Fractionation of human parotid salivary proteins by preparative isoelectric focusing

M. EAGLETON, F. HALLINAN and E. TEMpany
Children’s Research Centre, Our Lady’s Hospital for Sick Children, Crumlin, Dublin 12, Ireland

Parotid saliva is a particularly good system to study exocrine secretory processes in man. Various proteins from human parotid saliva that have been previously isolated and partially characterized include glycoproteins, acidic proteins that contain little or no carbohydrate, and smaller acidic peptides rich in histidine or tyrosine (Henkin et al., 1978). The secretory process in the parotid gland includes the synthesis transport and storage of these materials. The study of these secretory mechanisms requires the isolation and characterization of these proteins. Preparative flat-bed electrofocusing was used in an attempt to simultaneously purify a number of human parotid salivary proteins.

Citric acid (5%)-stimulated parotid saliva was obtained from three volunteers (with their informed consent) by using a Curby cup-collection device (Jenkins, 1978). The saliva was dialysed overnight against 1% glycine and then centrifuged. The dialysed saliva was mixed with the gel slurry and, after evaporation at room temperature, focusing was performed for 17h at 8W constant power as described previously (Winter et al., 1975). The voltage increased from 400 to 1700V on completion of focusing. Eight precipitates were visible in the focused gel. The addition of 0.01% Tween 20 to the saliva during dialysis and subsequent focusing did not alter the precipitation pattern. The precipitates were removed and the gel was then fractionated. Gel samples were eluted with 0.15 M-NaCl/0.15 M-Tris/HCl, pH 6.8. The eluates were subsequently analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Laemmli, 1970) as shown in Fig. 1.

In contrast with the normal blue staining pattern, some of the proteins stain pink-violet with Coomassie Blue, as indicated by dots in Fig. 1. At least four distinct groups of polypeptides have been isolated by this procedure: precipitates 1 and 2, precipitate 3, precipitate 4 and precipitate 8. We have not yet established if the proteins in the other precipitates are related to, or distinct from, these four groups. The irregular banding pattern observed in precipitates 1 and 2 appears to be due to incomplete denaturation. Precipitate 4 consists primarily of the major parotid salivary protein, amylase. The background staining observed in precipitates 7 and 8 disappears on prolonged destaining, revealing, more clearly in precipitate 8, seven main polypeptide bands. The identical violet-staining character of these bands suggests that these polypeptides are related. The yield of protein as determined by A450 was 70.5%.

These results indicate that this method enables the simultaneous isolation of several salivary proteins in a single step. In view of the simplicity and high-load capacity of this method, it offers a more convenient purification procedure than the previously used chromatographic techniques (e.g. Hay, 1975; Henkin, 1978).

We are indebted to the Medical Research Council of Ireland for their financial support.

Winter, A., Perlmutter, H. & Davies, H. (1975) LKB Application Note 198, LKB Productor, Bromma

Specific labelling of insoluble elastin with tetr phenylporphin sulphonate

ROBERT J. ELLIOTT
Department of Biochemistry, Queen’s University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, U.K.

After sulphonation, tetr phenylporphin (Fig. 1a) becomes water-soluble and exhibits an intense red fluorescence at an excitation wavelength of 418 nm (Kim et al., 1978). The binding of TPPS* without loss of its fluorescence by elastic tissue was a serendipic event reported by Winkelman & Spicer (1962).

Abbreviation used: TPPS, tetraphenylporphin sulphonate.

Winkelman (1962) had been using TPPS to examine the accumulation of porphyrins within some tumours. Specific labelling of elastic material by TPPS could be accomplished only when the porphyrin was used as a ‘vital’ stain. After its intravenous injection into rat tail vein, TPPS was found to be absorbed non-specifically by most tissue components. If, however, the animal was not killed until 24h after the injection, the TPPS was observed to have been metabolized and/or released from all but the elastin-containing tissues. Could TPPS be used in vitro on animal tissue?
Tissue sections were covered with a 1% (w/v) phosphomolybdic acid solution for $S_{\text{min}}$, after which the excess was washed off with 0.1 M-phosphate buffer, pH 7.0. The sections were then treated with 0.5% (w/v) TPPS in pH 7.0 buffer for 30 min, rinsed briefly in water, vacuum-dried in a desiccator and mounted in a non-fluorescent oil medium. The method provides sensitive detection of elastic material in situ. The finely distributed elastic material in lung tissue is clearly resolved against a dark background of unlabelled collagen. Excellent structural details can be obtained of the elastic lamina in cross-sections of arterial tissue.

To determine whether TPPS was bound to the cross-linked insoluble elastin protein, three preparations were prepared: (1) from bovine neck ligament (Sigma Chemical Co.), (2) and (3) from bovine ear cartilage, with (2) prepared by the alkaline extraction method of Lansing et al. (1952) and (3) by the milder guanidine/collagenase procedure of Field et al. (1978). The quantity of TPPS bound by the three elastin preparations is shown in Fig. 1(b).

Subsequent experiments have demonstrated that TPPS is bound to elastin in a molar ratio of about 6:1. The nature of the pH 7.0 and 20°C may occur as a dimer and exhibit out-of-plane reaction between elastin and TPPS is unknown. The TPPS at 23°C//17°C was suspended in 1 ml of water with 7 ml of 0.1 M-PO$_4$-buffer, pH 7.0, containing 2.5 mg of tetraphenylporphin sulphonate; it was shaken for 1 h, centrifuged, the residue washed with 0.5 M-NaOH and the elastin-bound porphyrin was recovered in methanol/NH$_3$ (10:1, v/v). Absorbance was measured at 418 nm.

Fig. 1. Labelling of insoluble elastin with tetraphenylporphin sulphonate

(a) Planar structure of tetraphenylporphin sulphonate (Rothmund, 1936; Schneider, 1975). (b) Tetraphenylporphin sulphonate bound by elastin. ▲, Commercial preparation; □, alkaline preparation: ■, using guanidine/collagenase. Elastin was suspended in 1 ml of water with 7 ml of 0.1 M-PO$_4$-buffer, pH 7.0, containing 2.5 mg of tetraphenylporphin sulphonate; it was shaken for 1 h, centrifuged, the residue washed with 0.5 M-NaOH and the elastin-bound porphyrin was recovered in methanol/NH$_3$ (10:1, v/v). Absorbance was measured at 418 nm.

Enzyme-immunoassay of human $\alpha$-foetoprotein

M. ISLA HALLIDAY and G. BRIAN WISDOM
Department of Biochemistry, The Queen's University of Belfast, Belfast BT9 7BL, Northern Ireland, U.K.

Neural-tube defects such as anencephaly and spina bifida may be diagnosed by the measurement of the $\alpha$-foetoprotein content in amniotic fluid and maternal serum (Brock, 1977). However, the determination of the low concentrations of this protein in maternal serum requires a sensitive and specific assay procedure such as enzyme-immunoassay (Schuurs & Van Weeman, 1977). The central reagent, in this form of immunoassay, is the enzyme-labelled antigen or antibody, and several different methods have been described for labelling proteins with various enzymes. The heterobifunctional reagent, m-maleimidobenzoyl-