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Fluorographic detection of radioactivity in polyacrylamide gels with 2,5-diphenyloxazole in acetic acid and its comparison with existing procedures

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A fluorographic procedure was optimized which utilized acetic acid as the solvent for 2,5-diphenyloxazole (PPO). This procedure was then compared with existing fluorographic procedures which utilize PPO in dimethyl sulphoxide or sodium salicylate in water, and a commercially available fluorographic solution, En^{3}Hance (New England Nuclear Corp.). A comparison of the four methods revealed that all of the procedures resulted in essentially the same efficiency of radioactivity detection. The acetic acid/PPO procedure was found to have several technical advantages. There is no need to pre-fix proteins in gels, and either agarose or acrylamide gels can be used. The acetic acid/PPO procedure was found to be a simple, sensitive and efficient alternative fluorographic method.

The use of polyacrylamide-gel electrophoresis to separate proteins or nucleic acids has become a commonly used biochemical technique. Therefore the detection of radioactivity in polyacrylamide gels has become a very useful procedure. Autoradiography is the procedure used to detect isotopes such as ^{125}I or ^{32}P; however, fluorography has been used to detect efficiently isotopes such as ^{3}H, ^{14}C or ^{35}S. Fluorography is the detection by an X-ray film of a photon that is produced when radioactivity in a polyacrylamide gel interacts with a scintillant that has been infused into the gel. The initial fluorographic method for polyacrylamide gels required PPO as the scintillant and Me_2SO as the solvent (Bonner & Laskey, 1974). Since then, another method has been published that uses sodium salicylate as a water-soluble scintillant (Chamberlain, 1979). Also a commercially made fluorographic solution, En^{3}Hance (New England Nuclear Corp.), has become available (New England Nuclear, 1981). Because of the time involved in the use of the PPO/Me_2SO method and concern over use of Me_2SO, the lack of resolution with certain applications of the salicylate method, and the expense involved with the En^{3}Hance method, a new fluorographic procedure was sought.

The present paper examines an alternative fluorographic method that utilizes PPO as a scintillant and acetic acid as the solvent. This study also presents qualitative and quantitative comparisons of this method with existing fluorographic procedures.

Abbreviations used: PPO, 2,5-diphenyloxazole; SDS, sodium dodecyl sulphate; Me_2SO, dimethyl sulphoxide.

Methods

Radioactive proteins

Newly synthesized radioactive proteins were harvested from the culture medium of Sertoli cells from 20-day-old rats. Sertoli-cell cultures and protein collection were done essentially as described previously (Skinner & Griswold, 1980). Either [^{35}S]methionine or [^{3}H]leucine was used for radioactive-amino acid incorporation.

Gel electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970). The gels were stained for 12h with 0.05% Coomassie Blue R in acetic acid/methanol/water (2:9:9, by vol.) solution and then destained in the same solution without the dye for 6h. Gel lanes were then cut from the slab and fluorographed by using the appropriate procedure.

Detection efficiency

A film scanner was used to compare the efficiency of the four fluorographic procedures to detect radioactivity under different experimental conditions. Radioactively labelled proteins were cast into

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a 10%-polyacrylamide slab gel, and then 2 cm × 2 cm squares were cut from the gel. These squares were then treated according to the appropriate protocol and fluorographed. Gel squares contained 10000 d.p.m. of labelled protein. Films were analysed on a Helena Laboratories Quick Scan automatic film-scanning densitometer and the absorbance was monitored. Integration of the total absorbance by the densitometer provided a quantitative way to compare different treatments of the gel pieces.

Drying

After infusion of the scintillant the gels were placed on filter-paper backing and covered with a plastic wrap or dialysis membrane, then dried on a gel dryer at 70°C for 5 h under 1.33 kPa (10 Torr) vacuum. Cooling of the gel dryer before the vacuum is released helps to prevent cracking of high-percentage-acrylamide gels. Also, the addition of glycerol to the final water incubation aids in the prevention of cracking. Extraneous spots were periodically detected on developed films when plastic wrap was used to cover gels. This was found to be a result of fluorescence produced when the plastic wrap pulled away from the gel during film exposure.

Filming

Dried gels were exposed to preflashed Kodak X-Omat X-ray film at −70°C. The use of pre-flashed film is necessary for a linear relationship of exposure densities (Laskey & Mills, 1975). It is possible when desired to remove half of the background of an exposed and developed film by washing the side opposite the side exposed to the dried gel with household bleach. The bleach is placed on the film for 30 s, then the bleach and emulsion are wiped off and the film rinsed with water.

Chemicals

En^{3}Hance, [^{35}S]methionine and [^{3}H]leucine were obtained from New England Nuclear Corp. (Boston, MA, U.S.A.) Acetic acid was obtained from Baker Chemical Co. Phillipsburg, NJ, U.S.A. Film and developing solutions were obtained from Eastman Kodak Co. Rochester, NY, U.S.A. All other chemicals were obtained from Sigma, St. Louis, MO, U.S.A.

Fluorographic procedures

The PPO/Me_2SO procedure was introduced by Bonner & Laskey (1974) and consists of the following steps.

(1) Pre-fix the gel (i.e., soak in acetic acid/methanol/water (2:9:9, by vol.) for 1 or 2 h); (2) soak the gel for 30 min in Me_2SO; (3) soak the gel for 3 h in 20% (v/v) PPO in Me_2SO; (4) soak the gel for 1 h in water; (5) dry the gel and expose it to film.

The sodium salicylate procedure has been demonstrated by Chamberlain (1979) to be an efficient fluorographic method with the following steps: (1) pre-fix the gel; (2) soak the gel for 30 min in water; (3) soak the gel for 30 min in 1 M-sodium salicylate (pH 5–7); (4) dry the gel and expose it to film. We found that with 1.5 mm-thick slabs gels, 1 h in the sodium salicylate gave better detection efficiency.

En^{3}Hance is a fluorographic solution available from New England Nuclear Corp. (New England Nuclear, 1981) and has the following prescribed procedure: (1) Pre-fix the gel; (2) soak the gel for 1 h in En^{3}Hance; (3)soak the gel for 1 h in water; (4) dry the gel and expose it to film. We found that with a 1.5 mm-thick gel, a 1 h En^{3}Hance incubation was approx. 20% less efficient than a 2 h incubation, which gave maximum detection efficiency.

Results and discussion

The detection of radioactivity was used as a quantitative measure to optimize the procedure that used PPO with acetic acid as the solvent. Slab-gel squares containing equal amounts of radioactive protein were treated according to the separate protocols and then fluorographed together on the same film to make a relative comparison. The exposure densities were determined on a film-scanning densitometer, and comparisons of different treatments were made. When the gels were fluorographed after incubation in different concentrations of PPO, it was found that 15% (w/v) PPO was optimal (Fig. 1*a*). The optimal time of incubation in 20% PPO or acetic acid was shown to be 1.5 h (Fig. 1*b*). A 20%-PPO solution was used instead of a 15% solution to avoid a decrease in detection efficiency due to any losses of PPO. The optimal time of incubation in water was determined to be 30 min (Fig. 1*c*). The optimal conditions for this method were found to be similar for both 0.7 mm-thick and 1.5 mm-thick gels.

As reported previously, the recrystallization and re-use of PPO makes the use of PPO much more cost-efficient (Bonner & Laskey, 1974). The recrystallization consists of dissolving any PPO precipitate in a minimal volume of 95% (v/v) ethanol then adding five times the volume of water to precipitate in a minimal volume of 95% (v/v) ethanol, allowed to air-dry. With the use of 200 ml of a 20% PPO/acetic acid solution, recrystallization was necessary every 2 months. At this time, addition of lost PPO to make a 20% solution was made. We found that, over a 12-month period, over 300 25 ml gels can be treated with the use of 100 g of PPO. It was also found that this solution of PPO, which had been recycled for a 12-month period, gave the same detection efficiency and resolution as a freshly prepared solution (Fig. 2, lanes A and E, and in Table 1).

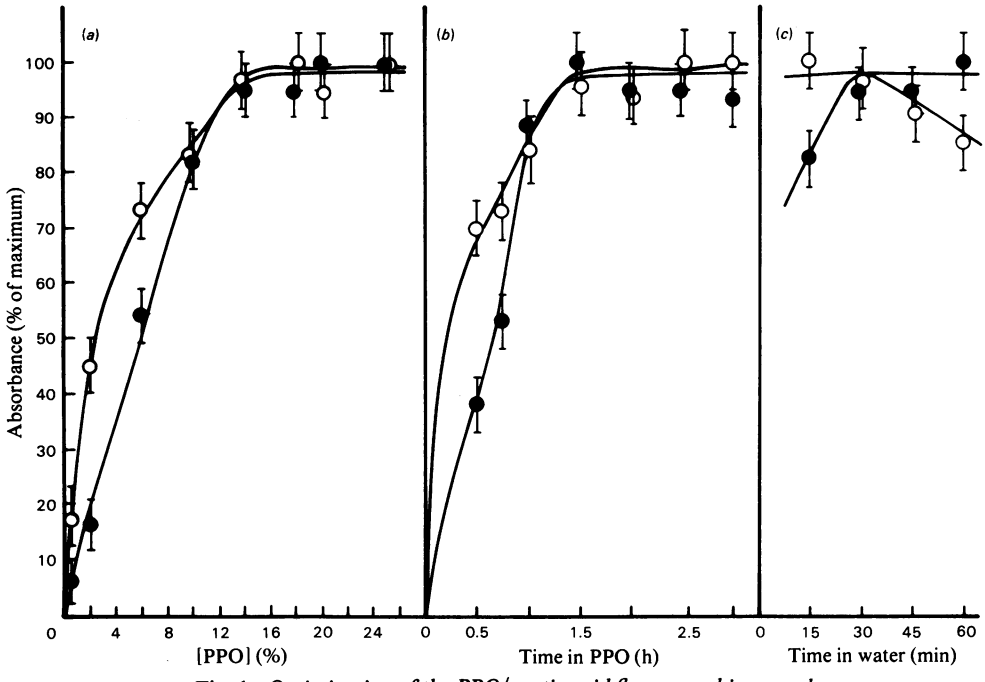

Fig. 1. *Optimization of the PPO/acetic acid fluorographic procedure*

Gel squares containing equivalent amounts of radioactivity (radioactivity labelled protein cast into the gel) were treated as indicated in the text, then fluorographed and analysed on an automatic film-scanning densitometer. Percentages of the maximum absorbance were determined and are expressed for 0.7 mm-thick (○) and 1.5 mm-thick (●) 2 cm × 2 cm gel squares. (*a*) Different concentrations (w/v) of PPO in acetic acid; (*b*) effect of time of incubation in 20% PPO in acetic acid; (*c*) effect of time of incubation in water. Each point represents the average ± S.D. for 12 determinations.

A quantitative comparison of the efficiency of the different fluorographic methods to detect radioactivity was done with 0.7 mm- and 1.5 mm-thick, 2 cm × 2 cm gel squares containing equivalent quantities of radioactivity. After treatment by a specific method, the gel squares from the different procedures were fluorographed on the same preflashed film. The film was then analysed on an automatic film-scanning densiometer and the exposure densities determined. Exposure densities below 1.0*A* were found to have a linear relationship; commonly observed densities were 0.2–0.9. As shown in Table 1, all the fluorographic methods had essentially the same efficiency for detection of radioactivity. It was found when using 1.5 mm-thick gels with the En^{3}Hance method that a 2 h incubation was necessary for maximum detection efficiency.

A qualitative comparison of the different methods is shown in Fig. 2. We have found that, with a 72 h exposure, the detection sensitivity would be approx. 150–200 d.p.m. of radioactively labelled protein. Optimum amounts of radioactivity per band in a 48–72 h exposure would be 500–2000 d.p.m. of radioactively labelled protein.

The sodium salicylate procedure provides a sensitive efficient fluorographic method; however, as Chamberlain (1979) suggests, the method does have poorer resolution. We have found in the analysis of two-dimensional gels with the sodium salicylate method that areas with multiple spots are not always resolved. This is not found with the PPO/acetic acid procedure.

Standard method

The standard method is as follows: (1) soak gel in acetic acid for 5 min; (2) soak gel in 20% (w/v) PPO in acetic acid for 1.5 h; (3) soak gel in water for 30 min; lay gel on thick paper and cover with plastic wrap or dialysis membrane; (4) dry under 1.33 kPa vacuum at 70°C for 3–5 h; cool before releasing vacuum; (5) expose to preflashed Kodak X-Omat X-ray film at −70°C.

The PPO/acetic acid procedure was found to be a simple, sensitive and efficient fluorographic procedure. The standard method presented applies to gels from 0.5 mm to 1.5 mm thick, with a need to increase to a 2 h PPO/acetic acid incubation with 2–5 mm-thick gels. The use of this method to fluorograph agarose gels was also found to be

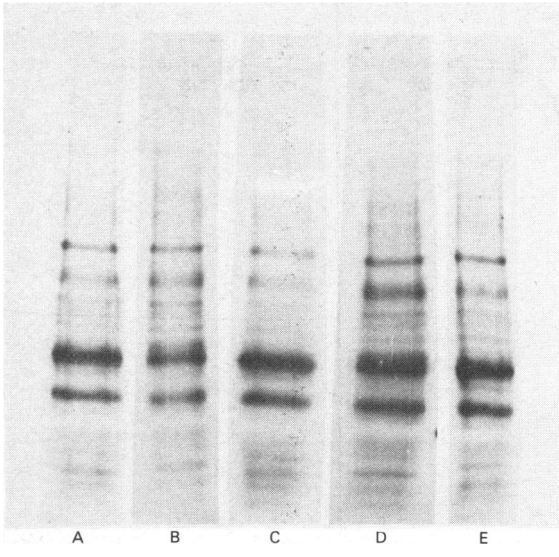

Fig. 2. *SDS/polyacrylamide-gel electrophoresis of ^{35}S-labelled Sertoli-cell-secreted proteins as detected by different fluorographic procedures*

Lanes indicated are: (*a*) freshly prepared PPO in acetic acid; (*b*) PPO in Me_2SO; (*c*) En^{3}Hance (2 h incubation); (*d*) sodium salicylate; (*e*) PPO in acetic acid (recycled over 12-month period). Equivalent amounts of radioactively labelled proteins (10000 d.p.m.) are detected in a 48 h exposure.

Table 1. *Relative comparison of radioactivity detection efficiency*

A relative comparison was made of different fluorographic methods using 0.7 mm- and 1.5 mm-thick 2 cm × 2 cm gel squares containing 10000 d.p.m. of radioactive protein. Analysis was done with an automatic film-scanning densitometer and integration of exposure densities. Results are expressed as a percentage of the maximum and represent an average for 12 determinations. A 5% coefficient of variation was calculated for each value.

Isotopic label ...	^{35}S		^3H	
Method / Gel thickness (mm) ...	0.7	1.5	0.7	1.5
PPO/acetic acid	97	100	98	100
PPO/Me_2SO	100	98	97	97
Salicylate	96	97	98	97
En^{3}Hance				
1 h	97	79	100	85
2 h	96	97	97	99
Used-PPO/ acetic acid	96	99	98	98

efficient when the same procedure was used. With the PPO/Me_2SO procedure, Me_2SO dissolves agarose. With the recrystallization and re-use of the PPO, this method is cost-effective without loss of detection efficiency. Also, with this procedure there is no need to pre-fix proteins in the gel, which is often necessary to prevent diffusion of small peptides.

The PPO/acetic acid fluorographic procedure provides an attractive alternative procedure to existing fluorographic methods.

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