ISOLATION AND IODINATING ABILITY OF APICAL POLES OF SHEEP THYROID EPITHELIAL CELLS

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The site of iodination of protein(s) in the thyroid follicle is still a matter of discussion. Autoradiographic investigations under the light or the electron microscope (EM) of thyroid glands of rat or mice sacrificed even a few seconds after ^{131}I or ^{125}I-injection failed to show any significant amount of iodine within the cells. Newly iodinated proteins were localized within the follicular lumen or upon the cell colloid interface and most likely associated with the thyroid cell microvilli which project into the lumen of the follicle (Nadler and Leblond, 1954 ; Wollman and Wodinsky, 1955 ; Stein and Gross, 1964 ; Pitt-Rivers et al, 1964).

The concept that the membrane of the microvilli is in fact the site for the iodination of thyroglobulin has been advocated and discussed recently (Wollman, 1965 ; Gross et al, 1966). It is strengthened by the finding that the precursor-protein first iodinated in vivo is the high molecular weight (S17 to S18) non-iodinated thyroglobulin dimer, the maturation of which is likely to occur in the lumen (Lissitzky et al, 1966). The well-differentiated apical pole of the thyroid cell is also important for the resorption of follicular colloid. The initial observation made by Dr. CARTOUZOU in this laboratory that structures resembling the apical portion of the acinar cell are present in homogenates of thyroid tissue prompted us to purify them and to investigate some of their properties in relation to a presumed role of the microvilli in the iodination processes within the thyroid follicle. We have termed these structures apical particles (AP).

Material and methods

Sliced sheep thyroid glands (40 g) were homogenized in 120 ml of Zalta's medium E (Zalta et al, 1962) with the Omni-mixer Servall for 1 min at 8-10,000 rpm and then in a glass-teflon Potter homogeneizer running at 1,500 rpm (2 strokes). After filtration through nylon gauze, the homogenate was centrifuged at 100 g for 5 min and the pellet discarded. The supernatant was separated into fractions sedimenting successively at 600 g (10 min), 12,000 g (10 min), 105,000 g (60 min) and 105,000 g supernatant. Each pellet was washed 2-times with 50 ml of Zalta's medium and resuspended in 10 ml 0.1M phosphate buffer pH 7.4. All processes were carried out at + 2°.

The peroxidase-dependent iodinating activity of the fractions was tested by incubation at 37° of an aliquot (10 to 50 µl) in 0.1M phosphate buffer pH 7.4 (final volume : 3 ml) containing D-glucose (5×10^{-3}M), glucose oxidase (16 µg), $Na^{127}I$ (1×10^{-5}M) and carrier free ^{125}I (2 µc) (complete system). The reaction was stopped by the addition of 2-mercaptoethanol (10^{-2}M final). The amount of organified ^{125}I was determined by the addition of 10 % TCA (3 ml) in the presence of carrier bovine serum albumin (BSA) (2 mg) and Na ^{127}I (0.5 mg/ml). TCA-insoluble material was washed 2-times and counted for ^{125}I in a Packard autogamma spectrometer. The results are expressed as % of iodide radioactivity organified or as specific iodinating activity (SIA) (µg ^{127}I incorporated per mg nitrogen (Kjeldahl) per minute).

Crystallized BSA was from Sigma (Saint-Louis, USA), RNAase free-DNAase from Worthington (Freehold, USA), glucose oxidase from N.B.C. (Cleveland, USA) and pure S19 sheep thyroglobulin was prepared in our laboratory.

For electron microscopy (Elmiskop I Siemens) the pellets were fixed in glutaraldehyde (2 % in PO_4 buffer pH 7.3) post-fixed in osmium tetroxide (2 %), dehydrated, embedded in Epon, sectioned (∿800 Å) and stained (uranyl acetate and lead citrate). Carbon-coated specimens were prepared for autoradiography by the technique of GRANBOULAN using Ilford L4 emulsion.

Results and discussion

Peroxidase-dependent iodinating activity has been found in all fractions except 105,000 g supernatant. The SIA is roughly 2-times

higher in the 12,000 g and the 105,000 g fractions than in the 600 g fraction (table I). Many experiments have given the same pattern of relative SIA. The iodinating properties of the different fractions studied are similar to those described by others for thyroid subcellular particles (see De Groot, 1965). No activity was found with the same fractions from rat liver.

Table I - Peroxidase-dependent iodinating activity of sub-cellular fractions of thyroid cells

fraction	amount of nitrogen (mg) in the sample	^{127}I incorporated in TCA-insoluble material (µg)	specific iodinating activity
600 g (3)	0.52	0.40	0.77
12,000 g (3)	0.40	0.59	1.47
105,000 g (3)	0.31	0.55	1.77
supernatant (3)	0.51	0.005	0.009
purified apical particles (2)	0.34	0.22	1.50

20 to 50 µl of each fraction incubated in the complete system for 5 min at 37°. AP purified by centrifugation over 2.2M sucrose (see text below). Figures in parentheses are number of separate fractions tested.

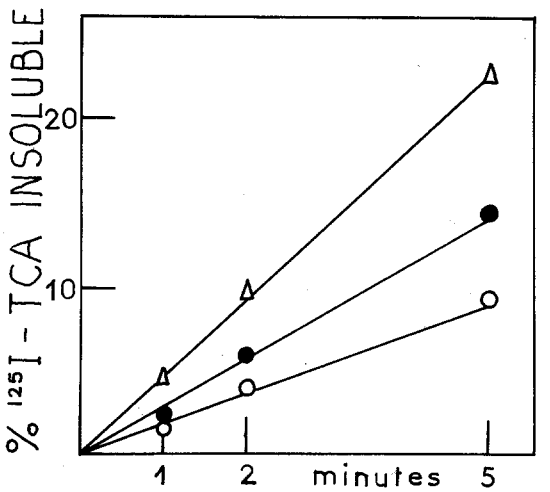

Fig.1 - Peroxidase-dependent iodinating activity of the 600 g fraction versus time and amount of material. 0.02 to 0.1 ml of fraction (0.65 to 3.25 mg protein) incubated in the complete system.
o-o, 0.65 mg N ;
●-●, 1.3 mg N ;
Δ-Δ, 3.25 mg N

The relations between iodinating activity and time or amount of material is illustrated in fig. 1 for the 600 g fraction. Similar results have been obtained with the 12,000 g and the 105,000 g fractions.

EM showed that the 600 g fraction contains mostly nuclei and AP (fig. 2A and B). These latter are ribbon-like in shape, of variable size (8 to 20µ length, 2 to 5µ width), bordered by

short and often broken microvilli. The villi are usually localized on one side of the AP. There are no mitochondria or endoplasmic reticulum to be found in the moderately dense material which makes up the bulk of the structure. In the absence of information on the possible contamination of the preparations with distal cytoplasm, we have prefered to refer to these structures as "apical particles" rather than "apical membranes".

The 12,000 g fraction contained a large percentage of mitochondria contaminated with microsomes but no nuclei. The 105,000 g contained mainly smooth and rough endoplasmic reticulum and ill-defined material.

To purify the AP, the 600 g fraction was frozen and thawed 2-times giving a suspension of high viscosity which was subsequently reduced by treatment with DNAase (5 µg/ml) for 30 min at room temperature. A pellet was recovered by centrifugation for 10 min at 2,000 g and 0°, and resuspended by gentle homogeneization in 0.1M phosphate buffer pH 7.4. 5 ml of the suspension were layered over 40 ml of 2.2 M sucrose in the same buffer and centrifuged at 0° for 5 hr at 17,000 g (rotor SS34 Servall). Two pellets sticking to the tube wall were found. The one at the top of the barrier, devoid of nuclei, contains mainly AP with evident microvilli and a little amorphous material, likely to be heterochromatin. Its SIA is 2-times higher than that of the 600 g fraction whereas that of the pellet at the bottom of the tube has no peroxidase activity (table I). The peroxidase-dependent iodinating activity of purified AP is destroyed by heating for 5 min at 100° and completely inhibited by 3-amino-1,2,4-triazole (10^{-4}M), SH-compounds or omitting glucose oxidase from the complete system. The iodide-peroxidase activity is firmly bound to the particles for it does not solubilize during a 30 min-preincubation of the 600 g fraction (table II). Addition of pure S19 sheep thyroglobulin (TG) or BSA to the complete system increases the total amount of TCA -insoluble iodine incorporated. The effect of TG addition is especially noticeable in the particulate fraction (table II). Centrifugation on a sucrose gradient (5-20 % ; rotor SW39 ; 39,000 rpm for 5 hr) of the soluble fraction after elimination of the particles shows the presence of labeled - S19 TG or - BSA.

These facts lend support to the idea that iodination of thyroglobulin occurs at the surface of the membrane limiting the

Table II - Non diffusibility of the peroxidase-dependent iodinating activity of the 600g fraction and iodination of added soluble proteins

		% TCA-insoluble ^{125}I in		
		particles	supernatant	total
A	complete system	3.0	0.8	3.8
	-id- + thyroglobulin (1.7 mg)	6.2	1.1	7.3
	-id- + BSA (2 mg)	3.4	1.6	5.0
B	complete system minus glucose oxidase	0.1	-	-
	supernatant incubated with glucose oxidase	-	0.3	-
	-id- + thyroglobulin (1.7 mg)	-	0.4	-
	-id- + BSA (2 mg)	-	0.7	-

A - 0.2 ml of 600g fraction (corresponding to 0.27g thyroid tissue) incubated for 20 min in the complete system with 2 µc ^{125}I.
B - 0.2 ml of the same preparation is pre-incubated for 20 min in the complete system minus glucose oxidase. The particles are separated by centrifugation and ^{125}I counted. The supernatant is incubated for 20 min at 37° in the presence of glucose oxidase (16 µg) and ^{125}I-TCA-insoluble radioactivity measured.

microvilli or beneath the apical membrane should further studies demonstrate that they are active *in vitro* in the resorption of thyroglobulin. That protein iodination carried out *in vitro* by the 600 g or the purified AP mimics the *in vivo* process is also indicated by the presence of ^{125}I-labeled DIT and MIT (DIT/MIT = 0.3 to 0.4) in the pronase digest (12 hr at 37°) of these particles labeled with ^{125}I for 10 min in the complete system. Autoradiography under the EM of the ^{125}I-labeled 600 g fraction shows significative amounts of iodine over the apical particles (fig. 2C).

It is obvious that apical particles of the thyroid cells are capable of iodinating pre-existing or added proteins with the formation of MIT *and* DIT after a short radioactive pulse *in vitro*. The existence of iodide-peroxidase activity in these structures devoid of mitochondria and of endoplasmic reticulum strongly suggests that they play an active and perhaps unique role in the iodination of protein(s) *in vivo* in the thyroid cell. That the so-called "microsomal" or "mitochondrial" fractions exhibit iodinating activity might be explained either by an intrinsic iodide-peroxidase activity or by their contamination with fragments of

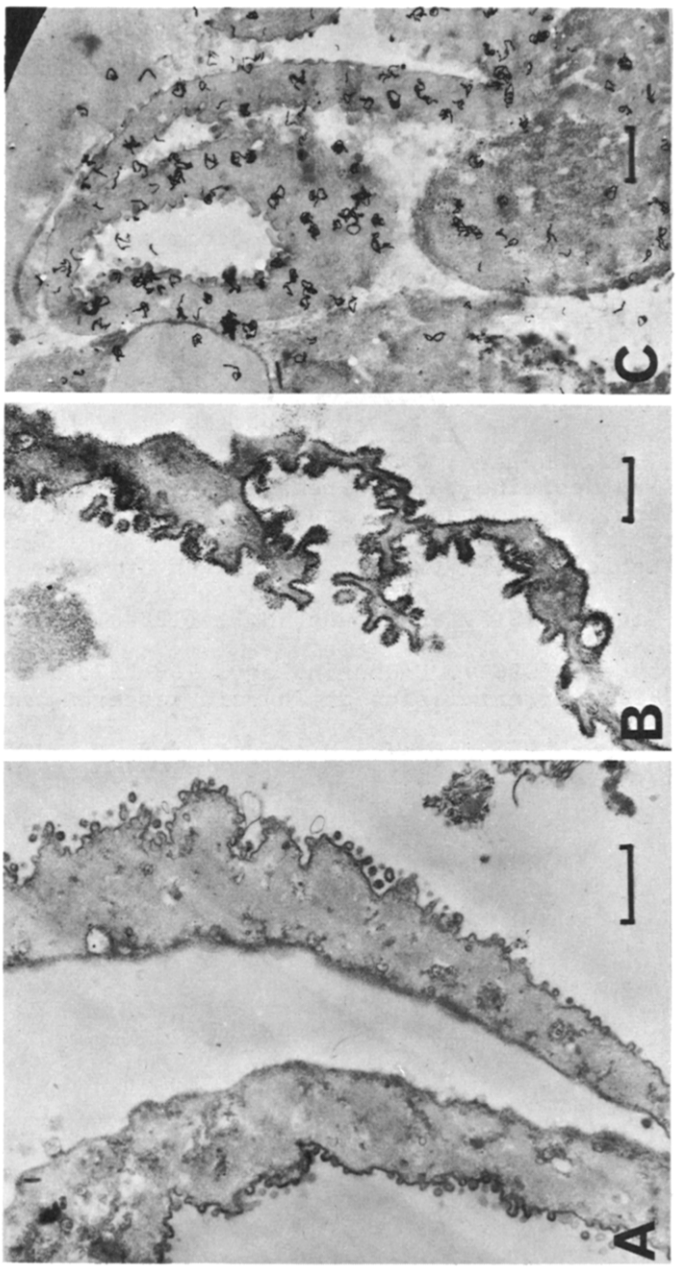

Fig. 2 - A and B : electron micrograph of a section of purified apical particles pellet obtained by centrifugation over 2.2 M sucrose. Two different aspects of apical particles. Note the well recognizable microvilli (A : 12,000 x ; B : 10,000 x). C : autoradiograph under the EM of a 800 Å section of labeled-600 g fraction (0.5 ml) incubated for 10 min in the complete system with 20 µc ^{125}I. Exposed 20 days. Note concentration of silver grains over apical particles (8,000 x). Scale marker = 1 µ

microvilli of several sizes generated during the homogeneization procedure. The latter explanation is strengthened when one compares the long filamentous microvilli observed on intact follicles to the short, flattened and often broken microvilli associated with AP isolated after homogeneization. Work is in progress to test this hypothesis. A full report of this work and further discussion will be published elsewhere.

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