THYROID
Volume 18, Number 7, 2008

DOI: 10.1089/thy.2007.0310

Factors Influencing the Study of Peroxidase-Generated Iodine Species and Implications for Thyroglobulin Synthesis

Jack Kessler, Ph.D.,[1] Christian Obinger, Ph.D.,[2] and Geoff Eales, Ph.D.[3]

A key issue in the mechanism of thyroglobulin (Tg) iodination by thyroperoxidase (TPO) is whether a TPO-bound iodine intermediate directly iodinates Tg-incorporated tyrosines (specific iodination) or whether reactive iodine species released from TPO effectuate Tg iodination (nonspecific iodination). We addressed these alternatives by (a) determining the aqueous equilibria of the iodine species potentially involved in the kinetic studies of TPO-mediated iodination, and (b) reviewing the structure of the substrate channel in mammalian peroxidases. Redox-potentiometric analysis of aqueous iodine combined with integrated mathematical modelling demonstrates that I_2 reacts with water to form several iodine species including hypoiodious acid (HOI). The HOI/I_2 ratio depends on time, iodide concentration, buffering agents, and pH varying dramatically from pH 4 to 7.4. These factors may confound the use of Michaelis–Menten kinetics to determine the mechanism of TPO-catalyzed iodination since both I_2 and HOI iodinate tyrosine but with different specificities and reaction rates. Consequently there is as yet no conclusive kinetic evidence that iodination occurs via formation of a TPO-bound iodinated intermediate. Furthermore, knowledge of TPO structure, gained from X-ray crystallographic studies indicates that access of Tg-bound tyrosyl groups to the active site of TPO is not possible. Thus the emerging conclusion is that the mechanism of Tg iodination is nonspecific. This is consistent with the occurrence of thyroid hormone formation in prevertebrate ascidians which exhibit TPO-like activity but lack the Tg gene.

Introduction

The iodination mechanism of thyroglobulin (Tg)-bound tyrosyl groups catalyzed by thyroid peroxidase (TPO) is unresolved. Two broad models have been advanced (1). One model is based upon the idea that iodination of Tg-bound tyrosyl groups occurs within the active site of TPO; this represents a "specific" iodination mechanism since TPO determines the stereospecificity for iodination of Tg-bound tyrosyl groups. The other model for iodination of Tg-bound tyrosyl groups is based on freely diffusing iodine species (iodination equivalents) that diffuse to Tg-bound tyrosyl groups after release from the active site of TPO. This model is termed "nonspecific" since the structure of Tg determines which Tg-bound tyrosyl groups form covalent bonds with iodine (iodination). The primary evidence used to evaluate these models comes from the kinetics of TPO-catalyzed iodide oxidation.

Kinetic studies of TPO-catalyzed iodide oxidation have primarily relied upon measuring formation of iodinated tyrosine, iodinated Tg-bound tyrosyl groups, or I_2 ("trapped" with excess iodide) (2). Several different iodinating agents have been advanced for TPO-catalyzed iodination including (a) iodide free radical, (b) iodinium ion, and (c) hypoiodite (OI^-) (3). Different conditions of pH and iodide concentrations have been employed in these studies. The interpretation of these kinetic studies therefore requires a knowledge of the behavior of the different aqueous iodine species that can potentially play a role in iodination. Iodine chemistry is generally thought of as an established field of study; however, our understanding of the various iodine species formed when iodide is oxidized and the inherent equilibrium that exists among them has expanded during the past two decades. A simultaneous solution for the thermodynamic and kinetic parameters describing these species (I^-, I_2, I_3^-, I_5^-, I_6^{2-}, hypoiodious acid [HOI], OI^-, IO_3^-, HI_2O^- , IO_2^-, and H_2IO^+) has been developed (4). The iodine species that exist in appreciable concentrations at pH 7.4 are iodide (I^-), triiodide (I_3^-), molecular iodine (I_2), hypoiodious acid (HOI), hypoiodite ion (OI^-), and the iodine anion (HI_2O^-). Gottardi's observations (4) may assist in the interpretation of the complex relationship among iodide concentration, pH, and

[1]Symbollon Pharmaceuticals, Inc., Framingham, Massachusetts.
[2]University of Natural Resources and Applied Life Sciences, Department of Chemistry, Division of Biochemistry, Vienna, Austria.
[3]Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada.

the concentration of iodination equivalents (I_2 and HOI) underlying interpretation of many TPO kinetic studies. While it is well known that TPO-catalyzed iodide oxidation generates freely diffusing I_2, the rate and extent of the reaction between I_2 and water to form HOI are not fully established. This is potentially significant because both I_2 and HOI effectuate nonspecific iodination. The influence of pH on the complex mixture of iodine species formed in an aqueous environment by the interaction of I_2 with water has not been fully integrated into the analysis of TPO iodination.

This manuscript examines the effects of pH and iodide on the concentration of I_2 and HOI using the model for predicting aqueous iodine equilibria previously developed by Gottardi (4). These results are discussed within the context of kinetic studies of TPO-catalyzed iodide oxidation which underlies the question of specific versus nonspecific Tg iodination. Also brought to bear on this question are recent X-ray crystallographic studies defining the spatial limits of the TPO active site as well as relevant comparative data.

Materials and Methods

Chemicals and materials

Sodium iodide (99.999%), iodine (beads 99.999%), and sodium phosphate were purchased from Sigma Chemical Company (St. Louis, MO).

Measurement of iodine species

Molecular iodine was measured according to the potentiometric method of Gottardi which relies upon measurement of iodide activity and the redox potential (5). Iodide activity was measured with an iodide ion selective electrode (Corning Model 476127, Woburn, MA) and the redox potential with the chain platinum/reference electrode (Fisher Cat. No. 13-620-115 and Fisher Cat. No. 13-620-51, Pittsburgh, PA) calibrated with an iodine/iodide redox buffer. Two pH meters (Corning Model 345, Woburn, MA) were used to measure the differences in electrode potential between the iodide and platinum electrode ($E_{I^-} - E_{Pt}$). Output from the pH meters was captured using a Signatech (Newport Beach, CA) board. Evaporation was minimized by performing experiments in glass containers with Teflon-lined screw-top lids. Lids were fabricated with orifices that (a) fit the corresponding electrodes to insure the solutions containing I_2 were not exposed to the environment and (b) allowed transfer of material into the reaction vessel without removing the lid. Experiments were conducted using a reaction volume equal to 85% of the bottle volume to minimize loss of I_2 to headspace in the reaction vessel. Solid iodine was crushed in a mortar and pestle and dissolved in acidified distilled water to saturation prior to transfer into the reaction vessel for measurements in buffers. The measurement of the loss of I_2 in water was made by transferring solid I_2 into the reaction vessel and initiating measurements once all I_2 was visually observed to be in solution.

Loss of I_2 in human plasma

The half-life of I_2 in human plasma was estimated by transferring 10 mL of I_2 (330 ppm) in 0.1 N HCl into 100 mL of plasma obtained from the American Red Cross (Washington, DC). Control experiments followed the loss of I_2 in normal saline.

Calculation of iodine species

Computer simulations were kindly conducted by Professor Waldemar Gottardi at the University of Innsbruck according to the methodology previously described (4).

Results

Figure 1 shows the effect of pH on the theoretical equilibrium distribution for I_2, HOI, I_3^- and iodide resulting from the interaction of 5.0×10^{-5} M I_2 with water. The primary effect of increasing pH on the concentration of iodination equivalents was to reduce the I_2 concentration, accompanied by corresponding increases in the concentrations of I_3^-, HOI, and iodide. The data suggest that the rate of I_2 hydrolysis into HOI is a variable of interest in the design of experiments that model Tg iodination with TPO since HOI iodinates tyrosine more effectively than I_2 (6).

Figure 2 models the influence of iodide on the equilibrium ratio of I_2/HOI in a starting solution of 10 μM I_2 at pH 7.5. The ratio of I_2/HOI increased by about 100 as iodide increased from 10^{-5} to 10^{-3} M; this change was primarily due to a reduction in HOI as there was only a modest (58%) reduction in I_2. The ratio of I_2/HOI exhibited a nonlinear response to pH. For example, a 10^{-3} M solution of I_2 dissolved in water yielded a concentration of HOI that was $10^{-3.9}$ M. 1f 10^{-3} or 10^{-2} M iodide is added to this solution the concentration of HOI will decrease to $10^{-4.8}$ and $10^{-6.5}$ M respectively. In comparison, the concentration of I_2 only decreases to $10^{-3.2}$ and $10^{-3.9}$ M, respectively. The reaction products of TPO-mediated iodide oxidation are a function of iodide concentration and these relationships may contribute to this observation (7,8). Figure 3 demonstrates the nonlinear relationship in the ratio of I_2/HOI as a function of pH and iodide.

Table 1 demonstrates the experimentally determined time-course of I_2 loss (hydrolysis) under different pH conditions. At pH 6.0 and lower the rate of I_2 loss was extremely slow but was rapid at pH 7.0 or 8.0. In the presence of human plasma the half-life of I_2 was less than 100 milliseconds (data not shown) consistent with prior observations in blood and

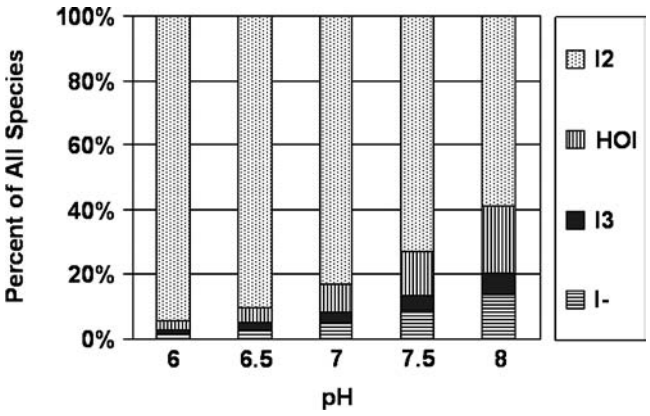

FIG. 1. Aqueous equilibrium distribution of iodine species of a 5.0×10^{-5} M solution of I_2. The effect of pH (0.050 M citrate-phosphate buffer) on the equilibrium distribution of I_2, HOI, I_3^-, and iodide derived from the reaction of 5.0×10^{-5} M I_2 with water.

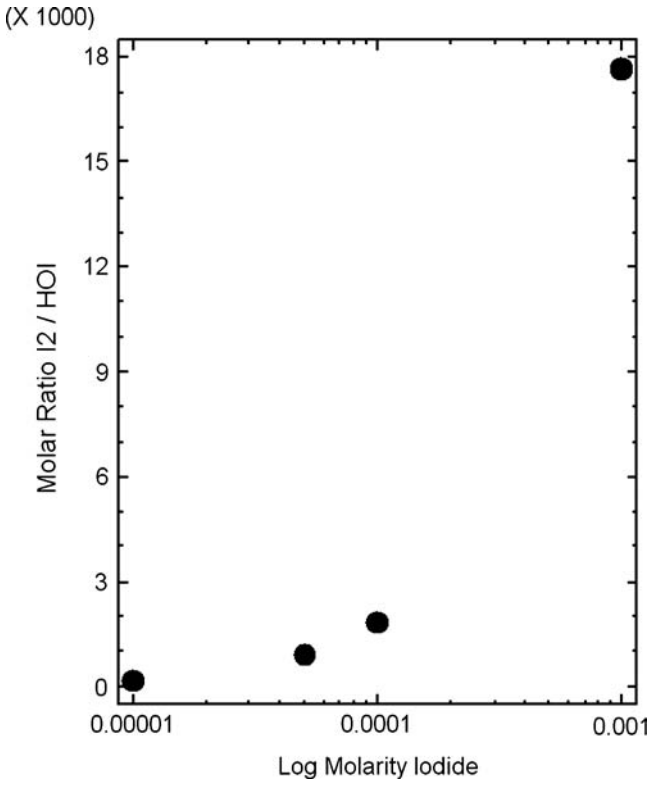

FIG. 2. Effect of iodide on ratio of I_2/HOI. The molar ratio of molecular iodine (I_2) to hypoiodious acid (HOI) is shown as iodide is added to an initial solution 1.0×10^{-5} M I_2 at a pH of 7.5.

plasma indicating that the reaction of I_2 with amino acids took place immediately (9).

Table 2 shows the exponential time-course of I_2 loss from water at a pH of 7.4, and demonstrates that in the presence of 0.05 M phosphate, a common buffer constituent in TPO kinetic studies, this rate was enhanced. Thus changes in both pH and phosphate have effects on I_2 loss (hydrolysis) in aqueous solution. Most importantly, these effects occurred in the probable physiologic range in the lumen for both pH and phosphate ion concentration. The loss of I_2 in water was observed to be slower than that observed in 0.05 M phosphate pH 7.4 consistent with earlier studies that show hydrolysis of I_2 in salt water begins in <1 second and that HOI is a significant species across the pH range of 4–9 (10). Prior studies also demonstrate that I_2 hydrolysis is a function of both the concentration and characteristics of the buffering agent (11).

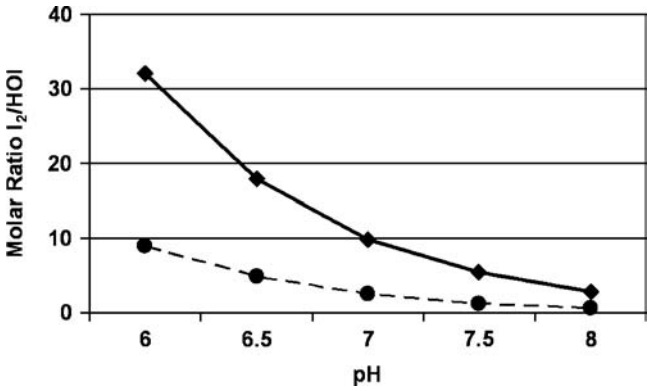

FIG. 3. Molar ratio of I_2/HOI vs. pH. The molar ratio of molecular iodine (I_2) to hypoiodious acid (HOI) at a pH of 7.4 (0.050 M phosphate) is shown as a function of pH at two different initial concentrations of I_2. The solid line (◆) represents a starting I_2 concentration of 1.0×10^{-3} M. The dashed line (●) represents a starting concentration of 5.0×10^{-5} M.

Table 1. Stability of I_2 in an Aqueous Environment

	Loss of I_2 (% lost)[a]				
Time (min)	*5*	*10*	*20*	*40*	*60*
0.05 M phosphate, pH 7.4	8.37	12.2	14.7	17.7	23.5
Water	4.97	5.19	6.24	6.83	7.52

[a]Mean ($n=5$) loss of I_2 measured potentiometrically (2) in a sealed container. The initial concentration of I_2 was 200 ppm.

Discussion

The above results demonstrate the complex relationships that exist among iodide, I_2, and HOI in an aqueous environment. Modest changes in iodine and iodide concentrations or pH can have profound effects on the final equilibrium state between iodine species. Furthermore, the rates at which I_2 undergoes conversion to other iodine species are influenced markedly by pH and by the presence of 0.05 M phosphate, a common buffer constituent. Thus, under conditions commonly employed in kinetic studies of TPO iodination, relatively minor changes in pH, iodide, and buffer may influence outcomes. The relationships displayed here can influence the interpretation of kinetic studies relating to TPO iodination and thyroid hormone biosynthesis.

Of particular significance in this regard is the iodination of Tg. Kinetic studies with TPO and tyrosine have evinced several different iodination mechanisms (3). The complex interrelationship among the various iodine species formed by iodide oxidation may contribute to this unsatisfying picture. The amount of I_2 formed from by TPO iodide oxidation has traditionally been estimated by "trapping" (12) I_2 with excess iodide to form I_3^-; that is, the substrate, iodide, reacts with the reaction product, I_2. In fact, I_2 also reacts with

Table 2. Stability of I_2 versus pH

	Loss of I_2 (percent lost)[a]				
	pH				
Time (h)	*3.0*	*5.0*	*6.0*	*7.0*	*8.0*
1	0.3	0	0	21.1	77.8
6	4.9	4.9	6.4	45.3	95.4
71	12.0	10.6	31.4	100	100

[a]Loss of I_2 measured potentiometrically (2) in a sealed container. The initial concentration of I_2 was 200 ppm in citrate-phosphate (0.050 M).

water to form HOI. Both I_2 and HOI iodinate tyrosine; however, they do so with different reaction kinetics (6). Complicating the picture is the fact that these underlying chemical relationships are sensitive to pH. Mechanistic conclusions from these kinetic studies are often applied to iodination of Tg using theoretical models that don't incorporate the extra-enzymatic reactions of these iodine species. It seems likely that the underlying relationships among the sundry species of iodine formed upon iodide oxidation confound the experimental design of many kinetic studies. Conceptual models addressing Tg iodination should (a) incorporate experimental limitations inherent to aqueous iodine equilibria and (b) examine the fact that molecules other than Tg are normally iodinated within the lumen (13,14).

Structural studies of the mammalian peroxidases myeloperoxidase and lactoperoxidase have advanced dramatically over the past two decades (15). Although the three-dimensional structure of human TPO is not available at present, its close phylogenetic relationship and high sequence homology with vertebrate and especially mammalian peroxidases (ranging from 40% to 70% residue identity) (16,17) allow important conclusions about substrate accessibility and halogenation pathway(s) in TPO. Together with recent kinetic data on the mechanism of halogenation of small and bulky molecules by myeloperoxidase (MPO) (18) these conclusions support nonspecific Tg iodination by TPO.

Generally, access to the active site in mammalian peroxidases is sterically hindered. The heme of mammalian peroxidases is located in a crevice, about 15 Å in depth, with access to the solvent via a single open funnel-shaped channel that ranges from approximately 10 to 15 Å in diameter. The substrate channel narrows before it discharges into the distal heme cavity that contains the amino acid triad histidine, arginine, and glutamine. This conserved triad participates in a rigid hydrogen bond network that involves water molecules and connects the heme cavity, i.e., the site of halide oxidation, with the exterior channel. At the immediate entrance to the distal cavity highly conserved phenylalanines are found in all mammalian peroxidases (15).

The 2.3 Å resolution X-ray structure of the MPO-salicyhydroxamic (SHA) complex indicates that the aromatic ring of SHA binds to this hydrophobic region (19). In detail, the aromatic ring of SHA is tilted about 20° with respect to that of the heme pyrrole ring D which forms the lower surface of the hydrophobic cavity, while the conserved arginine forms the upper surface (19). The hydroxamic acid moiety is hydrogen bonded to both the distal histidine and glutamine but is not coordinated to the heme iron. Additional van der Waals interactions occur with the adjacent conserved phenylalanines, each of which has an aromatic ring within 4.2 to 4.6 Å of the aromatic ring of the SHA molecule. A similar binding site has been found also for indole derivatives by computational docking (20). Melatonin (*N*-acetyl-5-methoxytryptamine) and serotonin (5-hydroxytryptamine) could be docked with their indole rings oriented parallel to the heme plane and close enough to the D pyrrole ring to achieve stacking. The distances of the closest indole atom to the center of the D ring were about 3.4 Å for both substrates. The side chain was never directed toward the distal cavity, whereas the indole substituent at position 5 was close to the heme center (20).

Both the structures of SHA-MPO complex and the indole-MPO complex models clearly suggest that a free tyrosine would bind in a similar way, which is underlined by the fact that tyrosine functions as an electron donor for mammalian peroxidases (15). However, the hydroxyphenyl group of a Tg-bound tyrosyl residue cannot reach this hydrophobic binding pocket. Thus, Tg-bound tyrosine would never approach the halide binding and oxidation site, thereby ruling out specific Tg iodination.

This conclusion is underlined by recent kinetic investigations of the mechanism of chlorination of taurine (21) and the artificial tripeptide Pro-Gly-Gly (22) by MPO. The kinetic data suggest two pathways for chlorination. One, for bulkier substrates, involves chlorination by free HOCl outside the heme cavity; ClO^- is released from the active center, diffuses away the heme cavity, and undergoes protonation to HOCl. The other implies the existence of a compound I-chloride complex, capable of directly chlorinating smaller substrates in the heme pocket. Computationally calculated structures of Pro-Gly-Gly and taurine show that the size of the tripeptide is twice that of taurine, and comparable to the available gap in the substrate channel, proving that access to the active site is difficult for Pro-Gly-Gly and impossible for the even bulkier compounds. Thus, chlorination is only possible by free HOCl outside the enzyme. The spatial constraints of the substrate channel prevent binding of Tg-bound tyrosyl groups and, therefore, TPO-catalyzed iodination of Tg can only occur outside of the active site in contrast to tyrosine.

The question of specific versus nonspecific iodination of Tg has been examined directly. Human Tg with few iodinated tyrosyl groups was used to compare TPO-catalyzed iodination with a corresponding chemical iodination using a mixture of I_2 and HOI (22). The identical hormonogenic sites are iodinated in both instances at low levels of iodination (approximately eight added iodine atoms per molecule). The only differences observed between the enzymatic and chemical iodination of Tg was a slightly higher di-iodotyrosine (DIT) content and a correspondingly lower mono-iodotyrosine (MIT) content in enzymatically iodinated Tg. The slight disparity observed between enzymatic and chemical Tg iodination was likely due to different ratios of HOI/I_2 in the enzymatic versus chemical iodination environment (a higher concentration of I_2 would be expected in the enzymatic iodination). The fact that the identical tyrosyl groups are iodinated via the enzymatic and chemical process indicates that TPO plays no role in determining which tyrosyl groups are iodinated. Thus these data strongly support a nonspecific model for Tg iodination. We are unable to identify another reaction where, at a pH of 7.4, an enzymatic and corresponding chemical reaction occur with equivalent rates and specificity.

Figure 4 summarizes the major pathways we propose for thyroid hormone biosynthesis and indicates the central role played by TPO following its oxidation to TPO-O (step 1). This scheme relies upon the ability of TPO to oxidize distinct substrates, e.g., iodide, tyrosine. Steps 2–6 are involved in the iodination of tyrosyls on Tg, while steps 7 and 8 in coupling adjacent pairs of iodinated tyrosyls to form the iodothyronyls that can be subsequently released as thyroid hormones following Tg proteolysis. This model relies largely upon extra-enzymatic chemical reactions.

The results from the present study are especially relevant to iodination and particularly conversion of I_2 to HOI (step 5). This is because both I_2 and HOI, the iodine equivalents

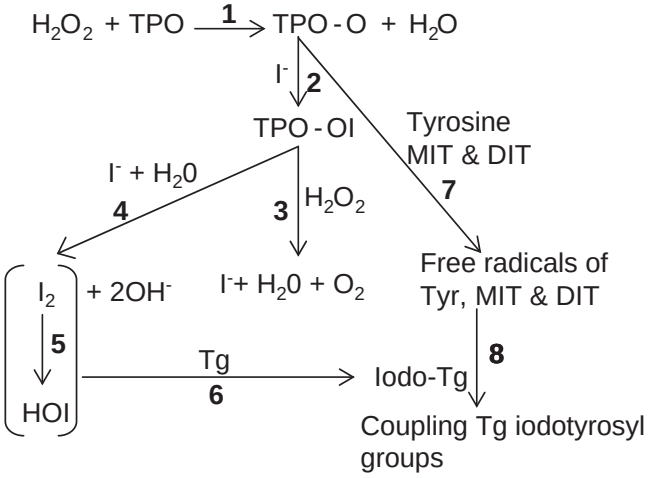

FIG. 4. Model of nonspecific thyroglobulin (Tg) iodination. The E-O intermediate formed from the reaction of thyroid peroxidase (TPO) with H_2O_2 (reaction 1) yields an intermediate that can initiate two distinct pathways. One pathway effectuate Tg iodination and the other coupling. The interaction of iodide with E-O (reaction 2) can lead to the formation of I_2 (reaction 4) and nonspecific iodination of Tg (reaction 6). The transfer of an electron from tyrosine to E-O (reaction 7) forms a free radical that catalyzes coupling of iodinated Tg-bound tyrosyl groups. Excess H_2O_2 leads to oxidation of hydrogen peroxide to O_2 (i.e. catalase activity, reaction 3). Hydrolysis of I_2 (reaction 5) may be significant in kinetic studies but not *in vivo*.

instrumental in iodination, have different specificities. Indeed HOI iodinates tyrosines more effectively than I_2. Consequently, any kinetic study of tyrosine iodination will depend on accurate predictions of, and allowance for, the proportions of iodine species, particularly I_2 and HOI, over the time-course of measurements. HOI is not anticipated to be a significant factor in Tg iodination *in vivo* due to the rapid reaction of I_2 with biological molecules.

Comparative studies also support nonspecific Tg iodination. Ascidians (tunicates or sea squirts) and amphioxus are the living representatives of protochordates from which vertebrates evolved. These species have a thyroid system; thyroid hormone (TH) receptors, deiodinases, and THs exert pharmacologic activity (23). However, THs are not formed in a follicular thyroid but in a more primitive homolog of the thyroid known as the endostyle. TPO is present in certain endostyle cells (24) but Tg cannot be synthesized since its gene is absent from the protochordate genome (25). Thus, any synthesis of TH occurring in the ascidian endostyle cannot depend on interaction between TPO and Tg. Presumably TPO-generated HOI and I_2 are responsible for iodination of either free tyrosines or tyrosyls on a different protein that couple to form TH.

If Tg is not obligatory for the formation of TH, the question arises as to why Tg, which was likely expressed by duplication of multiple domains (26) and synthesized at considerable metabolic expense with no evidence of recycling of its peptide components, ends up as a keystone of vertebrate TH synthesis in the thyroid follicle? An additional burden to this model exists, because <3% of synthesized TPO ultimately contributes catalytically to TH biosynthesis. The possible selective advantages of Tg have to be considered together with the structural aspects of the follicle itself and the chemistry that underlies synthesis of THs, i.e., iodination. These biological elements appeared simultaneously in cyclostomes, the most primitive extant vertebrates. This association suggests an advantage in storage of iodine and storage and release of THs in an iodine-deficient freshwater environment. While this may be true, the properties of biological iodination reactions impose evolutionary constraints on biological pathways that have a specific iodination product as an endpoint. Iodination in primitive organisms is nonspecific and a synthetic strategy for TH formation in a biological matrix is constrained by the potential of many organic species to react with oxidized iodide species.

In this regard, the exclusion of extraneous proteins and lipids from the lumen provides an environment that allows freely diffusing iodination equivalents to react primarily with Tg. Rather than evolve a pathway for TH synthesis, vertebrates evolved a physical structure that provides an environment which optimizes the specificity of nonspecific Tg iodination; that is, the lumen can be envisioned as a biological test tube. In addition, the lumen acts to protect the immune system from iodinated protein fragments that might otherwise be immunogenic. In essence the follicular lumen serves as a biological structure to provide specificity in the iodination of tyrosyl groups on Tg and to control the release of iodinated proteins and lipids that are potentially immunogenic.

Nonspecific iodination should lead to iodination of a variety of organic molecules other than Tg. Lemansky *et al.* (13) observed a substantial portion of iodine associated with cellular proteins other than Tg in primary cultures of porcine thyrocytes. The formation of iodinated lipids, key agents of thyroid autoregulation, has been shown to depend upon nonspecific iodination (27). All four double bonds in arachidonic acid are iodinated and multiple iodination of these molecules can also occur (28); a similar pattern exists with docosohexanoic acid. We conclude that the model of nonspecific Tg iodination is consistent with both clinical and biophysical observations and should be incorporated into the thought process of endocrinologists.

References

1. Dunford HB 1999 Heme Peroxidases. Wiley-VCH, New York, pp 425–427.
2. Magnusson RP, Taurog A, Dorris ML 1984 Mechanisms of thyroid peroxidase- and lactoperoxidase-catalyzed reactions involving iodide. J Biol Chem **259:**13783–13790.
3. Taurog A, ed. 1996 Hormone Synthesis and Secretion. Lippincott-Raven, Philadelphia, pp. 189–195.
4. Gottardi W 1999 Iodine and disinfection: theoretical study on mode of action, efficiency, stability, and analytical aspects in the aqueous system. Arch Pharm (Weinheim) **332:**151–157.
5. Gottardi W 1983 Potentiometric determination of equilibrium concentrations of free and complex bound iodine in aqueous solutions of polyvinylpyrrolidone iodine (PVP iodine). Fresenius Z Anal Chem **314:**582–585.
6. Dunford HB, Ralston IM 1983 On the mechanism of iodination of tyrosine. Biochem Biophys Res Commun **116:** 639–643.

7. Taurog A 1970 Thyroid peroxidase-catalyzed iodination of thyroglobulin; inhibition by excess iodide. Arch Biochem Biophys **139:**212–220.
8. Pommier J, Deme D, Nunez J 1973 Dissociation into subunits of thyroglobulin iodinated by thyroid and horse radish peroxidase. Biochimie **55:**263–267.
9. Gottardi W 1986 The decrease of efficiency of povidone-iodine preparations by blood. Presented at Third Conference on Progress in Chemical Disinfection, Binghamton, NY. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/9-87/031, pp. 357–371.
10. Truesdale V, Luther G 1995 Molecular iodine reduction by natural and model organic substances in seawater. Aquatic Geochem **1:**89–104.
11. Wyss O, Strandskov F 1945 The germicidal action of iodine. Arch Biochem **6:**261–268.
12. Pommier J, Deme D, Nunez J 1973 Effect of iodide concentration on thyroxine synthesis catalysed by thyroid peroxidase. Eur J Biochem **37:**406–414.
13. Lemansky P, Popp GM, Tietz J, Herzog V 1994 Identification of iodinated proteins in cultured thyrocytes and their possible significance for thyroid hormone formation. Endocrinology **135:**1566–1575.
14. Lemansky P, Brix K, Herzog V 1998 Iodination of mature cathepsin D in thyrocytes as an indicator for its transport to the cell surface. Eur J Cell Biol **76:**53–62.
15. Furtmuller PG, Zederbauer M, Jantschko W, Helm J, Bogner M, Jakopitsch C, Obinger C 2006 Active site structure and catalytic mechanisms of human peroxidases. Arch Biochem Biophys **445:**199–213.
16. Taurog A 1999 Molecular evolution of thyroid peroxidase. Biochimie **81:**557–562.
17. Zamocky M, Jakopitsch C, Furtmüller PG, Dunand C, Obinger C 2008 The peroxidase-cyclooxygenase superfamily: reconstructed evolution of critical enzymes of the innate immune system. Proteins **71:**589–605.
18. Ramos DR, Garcia MV, Canle ML, Santaballa JA, Furtmüller PG, Obinger C 2008 Myeloperoxidase-catalyzed chlorination: the quest for the active species. J Inorg Biochem **102:** 1300–1311.
19. Davey CA, Fenna RE 1996 2.3 A resolution X-ray crystal structure of the bisubstrate analogue inhibitor salicylhydroxamic acid bound to human myeloperoxidase: a model for a prereaction complex with hydrogen peroxide. Biochemistry **35:**10967–10973.
20. Hallingback HR, Gabdoulline RR, Wade RC 2006 Comparison of the binding and reactivity of plant and mammalian peroxidases to indole derivatives by computational docking. Biochemistry **45:**2940–2950.
21. Ramos DR, Victoria García M, Canle LM, Arturo Santaballa J, Furtmüller PG, Obinger C 2007 Myeloperoxidase- catalyzed taurine chlorination: initial versus equilibrium rate. Arch Biochem Biophys **466:**221–233.
22. Xiao S, Dorris ML, Rawitch AB, Taurog A 1996 Selectivity in tyrosyl iodination sites in human thyroglobulin. Arch Biochem Biophys **334:**284–294.
23. Sherwood N, Adams B, Tello J 2005 Endocrinology of protochordates. Can J Zool **83:**225–255.
24. Ogasawara M, Di Lauro R, Satoh N 1999 Ascidian homologs of mammalian thyroid peroxidase genes are expressed in the thyroid equivalent region of the endostyle. J Exp Zool **285:** 158–169.
25. Novinec M, Kordis D, Turk V, Lenarcic B 2006 Diversity and evolution of the thyroglobulin type-1 domain superfamily. Mol Biol Evol **23:**744–755.
26. Eales J 1997 Iodine metabolism and thyroid related functions in organisms lacking thyroid follicles: are thyroid hormones also vitamins? Proc Soc Exp Biol Med **214:**302–317.
27. Gartner R, Dugrillon A, Bechtner G 1996 Evidence that iodolactones are the mediators of growth inhibition by iodine on the thyroid. Acta Med Austriaca **23:**47–51.
28. Wolff J 1989 Excess iodide inhibits the thyroid by multiple mechanisms. Adv Exp Med Biol **261:**211–244.

Address reprint requests to:
Jack Kessler, Ph.D.
37 Loring Drive
Framingham, MA 01702

E-mail: jackkessler@hotmail.com