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Inulin-\(^{125}\)I-Tyramine, an Improved Residualizing Label for Studies on Sites of Catabolism of Circulating Proteins

Janet L. Maxwell†, John W. Baynes‡, and Suzanne R. Thorpe¶

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Residualizing labels for protein, such as dilactitol-\(^{125}\)I-tyramine (\(^{125}\)I-DLT) and cellobiitol-\(^{125}\)I-tyramine, have been used to identify the tissue and cellular sites of catabolism of long-lived plasma proteins, such as albumin, immunoglobulins, and lipoproteins. The radioactive degradation products formed from labeled proteins are relatively large, hydrophilic, resistant to lysosomal hydrolases, and accumulate in lysosomes in the cells involved in degradation of the carrier protein. However, the gradual loss of the catabolites from cells (\(t_\alpha\sim 2\) days) has limited the usefulness of residualizing labels in studies on longer lived proteins. We describe here a higher molecular weight (\(M_\text{r} \sim 5000\)), more efficient residualizing glycoconjugate label, inulin-\(^{125}\)I-tyramine (\(^{125}\)I-InTn). Attachment of \(^{125}\)I-InTn had no effect on the plasma half-life or tissue sites of catabolism of asialofetuin, fetuin, or rat serum albumin in the rat. The half-life for hepatic retention of degradation products from \(^{125}\)I-InTn-labeled asialofetuin was 5 days, compared to 2.3 days for \(^{125}\)I-DLT-labeled asialofetuin. The whole body half-lives for radioactivity from \(^{125}\)I-InTn-, \(^{125}\)I-DLT-, and \(^{125}\)I-labeled rat serum albumin were 7.5, 4.3, and 2.2 days, respectively. The tissue distribution of degradation products from \(^{125}\)I-InTn-labeled proteins agreed with results of previous studies using \(^{125}\)I-DLT, except that a greater fraction of total degradation products was recovered in tissues. Kinetic analyses indicated that the average half-life for retention of \(^{125}\)I-InTn degradation products in tissues is \(\sim 5\) days and suggested that in vivo there are both slow and rapid routes for release of degradation products from cells. Overall, these experiments indicate that \(^{125}\)I-InTn should provide greater sensitivity and more accurate quantitative information on the sites of catabolism of long-lived circulating proteins in vivo.

Residualizing labels are biologically inert radioactive tags used for studies on the sites of protein catabolism in vivo. These labels are designed to yield limit, hydrophilic degradation products of a sufficient size that they are retained in lysosomes following catabolism of the carrier protein. The sites of degradation of the labeled protein may then be determined either by measuring acid-soluble radioactivity in various tissues and cells or by autoradiography. Residualizing labels, such as \(^{3}H\)raffinose (2), dilactitol-\(^{125}\)I-tyramine (\(^{125}\)I-DLT)\(^{1}\), and cellobiitol-\(^{125}\)I-tyramine (4), have been used to identify the tissue and cellular sites of catabolism of plasma proteins, such as albumin (5-7), lipoproteins (4, 8), and immunoglobulins (9), and are also being increasingly used in studies on the uptake and catabolism of proteins by cells in culture (8, 10). One of the limitations of the use of these labels, however, is that whereas their rate of loss from cells is slow (\(t_\alpha \sim 2\) days for \(^{125}\)I-DLT in rat tissues), the rates of catabolism of plasma proteins are often equally slow or slower. Thus, a substantial fraction of degradation products is lost from tissues by the time significant amounts of the protein have been catabolized. Under these circumstances, it is not possible to assess rigorously the quantitative role of various tissues in catabolism of a protein since the distribution of degradation products in the body could be biased by differences in the rate of loss of the label from various cell types.

Because of the limited residualization of tetrasaccharide derivatives of tyramine and the fact that residualization is improved with increasing saccharide content or molecular weight of the label (3), we set out to design a higher molecular weight oligosaccharide derivative of tyramine, with the expectation that this label would be retained more efficiently in cells. We describe here the biological properties of inulin-\(^{125}\)I-tyramine (\(^{125}\)I-InTn), a residualizing glycoconjugate label derived from the inert fructan polymer inulin (\(M_\text{r} \sim 5000\)). The retention of protein degradation products containing the \(^{125}\)I-InTn label results from both their size and the absence of lysosomal fructofuranosidase activity (11). As shown below, \(^{125}\)I-InTn, the largest residualizing label for protein described thus far, has negligible effects on the kinetics or tissue sites of plasma protein catabolism and is retained in lysosomes more efficiently than is \(^{125}\)I-DLT. The data indicate that \(^{125}\)I-InTn should be widely applicable in studies on the catabolism of long-lived circulating proteins.

EXPERIMENTAL PROCEDURES AND RESULTS\(^{2}\)

The chemistry of synthesis and coupling of \(^{125}\)I-InTn to protein is outlined in Fig. 1 and described in detail under "Experimental Procedures." Also described in the Miniprint are a series of preliminary experiments validating the usefulness of InTn in studies on catabolism of circulating proteins such as asialofetuin and fetuin. The effectiveness of InTn as a residualizing label is clearly illustrated in Fig. 5, which shows both the plasma and whole body kinetics of clearance of \(^{125}\)I-InTn, \(^{125}\)I-DLT, and \(^{125}\)I-InTn-labeled RSA. Notably, as

\[^{1}\] The abbreviations used are: \(^{125}\)I-DLT, dilactitol-\(^{125}\)I-tyramine; \(^{125}\)I-InTn, inulin-\(^{125}\)I-tyramine; RSA, rat serum albumin; "I, \(^{125}\)I.

\[^{2}\] Portions of this paper including "Experimental Procedures," part of "Results," Figs. 2-4 and 6, and Table I-III, are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
Residualizing Labels: Inulin-\(^{125}\)I-Tyramine

**Fig. 1. Reaction scheme for synthesis of \(^{125}\)I-InTn and its coupling to protein.** Inulin is reduced with NaBH\(_4\), and the terminal alditol is oxidized with a limiting amount of periodate to generate an aldehyde. Tyramine is coupled to inulin aldehyde by reductive amination using NaBH\(_3\)CN, yielding InTn. InTn is then labeled with radioactive iodine using IODO-GEN and coupled to protein using cyanuric chloride.

with asialofetuin and fetuin, the plasma half-life of RSA was unaffected by the attachment of InTn. The \(^{125}\)I-InTn degradation products also residualized more efficiently, with a whole body half-life of about 7.5 days, compared to 4–5 days for \(^{125}\)I-DLT (Fig. 5 and Ref. 7). The tissue distribution of radioactivity at 4 days after injection of \(^{125}\)I-InTn-labeled RSA, shown in Table II, confirms previous evidence using \(^{125}\)I-DLT-labeled RSA that catabolism of RSA takes place primarily in muscle and skin (7). However, in these experiments, a significantly greater fraction of total degradation products was retained in the body. Thus, the circulating half-life of this preparation of \(^{125}\)I-InTn-labeled RSA was 1.8 days, i.e. 79% catabolism at 4 days; the loss of 27% of radioactivity from the body by 4 days indicates that about 66% (27:79) of the theoretical yield of \(^{125}\)I-InTn-labeled RSA degradation products was recovered, compared to 45% for \(^{125}\)I-DLT-labeled RSA (7). Overall, with all three proteins studied, the \(^{125}\)I-InTn label yielded results consistent with previous studies using other labels, but with substantially improved retention of degradation products in the body.

**Kinetic Modeling of Plasma Protein Catabolism**—As a first step toward understanding the biological behavior of residualizing labels, we have attempted to develop kinetic models for quantitative comparison of the rates of loss of the various labels from tissues. For this purpose, we have used SCoP and SCoPfit programs, which are simulation control and optimization programs developed for the IBM-PC/AT computer by the National Biomedical Simulation Resource at Duke University (Durham, NC). SCoP generates a graphical simulation of a kinetic process, given a series of differential equations (the kinetic model) and specified kinetic constants. The SCoPfit program accepts actual experimental data and, using the SCoP model, develops kinetic constants to optimize the fit of experimental data to the model. For the purpose of illustrating the results of calculations, experimental data from Fig. 5 (lower) are replotted in Fig. 7, showing the kinetics of whole body release of degradation products from \(^{125}\)I-DLT-labeled RSA (Fig. 7, upper) and \(^{125}\)I-InTn-labeled RSA (Fig. 7, lower). The various lines drawn on the graph represent different fits to the data using the SCoP or SCoPfit program with various models and assumptions, as described in detail in the Miniprint. In summary, the dotted lines are derived from the SCoP program using the three-compartment model described in Fig. 6 and assuming that RSA degradation products leak from all tissues at the same rate at which asialofetuin degradation products leak from liver. Because of the poor fit to the experimental data, SCoPfit was used with the same model to optimize the kinetic constants and to improve the fit to the data. However, this SCoPfit optimization (dashed lines) was also unsatisfactory, and systematic error was apparent, suggesting that the model was inappropriate. Since recent work by Buktenica et al. (30) indicated that degradation products were routed through both slow and fast release compartments in cells in vitro, the three-compartment model was revised to allow for a fraction of degradation products to be released rapidly from cells in vivo. The solid lines in Fig. 7 (upper and lower) are the results of SCoPfit optimizations to this revised model and yield good and consistent fits to the experimental data. The development, mathematical description and assumptions, and the kinetic constants obtained with the various models are described in detail in the Miniprint.

**DISCUSSION**

The need for residualizing labels which are more completely retained in tissues has been apparent since the earliest experiments using this technology to identify the sites of plasma
Residualizing Labels: Inulin-\textsuperscript{125}I-Tyramine

protein catabolism. Because of the gradual loss of degradation products from tissues, it has been necessary to terminate experiments at times when only a fraction of the protein has been catabolized and then to apply corrections for intact protein remaining in tissues, for example, by acid precipitation of the intact protein (3, 7) or by injection of a second, nonresidualizing tracer to estimate the amount of intact protein in the tissue (31). These manipulations are not only inconvenient, but they also ultimately affect the precision of estimates of protein degradation in tissues. Our previous work had revealed a relationship between the number of carbohydrate units in the label and its efficiency of residualization (3), so that the synthesis of a larger glycoconjugate label seemed a reasonable route for improving residualization.

There are obvious limits to this approach, however, since at some point the size or properties of the label itself will affect the mechanisms and sites of catabolism of the carrier protein. An inulin derivative was chosen as a reasonably sized target since the resulting molecular weight of the label would be, at most, about 10% of the mass of the smallest plasma protein. The synthesis of InTn was straightforward, and its iodination and coupling to protein proceeded with good efficiency, 30 and 70%, respectively. Thus, only nanomolar quantities are required to label proteins with high specific radioactivity. The inertness of underivatized inulin in the coupling reaction is also convenient because this inulin serves as a carrier to decrease losses during handling, does not appear to interfere with labeling of the protein, and is readily separated from labeled protein by gel exclusion chromatography. In all of the experiments described here, the average substitution of protein was limited to <1 mol of \textsuperscript{125}I-InTn/mol of protein in order to decrease the probability of multiple derivatization of carrier proteins. The addition of 1 mol of \textsuperscript{125}I-InTn/mol of protein had no detectable effect on the kinetics, mechanisms, or sites of catabolism of asialofetuin, fetuin, or RSA. This result is consistent with recent hypotheses on the regulation of protein catabolism. Thus, the kinetics of protein catabolism appear to be determined by genetically encoded molecular features of the protein molecule, such as the amino-terminal amino acid or a sequence or array of amino acids in the primary or tertiary structure of the protein, rather than by bulk physical characteristics such as hydrophobicity, subunit molecular weight, or isoelectric point (32).

The size of the radioactive products isolated from urine using the \textsuperscript{[3H]}raffinose (2), \textsuperscript{123}I-DLT (3), or \textsuperscript{125}I-InTn labels indicates that residualizing labels are excreted from the body largely in intact form. Thus, following catabolism of the carrier protein, the labeled degradation products appear to be released from cells by the process of exocytosis or regurgitation, rather than by deiodination or eventual hydrolysis to lower molecular weight products. The difference in whole body half-life of radioactivity from asialofetuin labeled with raffinose, DLT, InTn, and other labels (3) indicates that the structure of the label affects its rate of release from cells. In addition, however, kinetic analysis indicates that there are also differences in the routes of transport of these indigestible...
compounds within the cell. Thus, whereas some residualiza-
tion of these labels rapidly released from the body was not observed (Figs. 4 and 5, lower). Based on kinetic analysis, we have concluded that degradation products may be partitioned between slow and fast release compartments within the cell and that routing of the partially degraded protein or labeled degradation products to the fast release compartment may be an important factor limiting the long-term retention of catabo-
ism in the body. Our model makes no statement regarding the nature of the fast release compartment, although it is likely to be an early endocytic compartment, either prelysoso-
mal or in equilibrium with the lysosomal compartment. Whereas larger residualizing labels could theoretically prove more efficient, there is greater risk that they will affect the catabolism of the carrier protein. For most purposes, the InTn label should be suitable, for example, in studies on the catab-
olism of proteins.

REFERENCES

1. Maxwell, J. L., Baynes, J. W., and Thorpe, S. R. (1986) Fed. Proc. 45, 1540
2. Van Zile, J., Henderson, L. A., Baynes, J. W., and Thorpe, S. R. (1979) J. Biol. Chem. 254, 3547-3553
3. Strobel, J. L., Baynes, J. W., and Thorpe, S. R. (1985) Arch. Biochem. Biophys. 240, 656-663
4. Pittman, R. C., and Taylor, C. A., Jr. (1986) Methods Enzymol. 129, 612-628
5. Baynes, J. W., and Thorpe, S. R. (1981) Arch. Biochem. Biophys. 206, 372-379
6. Yedgar, S., Carew, T. E., Pittman, R. C., Bels, W. F., and Steinberg, D. (1983) Am. J. Physiol. 244, E161-E107
7. Strobel, J. L., Cady, S. G., Borg, T. K., Terracio, L., Baynes, J. W., and Thorpe, S. R. (1986) J. Biol. Chem. 261, 7989-7994
8. Daugherty, A., Thorpe, S. R., Lange, L. G., Sobel, B. E., and Schenfeld, G. (1985) J. Biol. Chem. 260, 14564-14570
9. Henderson, L. A., Baynes, J. W., and Thorpe, S. R. (1982) Arch. Biochem. Biophys. 215, 1-11
10. Maxwell, J. M., Baynes, J. W., and Thorpe, S. R. (1987) in The Pharmacology and Toxicology of Proteins (Wenkelske, J. L., and Hohenberg, J. S., eds.) pp. 58-72, Alan R. Liss, Inc., New York
11. Wattiaux, R. (1977) in Mammalian Cell Membranes (Jamison, G. A., and Robinson D. M., eds.) Vol. 2. pp. 165-184, Butterworth & Co., Ltd., London
12. Dulbecco, R., and Vogt, M. S. (1954) J. Exp. Med. 98, 267-173
13. Laemmli, U. K. (1970) Nature 227, 680-685
14. Aspinall, G. O. (1970) Polysaccharides, pp. 80-83, Pergamon Press, London
15. McFeeters, R. F. (1980) Anal. Biochem. 103, 302-306
16. Yedgar, G. (1969) Carbohydr. Res. 11, 113-118
17. Gregoriadis, G., and Sourkes, T. L. (1967) Can. J. Biochem. 45, 1541-1848
18. Avigdor, G. (1969) Biochemistry 8, 1-415
19. Ashwell, G., and Harford, J. (1983) Annu. Rev. Biochem. 51, 531-554
20. Baynes, J. W., Van Zile, J., Henderson, L. A., and Thorpe, S. R. (1980) Birth Defects Orig. Art. Ser. 16, 103-113
21. Martiashaw, M. H., Baynes, J. W., and Thorpe, S. R. (1983) Arch. Biochem. Biophys. 235, 266-283
22. Carew, T. E., Pittman, R. C., Marchand, E. R., and Steinberg, D. (1984) 1, 214-224
23. Cohn, Z. A., and Ehrenreich, B. A. (1969) J. Exp. Med. 129, 201-225
24. Beutman, J. M., Airdart, J. A., Wodworth, R. C., and Low, R. B. (1981) J. Cell. Biol. 91, 716-727
25. Hoppe, C. A., and Lee, Y. C. (1984) Birth Defects Orig. Art. Ser. 19, 394-399
26. Baynes, J. W., Van Zile, J., and Thorpe, S. R. (1985) J. Biol. Chem. 260, 9476-9476
27. Pittman, R. C., Carew, T. F., Glass, C. K., Green, S. R., Taylor, C. A., and Attie, A. D. (1983) Biochem. J. 212, 701-800
28. Rechstein, M., Rogers, S., and Rote, K. (1987) Trends Biotechnol. Sci. 12, 390-394

Experimental Procedures

Supplementary Material to Inulin-125I-Tyramine, an Improved Residualizing Label for Studies on Sites of Catabolism of Proteins by James L. Maxwell, John W. Baynes and Susan E. Thorpe

Materials: kit, O. tyramine, inulin (diballot beets, k.-32001), bovine serum albumin (BSA), and insulin labeling enzyme purchased from Sigma Chemical Co. Carrier-free 125I-tyramine (14; $100,000 Ci/1.25 M Bq) was prepared by C. A. Hoppe and Y. C. Lee from Soln Research Products. Tyramine iodide (130; Aldrich Chemical Co.) was synthesized from tyramine hydrochloride (2). Tyramine hydrochloride was isolated using the procedure described previously (3). Bovine serum albumin (BSA) was purified from fresh serum by affinity, ion exchange and gel exclusion chromatography and labeled using iodogen (as described previously (7)). Fetal (FET) was obtained from Gibson and a monomer fraction isolated by chromato-
graphy on Sephadex G-25 (Pharmacia, in Dulbecco's (2) phosphate-buffered saline (PBS) 537-542). Y. C. Lee (26) and R. C. Pittman (27) obtained BSA from the bovine serum. The iodination of the amino acid was catalyzed by sodium cyanoborohydride polyethylene glycol disulfite (EDS-250 K) under reducing conditions (15).

Synthesis and Purification of InTn: The scheme in Fig. 1 shows our route to synthesis and iodination of InTn and coupling of -125I InTn to protein. InTn is generally described as a non-reducing polysaccharide of low molecular mass. Methods for isolation and purification of InTn are described by fractionating Ins on Sephadex G-50 and pooling the fractions with high absorbance at 200 nm. First, the procedure of Fein et al. (22) was followed. The periodate-treated InTn was reductively aminated with 200-fold excess of excess solid N7BH4 in 7.75 M formaldehyde, with the mixture let stand for another hour in order to reduce the excess of solid NH4BH4. The pellet was then washed with 0.01 M NaCl and chromatographed on Sephadex G-25 in PBS. The fractions containing InTn were pooled, concentrated and desalted on Sephadex G-25 in PBS. The final InTn preparation contained $0.25 mol tyramine/mol inulin. The procedure for the coupling of InTn to protein is shown in Fig. 2. A 10-fold excess of InTn was added to the reaction mixture and the mixture let stand for another 1 hour in order to reduce the excess of solid NH4BH4. The resulting mixture was then added and reductive amination carried out overnight at room temperature with stirring. The procedure for the coupling of InTn to protein is shown in Fig. 2. A 10-fold excess of InTn was added and reductive amination carried out overnight at room temperature with stirring. Solid NaBH4 (25-fold excess) was then added and reductive amination carried out overnight at room temperature with stirring. Solid NaBH4 (25-fold excess) was then added and reductive amination carried out overnight at room temperature with stirring. Solid NaBH4 (25-fold excess) was then added and reductive amination carried out overnight at room temperature with stirring. Solid NaB

Acknowledgment—We thank Thomas G. Huggins (Department of Chemistry, University of South Carolina) for assistance in using the SCoP and SCoPfit programs.
Residualizing Labels: Inulin-125I-Tyramine

In the various proteins tested, the *I-InTn-protein was prepared from *I-InTn following dialysis and chromatography on Sephacryl S-200 as described previously (19). *I-InTn-ASF was prepared as previously described (21, 22) with one-half mol of *I-InTn-ASF immobilized per mol of Sepharose 4B. The purity of the material was determined by polyacrylamide gel electrophoresis as described previously (23). No protein radioreceptors were detected in these labeling experiments, since >90% of applied radioactivity was recovered in a single protein band on SDS-PAGE.

In Vivo Experiments. Protein preparations were carried out in male and female Sprague-Dawley rats, 100-150 g. Methods of animal care and maintenance are described in Materials and Methods. Radioactivity has been described previously (11). Unless otherwise indicated, all animal groups for clearance curves represent 5 animals and averages for at least 3 animals and errors of bars indicate that the coefficient of variation was 5%. Plasma and whole body half-lives were estimated by linear regression analysis. Total and acid soluble radioactivities in tissues were determined by precipitation with 2N HCl as described previously (11). In control experiments, *I-InTn, *I-InTn-ASF, or *I-InTn-FET was administered to rats of similar age and size as described in Materials and Methods. Total uptake of radioactivity was determined in tissues by precipitation with 2N HCl. Fractions 24-33 (bracketed in figure) were pooled for preparation of InTn.

In order to trace the disposition of *I-InTn radioactivity in liver, rats were subjected to a 20% hepatic resection to reduce the likelihood of radioactivity being returned to the circulation of the liver. Radioactivity was determined in the liver at all times: 1 h, 3 h, 5 h, 7 h, 9 h, and 1 d after injection of labeled proteins. Data points are average values for animals in each experiment. (20) (Bottom): Kinetics of whole body clearance of radioactivity from ASF labeled with *I (1). *I-DLT (4) or *I-InTn (A). For each experiment animals were injected with 2-5 x 10^6 cpm (50-150) labeled protein.

Chemistry of Coupling *I-InTn to Protein. One concern about coupling residualizing labels to proteins is that the modification of the protein may affect the biological properties of the protein. To minimize structural alterations in the protein the coupling reaction is normally designed to yield less than one labile group per molecule in order to decrease the probability that the label will modify the protein. It is also possible that the presence of the label would itself modify the function of the protein. This concern is unlikely because of the much higher pH required for reaction between Cy3 and cyanoacetamide (23), but the increase of radioactivity remaining was also confirmed experimentally. As shown in Table 1, Cy3 coupling of *I-InTn to BSA proceeded with an efficiency of 79%, compared to 11% for *I-InTn or control experiments without Cy3. The results are consistent with our previous work indicating that Cy3 couples Cy3-DT to protein via the tyrosine moiety (9).
Residualizing Labels: Inulin-\(^{125}\)I-Tyramine

Residualizing labels are important for understanding the dynamics of degradation products in tissues. The kinetic behavior of residualizing labels, such as Inulin-\(^{125}\)I-Tyramine, is critical for understanding the distribution and disposition of degradation products. In the context of the model shown in Fig. 1, the behavior of Inulin-\(^{125}\)I-Tyramine is considered in more detail.

### Kinetic Modeling of Inulin-\(^{125}\)I-Tyramine

The model shown in Fig. 1 assumes that inulin is a single compartment model for describing the residualization of Inulin-\(^{125}\)I-Tyramine. In this model, Inulin-\(^{125}\)I-Tyramine is considered as a single pool and assumes that the distribution of the kinetic model is not significant in its residualization behavior.

### Recovery of Radioactivity and Disposition of Degradation Products

The recovery of radioactivity and disposition of degradation products in tissues over 5 days after injection of Inulin-\(^{125}\)I-Tyramine is shown in Table I. The table summarizes the recovery of radioactivity in tissues over time, with the percentage of total radioactivity recovered at different times.

### Kinetic Constants

The kinetic constants, such as those described in Table I, include:

- **k1**: Rate constant for the fast release of degradation products.
- **k2**: Rate constant for the slow release of degradation products.
- **k3**: Rate constant for the third release compartment.

These constants are determined by fitting the model to the experimental data using optimization techniques such as the non-linear least squares method. The model then predicts the residualization behavior of Inulin-\(^{125}\)I-Tyramine in tissues.

### Summary of Kinetic Constants

A summary of kinetic constants determined by optimization procedures is shown in Table II. The table includes:

- **k1**: Rate constant for fast release.
- **k2**: Rate constant for slow release.
- **k3**: Rate constant for third release.

These constants are used to describe the residualization behavior of Inulin-\(^{125}\)I-Tyramine in tissues. The model is validated by comparing the predicted behavior with experimental data and adjusting the model parameters accordingly.

### Overall

Overall, the kinetic constants and model predictions for Inulin-\(^{125}\)I-Tyramine provide valuable insights into the residualization behavior of this label in tissues. The model allows for the prediction of residualization behavior and the determination of kinetic constants that are critical for understanding the dynamics of degradation products in tissues.