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Proteins iodinated by the chloramine-T method appear to be degraded at an abnormally rapid rate after endocytosis

(oocyte/fluid endocytosis/absorptive endocytosis/protein turnover)

L. OPRESKO, H. S. WILEY, AND R. A. WALLACE

University of Tennessee—Oak Ridge Graduate School of Biomedical Sciences, and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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ABSTRACT **Proteins labeled with either ^{3}H by reductive methylation or ^{125}I by the chloramine-T method were incubated with *Xenopus laevis* oocytes; the incorporation and acid precipitability of the proteins were then studied. The uptake rates of both specifically incorporated (vitellogenin) and nonspecifically incorporated proteins (bovine serum albumin and *X. laevis* serum proteins lacking albumin) were not influenced by the method of labeling. However, ^{125}I-labeled proteins were apparently degraded at rates far exceeding their ^{3}H-labeled counterparts, based on the generation of acid-soluble radioactivity. Thus, after a 3-hr incubation, 3–5 times more ^{125}I-labeled bovine serum albumin and *X. laevis* serum proteins lacking albumin were degraded than the corresponding ^{3}H-labeled proteins (95% compared to 30% and 75% compared to 15%, respectively), whereas after a 24-hr incubation, the degradation of ^{125}I-labeled vitellogenin was 15 times greater than that of [^{3}H]vitellogenin labeled *in vivo* (60% compared to 4%). Moreover, examination of the relative amounts of ^{3}H- compared to ^{125}I-labeled bovine serum albumin deposited into the exogenously derived yolk platelet compartment of the oocyte revealed 7 times more acid-precipitable ^{3}H-labeled protein, indicating that the observed discrepancies were not due to reincorporation of the ^{3}H-labeled ligands. Passage of dissolved oocytes previously exposed to ^{125}I-labeled bovine serum albumin (chloramine-T method) over a column of Bio-Gel P-10 revealed some breakdown of bovine serum albumin to intermediate molecular weight components and the presence of a large amount (≈90%) of labeled low molecular weight compounds, which analysis showed to be 72% free iodine. The evolution of either iodotyrosine or free iodine would nevertheless be perceived as protein degradation by most analytical procedures (e.g., acid precipitation or autoradiography). We conclude, therefore, that apparent degradation rates observed for endocytotically incorporated proteins may vary depending on the method used to label the protein and caution should be exercised when interpreting results obtained with labeled, particularly chloramine-T labeled, proteins.**

Much recent interest has been focused on the binding of peptide hormones to cell membranes and the subsequent internalization and fate of the bound hormone (1–5). In order to perform such studies, it is necessary to label the hormone to a high specific activity. This is most often achieved through iodination of the protein. Of the iodination procedures available, the one generally used is the chloramine-T method of Greenwood *et al.* (6). The resulting ^{125}I-labeled peptides are judged biologically unaltered if they retain their specificity of binding, immunoprecipitability with monospecific antibody, and activity in bioassays.

For the past decade our laboratory has been studying the incorporation of proteins by *Xenopus laevis* oocytes. It was found that the yolk precursor protein vitellogenin is taken up selectively by oocytes both *in vivo* (7) and *in vitro* (8, 9) at rates 20–50 times higher (on a molar basis) than other proteins tested. Because oocytes incorporate relatively large amounts of vitellogenin (>150 ng/oocyte per hr), vitellogenin labeled *in vivo* was used in these studies. Wallace *et al.* (10) used [^{3}H]vitellogenin labeled *in vivo* to study the macromolecular restructuring of vitellogenin into the yolk proteins lipovitellin and phosvitin. These workers demonstrated that 96% of the radioactivity contained in the sequestered [^{3}H]vitellogenin remained with the yolk proteins, which do not undergo turnover in the oocyte.

In order to study the compartmentation of proteins nonspecifically incorporated by oocytes, we found it necessary to use molecules labeled to a high specific activity. To achieve the required labeling we used the chloramine-T method for both bovine serum albumin (BSA) and a mixture of *X. laevis* serum proteins lacking albumin (XSP). As a control procedure we iodinated vitellogenin and compared its uptake and subsequent degradation with that obtained for [^{3}H]vitellogenin labeled *in vivo*. We found that the apparent rate of sequestered protein degradation was dependent upon the method used to radiolabel the molecule. This study documents these findings.

MATERIALS AND METHODS

X. laevis females were obtained from the South African Snake Farm (Fish Hoek, Cape Province, South Africa). The care and maintenance of the animals and the injection of hormones and isotopes were as described (11). BSA and other reagents were purchased from Sigma. XSP were obtained by the passage of normal male serum over a column of Affi-Gel Blue (Bio-Rad) to remove the albumin (12). The resulting protein mixture was dialyzed against distilled water and lyophilized. Vitellogenin was labeled *in vivo* with [^{3}H]leucine (Amersham) as described (7); the labeled protein was isolated from the serum by the method of Wiley *et al.* (13).

Labeling Procedures. Proteins were iodinated by a modification of the chloramine-T method (6, 14). One milligram of protein was treated with 1 mCi of $Na^{125}I$ (Amersham; 100 mCi/ml, carrier-free; 1 Ci = 3.7×10^{10} becquerels), resulting in a molar ratio of iodine:protein of 1:27 for BSA and 1:4 for vitellogenin. The final concentration of chloramine-T was 390 μg/ml, yielding a molar ratio of chloramine-T:protein of 3.7:1 for BSA and 25:1 for vitellogenin. The reaction (total volume, 47.5 μl) was carried out in a small vial for 1 min at 0°C with constant stirring and terminated by the addition of sodium metabisulfite and potassium iodide. The labeled protein(s) was isolated by passage over a small column (1 × 10 cm) of Sephadex G-25 (Pharmacia) saturated with unlabeled BSA and equilibrated with saline solution O-R2 (15). After the protein was dialyzed against several changes of solution O-R2, it was

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Abbreviations: BSA, bovine serum albumin; XSP, *X. laevis* serum proteins lacking albumin; Cl_3AcOH, trichloroacetic acid.

immediately used. (With the exception of [^{3}H]vitellogenin labeled *in vivo*, no labeled proteins were stored prior to use.) Specific activities obtained were 0.52 μCi/μg for BSA, 0.22 μCi/μg for XSP, and 0.006–0.06 μCi/μg for vitellogenin.

Proteins were labeled by reductive methylation with potassium [^{3}H]borohydride (Amersham; >3 Ci/mmol) by the procedure of Ascoli and Puett (16). Specific activities obtained were 0.82 μCi/μg for BSA, 0.36 μCi/μg for XSP, and 0.57 μCi/μg for vitellogenin.

Protein concentrations were determined by the method of Bramhall *et al.* (17) for BSA and XSP and by absorptivity for vitellogenin (18). Labeled proteins were examined by NaDodSO$_4$ gel electrophoresis (19) or by Ferguson plot analysis (20) of native polyacrylamide gels by the system of Ornstein (21) and Davis (22). In all cases, radioactive protein was >96% acid-precipitable. ^{125}I-Labeled BSA migrated as a single band on NaDodSO$_4$ gel electrophoresis, with mobility identical to that of unlabeled BSA. Ferguson plot analysis of ^{3}H-labeled BSA and ^{125}I-labeled vitellogenin revealed no change in retardation coefficient when compared to the authentic proteins. However, the ^{3}H-labeled BSA demonstrated a very slight increase in free electrophoretic mobility at pH 9.6. No such change in free electrophoretic mobility or aggregation was seen with ^{125}I-labeled vitellogenin.

Protein Incorporation by Oocytes. Ovaries were removed from animals, which were injected with 1000 International Units of human chorionic gonadotropin at least 24 hr prior to laparotomy. Under sterile conditions, oocytes (0.96 ± 0.04 mm in diameter) were dissected from their follicles into solution O-R2 and maintained at 20°C in sterile 50% Liebovitz L-15 medium (GIBCO) containing 1 mM glutamine, 15 mM Hepes/NaOH buffer, 50 μg of gentamycin per ml, and 50 International Units of nystatin per ml (23). Oocytes were then incubated in solution O-R2 containing the labeled protein and 50 μg of gentamycin per ml. All cultures, except those containing labeled vitellogenin, also contained 3–5 mg of unlabeled vitellogenin per ml. At appropriate time intervals, oocytes were removed from culture, rinsed in three changes of O-R2, and processed for determination of radioactivity.

Several different procedures were tried in the processing of oocytes exposed to ^{125}I-labeled proteins. These included disruption of individual oocytes by mixing on a Vortex followed by the addition of 10% trichloroacetic acid (Cl_3AcOH). The resulting supernatants and precipitates were separated by centrifugation and radioactivity was measured in a γ counter. A second procedure was dissolution of individual oocytes in 2% NaDodSO$_4$/10 mM dithioerythritol, pH 8, by boiling followed by application of the samples to Whatman 3 MM discs (24). Total radioactivity of the discs was measured; they were then processed to remove Cl_3AcOH-soluble radioactivity (24, 25). The remaining radioactivity then was measured. The third procedure was placement of intact oocytes into 10% Cl_3AcOH followed by washing with alcohol/ether, 3:1 (vol/vol), and ether. Radioactivity in aliquots of the Cl_3AcOH and in the processed oocytes was then determined. The fourth procedure was placement of intact oocytes into 100% acetone. Radioactivity in both oocytes and acetone was then determined. In all cases, essentially identical results were obtained and the second procedure was routinely used. For all of the experiments using ^{3}H-labeled proteins and [^{3}H]vitellogenin, groups of 10 washed oocytes were placed into 0.5 ml of cold 10% Cl_3AcOH overnight to remove acid-soluble radioactivity. Duplicate 50-μl samples of Cl_3AcOH were removed for measurement of radioactivity; the oocytes were washed successively with two changes each of 10% Cl_3AcOH, alcohol/ether (3:1), and ether and then air dried. Individual oocytes were placed in scintillation vials and dissolved with 0.5 ml of Protosol (New England Nuclear) for 2 hr at 50°C. After digestion and cooling, 10 ml of 0.6% 2,5-diphenyloxazole (New England Nuclear) in toluene was added to each vial; radioactivity in the vials was measured in a Beckman scintillation spectrometer.

Characterization of Oocyte-Incorporated ^{125}I-Labeled BSA. The degradation products of internalized ^{125}I-labeled BSA were evaluated by thin-layer chromatography with polyamide thin-layer strips (Baker) and an *n*-butanol/acetic acid/water, 4:1:1 (vol/vol), solvent system as described (26). Oocytes were incubated in ^{125}I-labeled BSA for 12 hr, washed three times, and placed in ice-cold acetone (100 μl/oocyte). After 12 hr the acetone was removed and evaporated with N_2, and the residue was dissolved in acetone/acetic acid, 3:2 (vol/vol), and spotted onto the thin-layer strips. [^{125}I]Monoiodotyrosine and [^{125}I]-diiodotyrosine standards were synthesized with Na^{125}I, L-tyrosine, and chloramine-T. After development, the strips were dried and cut into fractions; radioactivity was measured in a γ counter.

RESULTS

Oocytes were incubated for 1–24 hr in ^{125}I-labeled BSA, and the total and Cl_3AcOH-precipitable radioactivity incorporated was determined (Fig. 1*A*). As can be seen, >90% of the label present in the oocytes was acid soluble, implying extensive degradation of the incorporated BSA. Although the amount of Cl_3AcOH-precipitable radioactivity slowly increased with time, the total radioactivity incorporated leveled off during the final 12 hr of incubation, indicating a steady-state condition in which the released label is lost from the oocyte at the same rate that ^{125}I-labeled BSA is incorporated. [In contrast to what has been reported for smaller, mammalian cells (27), *Xenopus* oocytes

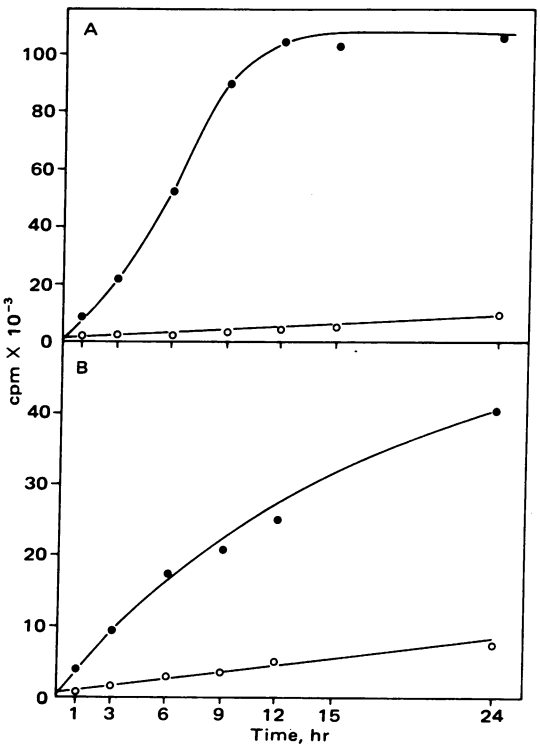

FIG. 1. Incorporation and degradation of ^{125}I-labeled proteins by *Xenopus* oocytes. Oocytes were incubated with the labeled proteins (0.7–1 mg/ml) for the indicated times. Then oocytes were evaluated for the presence of total (●) and Cl_3AcOH-precipitable (○) radioactivity. Each point is the mean of 10 oocytes. (*A*) Data obtained with ^{125}I-labeled BSA (0.52 μCi/μg); (*B*) data obtained with XSP (0.22 μCi/μg).

lose relatively small amounts (≈5%/hr) of acid-soluble radioactivity back to the surrounding medium.] Passage of dissolved oocytes over a column of Bio-Gel P-10 (Fig. 2) revealed the partial breakdown products of the BSA and the presence of ≈90% of low molecular weight material that is substantially retarded by the column. Thin-layer chromatography indicated that 72% of the acetone-soluble (Cl_3AcOH-soluble) compounds remain at the origin (as does free iodine) whereas 26% comigrate with iodotyrosine (Fig. 3). Results similar to these have been reported by Williams *et al.* (27) for rat yolk sac cells exposed to ^{125}I-labeled BSA (labeled by a modification of the chloramine-T procedure).

To determine whether other serum proteins were apparently degraded to the same extent as ^{125}I-labeled BSA, we labeled XSP (see *Materials and Methods*) with ^{125}I. Oocytes incubated in this labeled protein mixture were evaluated for total and Cl_3AcOH-insoluble radioactivity (Fig. 1*B*). As for ^{125}I-labeled BSA, most of the incorporated label (>75%) became Cl_3AcOH-soluble.

The results of these two experiments do not agree with the findings of Wallace and Hollinger (24), who found that iodinated BSA, labeled at lysine groups by the Bolton–Hunter reagent, accumulated in oocytes 6 times faster than its rate of degradation. The dichotomy between our results and those of Wallace and Hollinger could best be explained if the observed rates of degradation were dependent upon the protein-labeling procedure. Therefore, BSA and XSP were labeled with ^3H by reductive methylation (16), and the experiments were repeated with the ^3H-labeled proteins. As seen in Fig. 4, the extent of apparent degradation of the ^3H-labeled proteins was much less than that observed for the corresponding ^{125}I-labeled protein (30% compared to 95% for BSA and 15% compared to 75% for XSP).

To ensure that the observed disparity between the results obtained for ^3H- and ^{125}I-labeled proteins was not due to reincorporation of the ^3H-labeled ligand into oocyte proteins, we examined the relative amounts of labeled BSA deposited into the oocyte yolk platelets. The yolk platelet compartment was chosen for several reasons. First, our studies on the compartmentation of oocyte-incorporated substances (e.g., BSA, vitellogenin, and DNA) have shown that a large proportion of these diverse materials are deposited into the yolk platelets (for DNA, see ref. 29). Second, previous work (10, 30, 31) has shown that the yolk is almost exclusively derived from exogenously synthesized material. In addition, we have found that, of the acid-insoluble radioactivity present in oocytes incubated for 1.5 hr in [^3H]leucine, <0.4% is found in the yolk platelets. Thus,

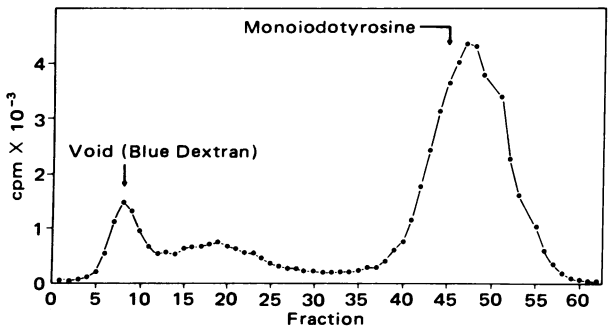

FIG. 2. Elution profile of internalized ^{125}I-labeled BSA. Ten oocytes were incubated for 3 hr in labeled protein, washed, and dissolved in 1.0 ml of 5% $NaDodSO_4$/10 mM dithioerythritol. The entire sample was applied to a 50 × 1 cm column of Bio-Gel P-10 and eluted with 10 mM Tris buffer, pH 7.5/0.5% $NaDodSO_4$/5 mM EDTA/1 mM dithioerythritol (28). One-milliliter fractions were collected; radioactivity was measured in a γ counter.

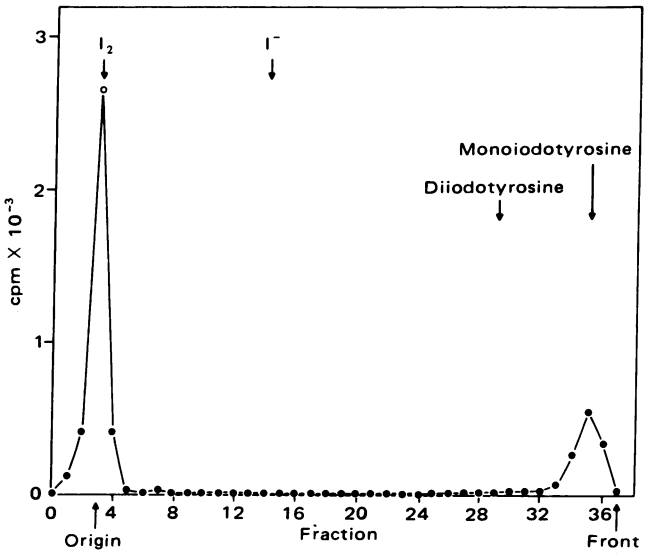

FIG. 3. Thin-layer chromatography of acetone-soluble material from *X. laevis* oocytes exposed to ^{125}I-labeled BSA for 12 hr. The sample was evaporated with nitrogen and the residue was dissolved in acetone/acetic acid, 3:2, and spotted onto polyamide strips. After development in *n*-butanol/acetic acid/water, 4:1:1, the strips were cut into 2-mm fragments and radioactivity was measured in a γ counter.

any Cl_3AcOH-insoluble label that appears in the yolk platelets of oocytes exposed to radioactive proteins must arise from the direct deposition of the material into the yolk and not via incorporation of the label into oocyte-synthesized proteins. Accordingly, oocytes were incubated in ^{125}I- or ^3H-labeled BSA for 2 hr prior to fractionation on sucrose gradients (31) and the proportion and distribution of acid-precipitable radioactivity were determined. The results in Table 1 show that there is proportionally 7 times more Cl_3AcOH-insoluble labeled material in the yolk platelets of oocytes exposed to ^3H-labeled BSA than in oocytes exposed to ^{125}I-labeled BSA.

It was not possible in either of these experiments to determine

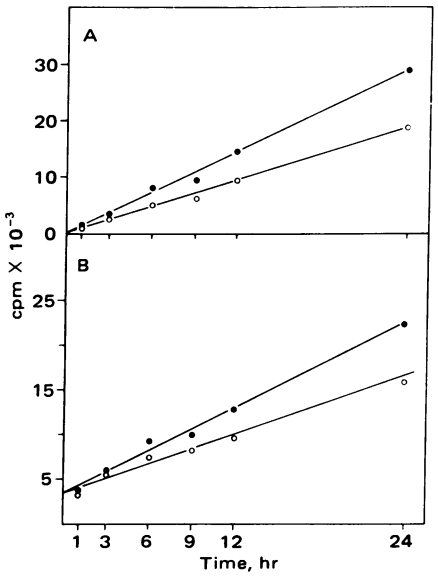

FIG. 4. Incorporation and degradation of [^3H]methylated proteins by *Xenopus* oocytes. Oocytes were incubated with either labeled BSA (0.82 μCi/μg) (*A*) or XSP (0.36 μCi/μg) (*B*) for the indicated lengths of time and then evaluated for total (●) and Cl_3AcOH-precipitable (○) radioactivity. Each point is the average of 10 oocytes; protein concentrations, 0.5–1 mg/ml.

Table 1. Relative amounts of acid-precipitable, labeled material present in yolk platelets of oocytes exposed to either ^{125}I-labeled BSA or ^{3}H-labeled BSA for 2 hr

BSA	Internalized, Cl_3AcOH-precipitable label, % of total	% total label Cl_3AcOH-precipitable in yolk
^{125}I-labeled	5	4
^{3}H-labeled	60	29

The intracellular distribution of acid-precipitable label was determined by sucrose gradient fractionation by a modification of the gradient system of Jared *et al.* (31) and Opresko *et al.* (29). The original 4.5-ml 20–60% linear gradient was expanded to 11 ml and contained 19–50% sucrose plus a 1-ml cushion of 60% sucrose (other components of all sucrose solutions remain as described by Jared *et al.*). Thirty oocytes, homogenized in 1 ml of 15% sucrose, were placed on each gradient (in these experiments each oocyte incorporated a minimum of 1000 cpm) immediately prior to centrifugation at 40,000 rpm in an SW 40 rotor (202,000 × g_{av}) for 12 hr. The gradient was fractionated on an ISCO model 185 density gradient fractionator and 180-μl fractions were spotted onto Whatman 3 MM 2.3-cm filter paper discs. The discs were processed as described (31). The yolk platelet region of the gradient was determined both by radiotracer studies with oocyte-incorporated [^{32}P]vitellogenin and by electron microscopic examination. The data were corrected for 80% recovery of yolk-associated protein, as determined by [^{32}P]vitellogenin incorporation.

whether the apparent rates of degradation observed were equivalent to those of unmodified molecules because the high specific activities required for these studies precluded the use of proteins labeled *in vivo*. However, the specifically incorporated yolk precursor protein vitellogenin can be labeled *in vivo* to a sufficient specific activity. In addition, the extent of degradation of the micropinocytotically incorporated vitellogenin (<4%) has previously been determined (10). Therefore, we labeled vitellogenin with ^{125}I by the chloramine-T procedure and compared its uptake and degradation to vitellogenin labeled *in vivo* with [^{3}H]leucine (Fig. 5). The uptake of ^{125}I-labeled vitellogenin was essentially identical to that obtained for [^{3}H]vitellogenin, demonstrating that the specific binding properties of the iodinated vitellogenin were unaltered. However, ^{125}I-labeled vitellogenin appeared to be extensively degraded (≈60%; Fig. 5*A*) after entrance into the oocyte whereas [^{3}H]vitellogenin was stable (≈4% degraded; Fig. 5*B*).

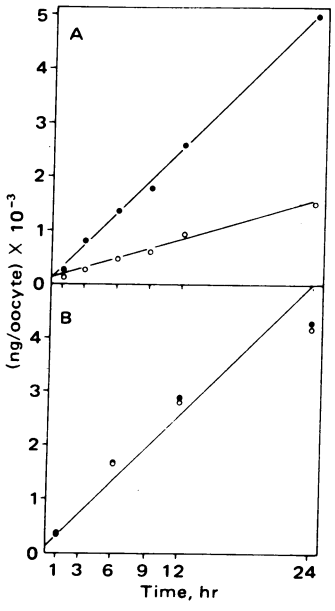

FIG. 5. Incorporation and degradation of labeled vitellogenin by *Xenopus* oocytes. Oocytes were incubated with either ^{125}I-labeled vitellogenin (1 mg/ml) (*A*) or [^{3}H]vitellogenin labeled *in vivo* (2.5 mg/ml) (*B*) for the indicated lengths of time and then evaluated for the presence of total (●) and Cl_3AcOH-precipitable (○) radioactivity. Each point is the average of 10 oocytes. The radioactivity measurements have been converted into ng of vitellogenin in order to allow comparison between the two experiments, and uptake of ^{125}I-labeled vitellogenin was corrected to a concentration of 2.5 mg/ml (9).

Table 2. Comparison of uptake and degradation of ^{125}I-labeled [^{3}H]vitellogenin, I-labeled [^{3}H]vitellogenin, and [^{3}H]vitellogenin by *X. laevis* oocytes after a 1-hr incubation

Vitellogenin	Isotope measured	Uptake, ng/oocyte	Cl_3AcOH-soluble radioactivity, % of total
^{3}H- and ^{125}I-labeled	^{125}I	110.0	34
^{3}H- and I-labeled	^{3}H	102.2	1
^{3}H-labeled	^{3}H	96.6	3

It was possible that the observed degradation of ^{125}I-labeled vitellogenin was due to molecular alterations caused by exposure to chloramine-T and not the presence of the radioactive ligand. To distinguish between these two possibilities, we divided a sample of [^{3}H]vitellogenin into three parts and treated them as follows. One aliquot was iodinated with chloramine-T such that <1% of the total molecules was labeled with ^{125}I. A second aliquot was iodinated by the same procedure but KI was substituted for Na^{125}I. The third aliquot remained untreated. Oocytes were then exposed to the three types of [^{3}H]vitellogenin, and the incorporation and degradation of the three were compared (Table 2). As previously observed, whereas iodination did not alter the rate of [^{3}H]vitellogenin incorporation, the ^{125}I-labeled [^{3}H]vitellogenin demonstrated an apparent high degree of degradation. However, the loss of acid-precipitable radioactivity reported for this vitellogenin preparation is based solely on the examination of ^{125}I. It was not possible to follow both the ^{3}H and ^{125}I labels contained in this sample because the respective specific activities were similar and both isotopes have similar energy spectra in a scintillation counter. In contrast to the 34% degradation observed for ^{125}I-labeled [^{3}H]vitellogenin, <4% of the vitellogenin that was treated with KI and chloramine-T was degraded. We therefore conclude that the labeling conditions themselves are not detrimental to the vitellogenin molecule.

DISCUSSION

Radioiodinated proteins have been used extensively in hormone–receptor binding studies (32) because of the high specific activities obtainable. Labeling by the original chloramine-T procedure (6) or some modification thereof (33) is most often used because of the simplicity and efficiency of the procedures. To ensure that the labeled hormone remains functionally unaffected by either the labeling conditions or the presence of iodinated residues, the iodinated samples are examined for the retention of binding specificity, biological activity, and immunoreactivity. Nonmodification of these variables has been regarded as evidence for the suitability of the iodinated protein for use in biological or biochemical studies. Recently, iodinated proteins (particularly hormones) have also been used for the investigation of the proteolytic breakdown of compounds entering cells by micropinocytosis. However, for hormones and nonenzymatic proteins, it remains to be demonstrated that there is a relationship between the retention of normal biological activity and proteolytic susceptibility.

An assumption implicit in studies of the degradation of incorporated proteins is that rates of degradation are independent of the method used to label the molecule. We have found, at least for *X. laevis* oocytes, that this assumption is not valid. In all three proteins tested here (BSA, XSP, and vitellogenin), proteins iodinated by the chloramine-T method are apparently degraded at rates far exceeding those observed for proteins labeled *in vivo* or by reductive methylation. For BSA and XSP, it is not known which set of results reflects the "true" situation

in vivo because measurements of degradation are dependent upon the release of a radioactive ligand placed on the molecule by *in vitro* procedures. However, because we are able to use vitellogenin labeled *in vivo* in degradation studies, we can conclude that vitellogenin labeled by the chloramine-T procedure is degraded at an artificially high rate. Moreover, although ^{125}I-labeled vitellogenin is degraded at least 10 times faster than [^{3}H]vitellogenin, its rate of uptake is identical to that of [^{3}H]vitellogenin. In a separate experiment (data not shown) we have found that vitellogenin labeled by reductive methylation is degraded to a much lesser extent than ^{125}I-labeled vitellogenin (15% compared to 60% after a 3-hr incubation) even though the specific binding capability of the vitellogenin was completely abolished by this procedure. Therefore, for vitellogenin there is no relationship between retention of biological binding activity and susceptibility of the protein to degradation. It is possible that a similar situation may also exist for other specifically incorporated proteins.

The mechanism by which proteins labeled by the chloramine-T method are preferentially degraded is not known. However, our preliminary evidence indicates that it may involve recognition of a specific modification of the protein and not the oxidizing conditions to which the proteins are exposed. In one of our experiments (Table 2), <1% of the total vitellogenin population contained a substituted tyrosine residue. When we followed the fate of this labeled subpopulation, we found it was degraded at a rate 10 times greater than that for the remainder of the population. However, we have no conclusive evidence as to whether this subpopulation of molecules was preferentially iodinated due to their possible abnormal configuration or preferentially degraded due to the presence of iodotyrosine. Evidence supporting the latter supposition comes from the work of Wallace and Hollinger (24), in which they found that ^{125}I-labeled BSA labeled by the Bolton–Hunter reagent (which places the radioactive ligand onto lysine residues) is not immediately degraded by oocytes. The marked divergence between the fates of these incorporated ^{125}I-labeled molecules implies that a specific biological recognition system for iodotyrosine may exist in the cell.

Alternately, the liberation of Cl_3AcOH-soluble radioactivity from iodinated proteins could represent a deiodination of the conjugated tyrosine rather than the total degradation of the protein molecule. For oocyte-internalized ^{125}I-labeled BSA, column chromatography revealed the presence of some intermediate molecular weight material (Fig. 2); however, the large majority of label resided in low molecular weight compounds, of which 72% appear to be free iodine. Similar results have been reported for rat hepatocytes exposed to ^{125}I-labeled insulin (4, 5) and for human fibroblasts incubated in ^{125}I-labeled low-density lipoproteins (34). Almost all of the insulin degradation products coeluted from columns with Na^{125}I whereas 35% of that from the labeled low-density lipoproteins was free iodide. More recently, Willinger *et al.* (35) observed that rabbit polymorphonuclear leukocytes are able to transfer ^{125}I from one internalized protein to another. In all of these cases the proteins were labeled on the tyrosine residues, and the presence of free iodide or its transfer may result from either deiodination of iodotyrosine liberated from a degraded molecule or from direct deiodination of tyrosine from an intact molecule. A given cell type may be capable of one or both of these mechanisms, and either one has been equated with degradation.

In conclusion, we have found that proteins labeled with ^{125}I by the chloramine-T method are apparently degraded at an unusually high rate by growing oocytes, cells that are specialized for micropinocytotic uptake (30). Although our results do not prove that a similar situation exists with respect to other cell types, they do show the necessity of using extensive controls in these types of experiments. Unless the degradation of incorporated proteins can be shown to be independent of the method used to label the proteins, any reported rates of degradation are speculative at best.

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