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FLUORESCENT LABELLING OF PROTEINS ON THIN LAYERS OF SOLID DANSYL CHLORIDE

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This report describes a simple procedure for the dansylation of membrane proteins on a thin layer of solid dansyl chloride.

Dansyl chloride has been widely used for fluorescent labelling of proteins [1–3]. Because of its insolubility in aqueous media, the dansylation solutions usually include an organic solvent. Several authors have been successful in minimizing or even excluding the organic solvent (mainly acetone) from the reaction mixture [4–6]. Schmidt-Ullrich et al. [7] described a gentle method of dansylation of erythrocyte membranes employing dansyl chloride dispersed in phosphatidylcholine – cholesterol vesicles. Kinoshita et al. [8] applied the complex of dansyl chloride with cyclodextrin for dansylation of proteins and plasma membranes. A modification of this method was used by Lengrum et al. [9] for the studies on anion transport upon modification of integral membrane proteins with dansyl chloride.

In this communication we present a simple procedure for the dansylation of standard proteins in solution and membrane proteins on a thin layer of solid dansyl chloride.

Recently outdated human blood was obtained from Wrocław Blood Bank. Erythrocyte membranes were isolated according to the method of Dodge et al. [10]. Inside-out vesicles were obtained by extraction of erythrocyte ghosts with water at 37°C for 30 min [11]. The extract was concentrated in Amicon ultrafiltration apparatus, dansylated and passed through a Sephadex G-150 column in order to purify dansyl-labelled spectrin.

A thin layer of solid dansyl chloride was prepared by evaporation of acetone from a solution containing 5–10 mg of dansyl chloride. The dansyl chloride layer was formed at the bottom of glass test tubes (at least 3 cm in diameter) or in small round bottom flasks. To the flask with the dansyl chloride film were added 1 ml of protein solution (10–20 mg of protein) in 0.1 M phosphate buffer, pH 7.4 or 1 ml of membrane or cell suspension in appropriate Dodge buffer (310 or 20 imosM). The flask was gently shaken for 1–3 h at a temperature of 4°C. The reaction was stopped by removal of the solution from the flask. Unbound dansyl chloride and its derivatives were removed from the protein solution by gel filtration on a Bio-Gel P-6 column (1 × 40 cm) equilibrated with the same buffer. Unbound dansyl chloride was removed from the membrane or cell suspension by repeated washing with either 0.5% cyclodextrin or 0.5% soluble starch in appropriate Dodge buffer (310 or 20 imosM) and then with the same buffer containing neither cyclodextrin nor starch. The content of unbound dansyl chloride and its derivatives in the

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Abbreviations: dansyl chloride, 5-dimethylaminonaphthalene-1-sulfonyl chloride; SDS, sodium dodecyl sulfate; imosM, ideal milliosmomolar.

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TABLE I

DEGREE OF LABELLING AND EMISSION MAXIMA OF PROTEINS DANSYLATED FOR 3 h AT 4°C

Dansylation was carried out in 0.1 M phosphate buffer pH 7.4, 10 mg of dansyl chloride were used for the preparation of each film, 20 mg of each protein in each sample. Emission spectra were taken in the same buffer at an excitation wavelength of 365 nm.

Protein	Degree of labelling		Emission maximum (nm)
	mol dansyl per mol protein	μmol dansyl per g protein	
Bovine serum albumin	5.35	78.6	530
α-Chymotrypsinogen A	0.35	14.3	510
Human serum acid α_1-glycoprotein	1.66	37.8	530
Pepsin	0.60	17.9	475
Spectrin	6.24	27.1	500

supernatants was determined by measuring absorbance at 320 nm after addition of an equal volume on 1 mM NaOH [8]. The dansylated membranes were treated with 0.5% Triton X-100 for 6 h at room temperature, centrifuged for 1 h at 90000 $\times g$ and the fluorescence measurements of the supernatants were made.

The degree of labelling for dansylated standard proteins was calculated from the absorbances at 280 and 335 nm. A ratio of absorbances at 280 and 335 nm of 0.34 [12] and molar absorptivity of 3400 at 335 nm for the dansyl group bound to protein [13,14] were used. Electrophoresis was carried out in 5.6% polyacrylamide gel in the presence of 0.1% SDS according to Fairbanks et al. [15]. Gels were fixed with 25% isopropanol in 10% acetic acid. Protein was estimated according to Lowry et al. [16] and in the samples containing Triton X-100 according to the method of Dulley and Grieve [17].

Reagents. Dansyl chloride was purchased from BDH Biochemicals, bovine serum albumin, pepsin

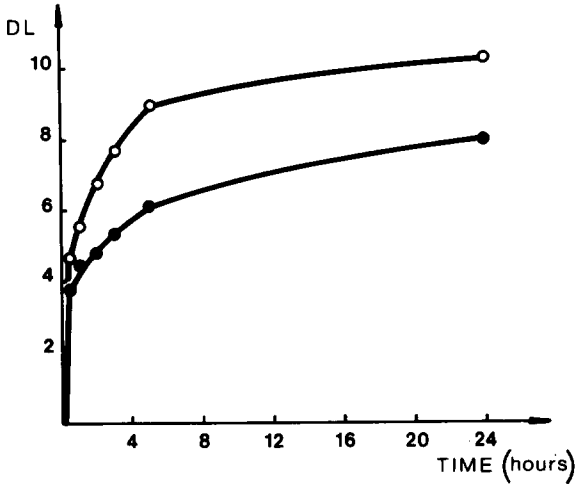

Fig. 1. The effect of time on the degree of labelling (DL, in mol dansyl per mol protein) of bovine serum albumin in 0.1 M phosphate buffer, pH 7.4. ●———●, Dansylation carried out at 4°C; ○———○, dansylation carried out at 22°C.

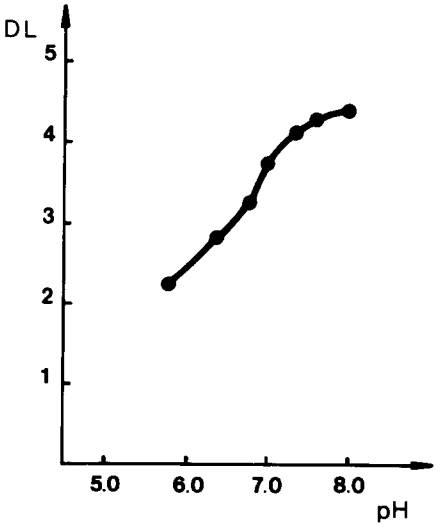

Fig. 2. The effect of pH of the reaction buffer on the degree of labelling (DL) of bovine serum albumin. In this experiment 0.1 M sodium phosphate buffers were used.

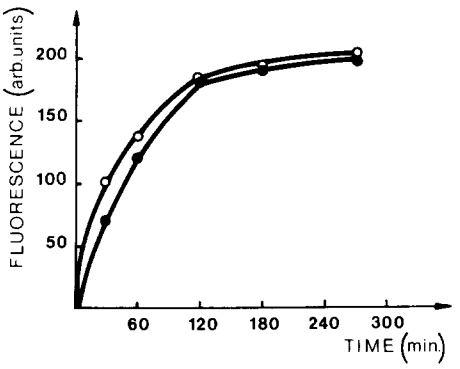

Fig. 3. The effect of time on the fluorescent labelling of erythrocyte ghosts dansylated in 20 imosM phosphate buffer, pH 7.4. ●———●, Dansylation carried out at 4°C; ○———○, dansylation carried out at 22°C.

and Triton X-100 from Serva Feinbiochemica and chymotrypsinogen A from bovine pancreas from Sigma Chemical Co. Human serum α_1-acid glycoprotein was a gift from Dr. Z. Wróblewski.

Table I shows the degree of labelling and the emission maxima of some standard proteins dansylated for 180 min on solid dansyl chloride layer. Values presented are comparable to other methods published [1,5,8]. As in the other methods bovine serum albumin is the most efficiently labelled protein among the chosen standards.

In Fig. 1 the time-course of labelling of bovine serum albumin in 0.1 M phosphate buffer pH 7.4 is shown. A period of 3 h seems to be proper for the dansylation of proteins in the solutions. When bovine serum albumin was dansylated at room temperature the degree of labelling was about 30% higher than at 4°C (Fig. 1).

The influence of the pH of the dansylation mixture, in the range 5.5–8.0, on labelling bovine serum albumin is shown in Fig. 2. These results are in good agreement with those of Mihalyi and Albert [12].

The erythrocyte membrane proteins were labelled when membrane or cell suspension was shaken on a thin layer of solid dansyl chloride. The reaction was almost completed within 2 h (Fig. 3). Extension of the time of the reaction up to 24 h resulted only in a 10% increase in fluorescence measured in Triton X-100-solubilized membrane solutions. It should be noted that unreacted dansyl chloride was removed with the same efficiency when membranes or cells were washed either with cyclodextrin [8] or soluble starch in phosphate buffer. The measured fluorescence intensities of different kinds of dansylated erythrocyte membrane preparations (solubilized in 0.5% Triton X-100) were: for membranes isolated from labelled intact erythrocytes 161.5, for labelled erythrocyte ghosts 228.7 and for inside-out vesicles 301.6. Thus, inside-out vesicles were the most efficiently labelled ones.

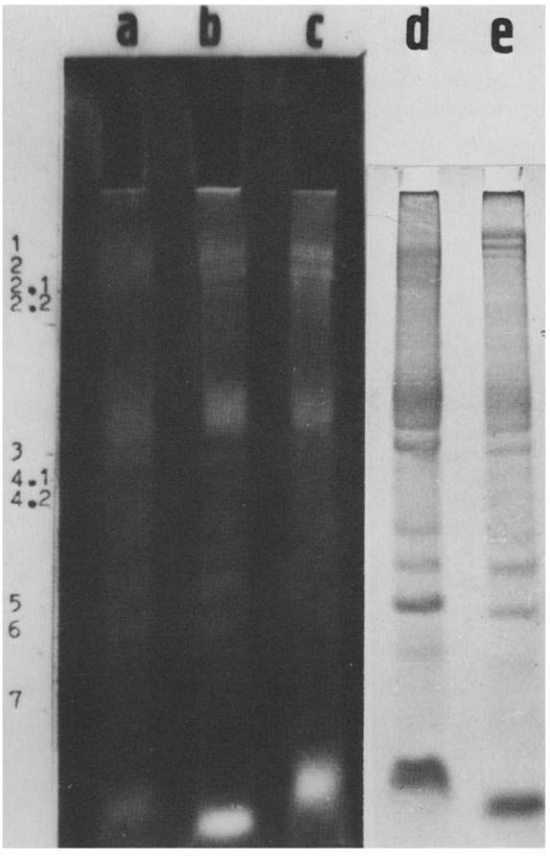

Fig. 4. SDS-polyacrylamide gel electrophoresis of erythrocyte membranes. (a) Ghosts isolated from dansylated erythrocytes (100 μg of protein). (b) Dansylated erythrocyte ghosts (50 μg of protein). (c) Dansylated inside-out vesicles (25 μg of protein). Photograph taken in the light of a mercury lamp equipped with a 366 nm filter. (d) and (e) Coomassie blue stained gel of dansylated inside-out vesicles and erythrocyte ghosts (25 μg of protein). Electrophoresis was carried out in 3-mm slabs in 5.6% gel in the presence of 0.1% SDS. Nomenclature of human erythrocyte membrane proteins was taken from Steck [18].

The same preparations were subjected to SDS-

polyacrylamide gel electrophoresis (Fig. 4). In intact erythrocytes, polypeptides which are known to be integral were labelled. Among them the component 3 band was always very well visible. To obtain the pattern presented in Fig. 4a it was necessary to apply twice as much of the ghost protein to the gel than in the case of labelled erythrocyte ghosts (Fig. 4b). When the same amount of protein was applied only the component 3 band was visible (not shown). In the isolated and dansylated ghosts, or inside-out vesicles, all major polypeptides recognizable by Coomassie blue staining were labelled with dansyl chloride. These results are in good agreement with ones obtained by Schmidt-Ullrich et al. [7].

Since the procedure presented here is simpler and at least as efficient when compared to the other methods it could find applications for the dansylation of proteins in other membranes, as well as for gentle labelling of proteins in solutions.

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