Localization of amylase and mucins in the major salivary glands of the mouse

A. P. VREUGDENHIL\(^1\), A. V. NIEUW AMERONGEN\(^1\),
G. L. DE LANGE\(^2\) and P. A. ROUKEMA\(^1\)

Departments of \(^1\)Oral Biochemistry and \(^2\)Oral Histology, Faculty of Dentistry, Vrije Universiteit, Van der Boechorststraat 7, P.O. Box 7161, 1007 MC Amsterdam, The Netherlands

Received 2 March 1981 and in revised form 25 November 1981

Summary

Antibodies against murine submandibular and sublingual mucins have been raised in rabbits. Both antisera appeared to be specific. Using these antibodies, the mucins were localized in the acinar cells of the submandibular and sublingual glands respectively.

The dyed amylopectin method was used to estimate the activity of amylase in the salivary glands. The enzyme was localized either by a starch-substrate film method or with antibodies against purified parotid amylase. The activity of amylase in parotid homogenates is about 1000-fold higher than that in homogenates of either submandibular or sublingual glands, in which the activity was comparable. Amylase was localized in the acinar cells of the parotid gland with both localization techniques. In the sublingual gland, amylase was found predominantly in the stroma around the acini, and there was some evidence that amylase was present in the demilune cells as well. In the submandibular gland, contradictory results were obtained with both techniques. With the starch-substrate film method, amylase activity was found in the granular convoluted tubular cells, whereas immuno-reactive amylase could only be demonstrated in the acinar cells of this gland. It is concluded that in the submandibular gland amylase and mucin are present in the same cell type.

Introduction

In our studies on the regulation of the secretory processes in the salivary glands of the mouse, amylase and sialomucin have been used as markers for the secretion. Amylase comprises about 40% of the secretory proteins in the parotid gland of the mouse (Nieuw Amerongen et al., 1980a), whereas a sialomucin is the major secretory product of the sublingual gland (Spicer & Warren, 1960; Roukema et al., 1976).

A sialomucin (Roukema et al., 1976) and amylase (Nieuw Amerongen et al., 1980a) are also present in the submandibular gland of the mouse. Because salivary glands contain several types of secretory cells, it is necessary to know the cellular localization of amylase and the sialomucins within the glands, in order to correlate the biochemical
(Vreugdenhil & Roukema, 1975; Nieuw Amerongen et al., 1978a, 1980b) and morphological phenomena (Vreugdenhil et al., 1980a,b,c, 1981) that arise during secretion under influence of various secretagogues. Histochemically, amylase has been demonstrated in parotid acinar cells and in the convoluted tubules of the submandibular gland of the mouse (Shear, 1972), and a sialomucin in murine submandibular and sublingual acinar cells (Spicer & Duvenci, 1964). In this paper, we report the immunohistochemical localization of submandibular and sublingual mucin in these glands. The localization of amylase with the starch-substrate film method according to Shear & Pearse (1963) was confirmed and extended by immunohistochemical observations.

Methods

Preparation and tests of the antisera
Amylase from the parotid and submandibular glands of female mice (Swiss random) was isolated and characterized as described by Nieuw Amerongen et al. (1980a). The purified parotid amylase was used as antigen for the preparation of specific antibodies. The titre and specificity of the antisera, raised in rabbits, were tested with various immunohemical precipitation tests (Nieuw Amerongen et al., 1980a).

Submandibular and sublingual sialomucins, isolated and characterized by Roukema et al. (1976), were also used for evoking specific antibodies. The immunization of a few rabbits with these mucins was performed as described by Oemrawsingh & Roukema (1976) for human submandibular mucins. The titre and specificity of the antisera were tested with the indirect hemagglutination test according to Boyden (1951), as described by Oemrawsingh & Roukema (1974) for human submandibular mucins. This test is based on the adsorption of proteins onto erythrocytes pretreated with tannic acid, and subsequent haemagglutination by antiprotein sera. In these experiments, the erythrocytes were coated with either submandibular or sublingual mucins.

Preparation of the tissues for immunohistochemical experiments
Fresh frozen tissues were preserved in liquid nitrogen until required for use. Sections were cut (8 μm thick) in a cryostat at −15°C, mounted on glass slides and dried at room temperature.

For the localization of the mucins with the indirect immunofluorescence technique, the sections were either fixed in 4% formaldehyde at 4°C for 10 min, or in 4% formaldehyde with 0.5% cetyl pyridinium chloride at 20°C for 10 min (Williams & Jackson, 1956). Unfixed sections were also used. To localize amylase, the sections were fixed in 10% formaldehyde at 4°C for 10 min.

Indirect immunofluorescence technique
For the localization of antigenic components, the immunofluorescence technique, developed by Coons et al. (1941), was applied. Fixed and unfixed sections of the salivary glands were incubated with a few drops of 5-10-fold diluted antisera at room temperature for 30 min. After removing excess antiserum, the slides were washed three times in phosphate-buffered saline (PBS). Subsequently, the sections were incubated with a few drops of a 60-fold PBS-diluted conjugate, consisting of horse anti-rabbit IgG-IgM-IgA serum labelled with Fluorescein isothiocyanate (FITC), for 30 min as described by Feltkamp & van Rossum (1968). After washing with PBS, the sections were covered with a cover-slip and preserved at −20°C until needed for examination. Photomicroscopy was carried out with a Leitz fluorescence microscope and Kodak Tri-X pan film. Four mice were used for the localization of mucins. The anti-amylase serum was tested on the glands of at least 12 mice.