared with standard curves of protein concentration plotted against optical density at the two wavelengths, obtained by treating aliquots of a standard solution of protein (wool root low-S protein[2], 5 mg/ml in urea buffer) in exactly the same way. A blank starch gel was used in this case and the protein solutions equivalent to 5–2000 μg were added after sonication.

Results and discussion

Protein determination. The direct determination of protein concentration in starch gels by the method of LOWRY *et al.*[1] was not successful due to the opacity of the

solution and consequent light scattering. The CTAB in butanol quantitatively separated the protein from the starch as the cetyl trimethylammonium salt, soluble in butanol. The colour was stable in this solvent for at least 2.5 h. The absorption maximum was at 750 nm and the minimum at 420 nm; the use of these two wavelengths covered the range of protein weights 50–2000 μg (750 nm, 50–125 μg; 420 nm, 125–2000 μg). The blank values measured against butanol-CTAB alone were relatively high, presumably because of the high concentration of urea affecting the colour reaction[1] and it was essential to determine the standard curves under identical conditions. A least squares regression analysis of optical density against standard protein weights was made on the accumulated data from several "standard runs". Fig. 1 shows the resulting standard curves and the average error estimates at both wavelengths.

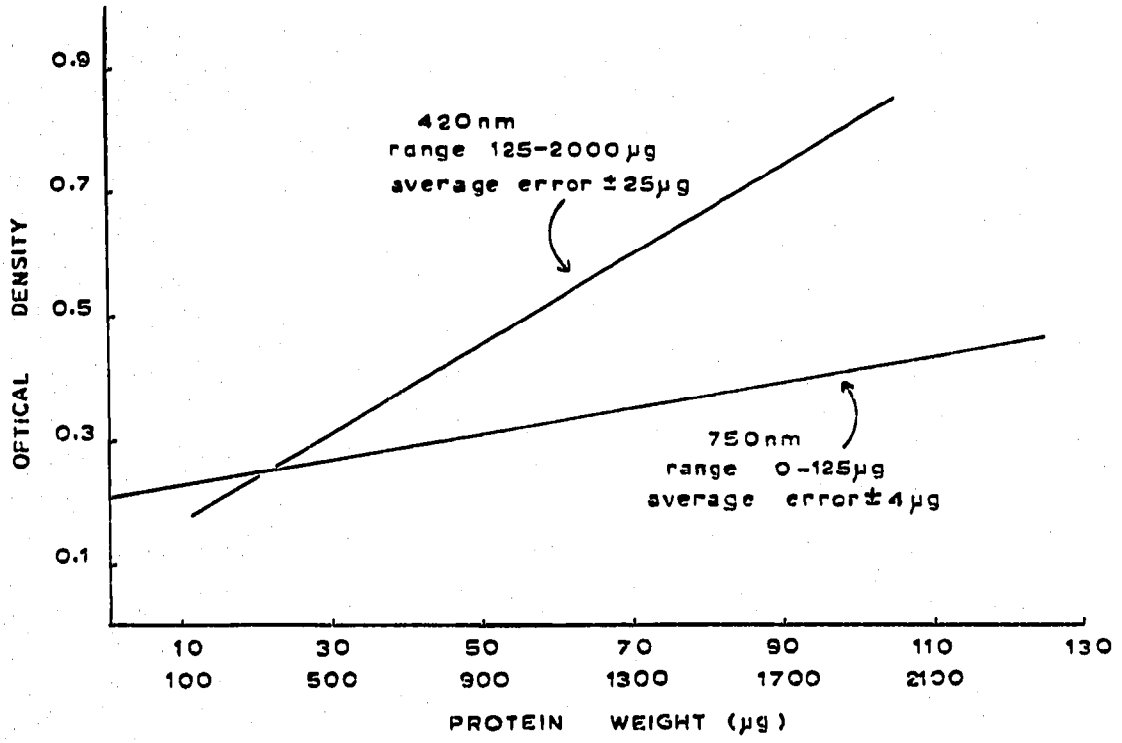

Fig. 1. Standard curves of optical density at 750 nm and 420 nm as a function of protein weight covering the ranges 0–125 μg and 125–2000 μg of protein.

TABLE I

COUNTING EFFICIENCIES

Scintillation fluid	*Additives*	*Counting efficiency* (%)	
		^{3}H	^{14}C
Xylol-based/Triton X100	—	16	79
Xylol-based/Triton X100	1.8 ml starch mixture	10	63
Xylol-based/Triton X100	2 ml BuOH/CTAB	13	68
BRAY's solution	—	21	79
BRAY's solution	2 ml BuOH/CTAB	17	78

Radioactivity counting. The xylol-based scintillating fluid containing Triton X100 was well tolerated by the starch–protein mixtures. The finely dispersed starch tended to settle to the bottom of the counting vial with time but with no change in counting efficiency. An alternative method, that of counting the protein in an aliquot of the butanol phase prior to the colour reaction, was successful and may be preferred in some cases. Under these circumstances, improved counting efficiency was obtained using BRAY's solution[6].

Table I shows counting efficiencies for ^{3}H and ^{14}C using these methods.

Separation of the complex mixture of proteins of the wool root into well defined fractions is reproducibly accomplished by starch gel electrophoresis in the presence of 8 *M* urea[3].

The techniques described above, which allow the ready quantitation of protein fractions and determination of specific activity are being currently applied in time-course studies of the incorporation of labelled amino acids into wool root proteins.

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