Localization of Thyroid Peroxidase and the Site of Iodination in Rat Thyroid Gland

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The iodinated protein was localized in thyroid tissue slices by using radioautography. In unfixed tissue, the labelled protein was localized in the colloid, whereas, in tissue that was fixed before the ^{125}I addition, the label was within the follicular cell. This localizes thyroid peroxidase largely on the endoplasmic reticulum of the cell.

Cytochemical (Strum & Karnovsky, 1970; Nakai & Fujita, 1970; Novikoff *et al.*, 1971; Hosoya *et al.*, 1972; Tice & Wollmann, 1972) and biochemical studies (Alexander, 1965; Maloof & Soodak, 1965; Mahoney & Igo, 1966; Hosoya & Morrison, 1967*a,b*; Coval & Taurog, 1967; Ljunggren & Akeson, 1968; Taurog *et al.*, 1970; Hosoya *et al.*, 1971; Danner & Morrison, 1971; Nagasaka *et al.*, 1971; Valenta *et al.*, 1973) have consistently shown that the thyroid peroxidase is localized largely on the endoplasmic reticulum of the thyroid follicular cells. In addition to the endoplasmic reticulum, peroxidase has been localized (Strum & Karnovsky, 1970; Tice & Wollmann, 1972) on the perinuclear cisternae, the lamellae of the Golgi complex, the interior surface of the apical vesicles and the external surface of the apical border microvilli. However, there is no evidence that peroxidase is in the colloid.

Despite the apparent intracellular localization of the peroxidase, it is not clear that the enzyme within the cell is involved in the iodination of thyroglobulin. Although one study (Croft & Pitt-Rivers, 1970; Pitt-Rivers, 1971) has found radioautographic evidence for intracellular iodinated proteins, other attempts to localize the site of iodination by radioautography (Wollmann & Wodinsky, 1955; Stein & Gross, 1964; Ibrahim & Budd, 1965; Ekholm & Wollmann, 1975) have failed to show any appreciable iodinated protein within the cell; the iodine-labelled protein was found in the colloid within seconds after the iodide was introduced into the animals. This has led to Ekholm & Wollmann (1975) to suggest that thyroid peroxidase is largely on the apical border of the follicular cell and that pseudo peroxidases may be responsible for the histochemical stains within the cell.

In the present radioautographic investigation with thyroid tissue slices, we have shown that, if the thyroid tissue is aldehyde-fixed before iodination, the iodo-proteins are found within the follicular cell, confirming the histochemical (Strum & Karnovsky, 1970;

Nakai & Fujita, 1970; Novikoff *et al.*, 1971; Hosoya *et al.*, 1972; Tice & Wollmann, 1972) and biochemical reports (Alexander, 1965; Maloof & Soodak, 1965; Mahoney & Igo, 1966; Hosoya & Morrison, 1967*a,b*; Coval & Taurog, 1967; Ljunggren & Akeson, 1968; Taurog *et al.*, 1970; Hosoya *et al.*, 1971; Danner & Morrison, 1971; Nagasaka *et al.*, 1971; Valenta *et al.*, 1973) on the location of the thyroid peroxidase. As in previous radioautographic studies (Wollmann & Wodinsky, 1955; Stein & Gross, 1964; Ibrahim & Budd, 1965; Ekholm & Wollmann, 1975), if the tissue is not fixed before iodination, the iodoproteins are found in the lumen of the follicle.

Methods

After rats (about 300g) were anaesthetized by the injection of 20mg of pentobarbital sodium, the thyroid was immediately excised and placed in cold Hanks (1949) solution without glucose. The tissue was then sliced into small cubes (0.2–0.5 μl in volume) either in the Hanks solution or in the Hanks solution plus formaldehyde or glutaraldehyde. The pre-fixed tissues were left in the desired concentration of aldehyde for 30–60min at 22°C and then rinsed repeatedly to remove the excess of fixative. For iodination, samples were incubated in 0.25ml of Hanks solution and 0.25mCi of Na^{125}I was added to a final concentration of 0.1 μM .

Iodination was carried out under four different conditions: (a) glucose; (b) H_2O_2 ; (c) control; (d) CN^- control. In group (a) 1g of glucose/litre was added. In group (b) 5 μl of 80 μM - H_2O_2 was added four separate times at 1min intervals after addition of the Na^{125}I . In group (c) neither peroxide nor glucose was added. In group (d) KCN was added to give a final concentration of 7.5mM.

After 5–15min incubation, the samples were rinsed three times over a 10min period with Hanks solution containing 10 μM -KI. After 30min the

tissues were rinsed in the same solution and then fixed with 3% (v/v) glutaraldehyde in 0.15M-cacodylate buffer, pH 7.3, for 1 h at room temperature (22°C). After addition of fresh glutaraldehyde solution, the samples were placed in the refrigerator overnight.

The samples were post-fixed with 1% (w/v) OsO_4 in 0.15M-cacodylate buffer, dehydrated, and embedded in Epon 812 (E.M. Sciences, Fort Washington, PA, U.S.A.).

Light gold sections of the samples were cut on an LKB Ultratome I with a diamond knife, placed on a collodion-coated glass slide, stained with uranyl acetate for 5–10 min, followed by lead citrate for 3–5 min, carbon-coated in a vacuum evaporator, coated with Ilford L-4 emulsion (Ilford, Essex, U.K. as described by Salpeter & Bachmann (1972). The emulsion was developed with Microdol X (Kodak) after periods varying from 5 days to 3 weeks. After mounting of the sections on bare grids, they were viewed on a Siemens Elmiskop 1 electron microscope.

Results and Discussion

If the rat thyroid tissue was incubated with Na^{125}I before fixation, radioautography reveals that the great majority of the grains appear in the colloid (Plate 1a). This distribution of grains is typical of that obtained with a similarly treated thyroid of an animal that had been injected with radioactive iodide shortly before death.

When the tissue is incubated with Na^{125}I after fixation in 0.5% glutaraldehyde, then the grains appear within the cell (Plate 1b). The majority of these grains are over the cytoplasm of the cell. Similar results were obtained when the tissue was pre-fixed with 2% (v/v) glutaraldehyde or 4% (w/v) formaldehyde.

If a low concentration of fixative was used, such as 0.05% glutaraldehyde or 0.5% formaldehyde, a significant fraction of the grains appeared over both the colloid and the follicular cell. The distribution of grains in these samples seemed to indicate a non-homogeneous fixation, since there was no consistent pattern of grain distribution between the colloid and the follicular cells.

CN^- effectively stopped iodide incorporation. Radioautographs of CN^- -treated samples had the same grain density as the non-iodinated samples (less than 0.1% of the iodinated samples).

Other incubation conditions had no significant effect on the grain distribution in the radioautographs. The endogenous peroxide-generating ability of the follicular cell gave adequate incorporation of iodide without added peroxide or glucose, although the total incorporation was somewhat lower. The pre-fixed cells incorporated only about one-tenth as much radioactivity as the fixed cells. This could be caused by several factors: (1) the inability of a polypeptide to either leave or move to the enzyme in a

fixed sample; (2) the decreased rate of diffusion of peroxide and iodide in a fixed sample; (3) a possible decrease of activity of the enzyme in a fixed sample (see Tice & Wollmann, 1972; Hosoya *et al.*, 1972).

As shown in Plate 1(a), protein iodination occurs largely in the cytoplasm of the follicular cell. By using the method of Nadler (Haddad *et al.*, 1971) about 300 grains from several randomly selected radioautographs were analysed to determine the cell organelle with which they were associated: 3% of the grains were in the colloid. Of the remaining grains, 75% were associated with the endoplasmic reticulum, 8% with cytoplasmic vesicles, 3% with the nuclear membrane, 3% with the apical border membrane, 2% with the lateral plasma membrane, 3% with the Golgi apparatus, and 3% were scattered among mitochondria and the nucleus of the cell.

Of the 500 grains counted in radioautographs from unfixed tissue, 95% were associated with the colloid. Since thyroglobulin is the main constituent of the colloid (Smed, 1970), it is clear that the use of tissue slices instead of the intact gland has not eliminated the ability of the tissue to iodinate thyroglobulin.

Also, the decided difference in labelling patterns seen between Plate 1(a) and 1(b) indicated that the grains cannot be due to free $^{125}\text{I}^-$ that has not washed out of the tissue. This is also confirmed by the absence of radioactivity from CN^- -treated samples. CN^- has multiple sites of action on the follicular cell, but it does not inhibit the influx of iodide into the cell (Bagchi & Fawcett, 1973). The lack of iodination in the presence of CN^- confirms that the observed iodination is mediated by a haemoprotein peroxidase, the thyroid peroxidase. Since thyroid peroxidase, like lactoperoxidase, probably catalyses the iodination of protein directly through an enzyme complex (Morrison, 1973), the proteins iodinated in the fixed tissue are at or very close to the site of the peroxidase.

Thus the membrane-bound peroxidase, which is the presumed enzyme responsible for iodination, is primarily in the cell, but the product appears to be produced outside the cell. To account for this paradox, it has been suggested that the site of iodination is the apical vesicles, which are on the surface facing the colloid of the follicular cell microvilli (Strum & Karnovsky, 1970; Tice & Wollmann, 1972). This view carries the implication that most of the peroxidase in the cell is inactive or the enzyme would either iodinate proteins with which they are in contact or would generate I_2 that would itself act as a non-discriminating iodinating agent. The latter does not appear to take place.

An alternative suggestion is that the control of peroxidase activity may be at the level of peroxide generation and only the apical vesicles generate the peroxide necessary for iodination. This does not appear to be true, since the addition of peroxide was not required for intracellular iodination of pre-fixed

EXPLANATION OF PLATE I

Radioautographs of rat thyroid tissue showing portions of the colloid region (C), the follicular cells and adjacent capillary with erythrocyte (E)

(a) Tissue incubated in Hanks solution with Na^{125}I and H_2O_2 before fixation. (b) Tissue fixed with 0.5% glutaraldehyde before incubation as in (a). The bar is $2\mu\text{m}$ long.

cells, nor did it cause iodinated proteins to appear in the cell in unfixed tissue.

Thus either the fixing agent directly or indirectly activates the peroxidase within the cell, or the exocytosis of thyroglobulin from the cell is a very rapid process that is triggered by iodination.

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