Preparation and Properties of a Selenium-containing Catalytic Antibody as Type I Deiodinase Mimic*

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Conversion of thyroxine (T₄) to 3,5,3’-triiodothyronine is an essential first step in controlling thyroid hormone action. Type I deiodinase (DI) can catalyze the conversion to produce the bulk of serum 3,5,3’-triiodothyronine. Acting as a mimic of DI, a selenium-containing catalytic antibody (Se-4C5) prepared by converting the serine residues of monoclonal antibody 4C5 endowed 4C5 with enzymatic activity. To probe the catalytic mechanism of the catalytic antibody, detailed kinetic studies were carried out in this paper. Investigations into the deiodinative reaction revealed the relationship between the initial velocity and substrate concentration. The characteristic parallel Dalziel plots demonstrated that Se-4C5-catalyzed reaction mechanism was ping-pong one, involving at least one covalent enzyme intermediate. The kinetic properties of the catalytic antibody were similar to those of DI, with Kₘ values for T₄ and DTT of approximately 0.8 μM and 1.8 mM, respectively, and a Vₘ value of 270 pmol per mg of protein per min. The activity could be sensitively inhibited by 6-propyl-2-thiouracil (PTU) against GSH. The coupling of selenocysteine with the combining pocket of antibody 4C5 endowed Se-4C5 with enzymatic activity. To probe the catalytic mechanism of the catalytic antibody, detailed kinetic studies were carried out in this paper. Investigations into the deiodinative reaction revealed the relationship between the initial velocity and substrate concentration. The characteristic parallel Dalziel plots demonstrated that Se-4C5-catalyzed reaction mechanism was ping-pong one, involving at least one covalent enzyme intermediate. The kinetic properties of the catalytic antibody were similar to those of DI, with Kₘ values for T₄ and DTT of approximately 0.8 μM and 1.8 mM, respectively, and a Vₘ value of 270 pmol per mg of protein per min. The activity could be sensitively inhibited by 6-propyl-2-thiouracil (PTU) with a Kᵢ value of ~120 μM at 2.0 μM T₄ concentration. The PTU inhibition was progressively alleviated with the increasing concentration of added DTT, revealing that PTU was a competitive inhibitor for DTT.

The thyroid hormone 3,3’,5’-triiodothyronine (T₃) exerts important influence on metabolic-energetic homeostasis, differentiation, general hormonal balance, and mammalian development (1–3). The majority of T₃ in plasma is derived from the peripheral tissues rather than directly from the thyroid. The enzymes responsible for the deiodination of T₄, the iodothyronine deiodinases, are a family of homogenous selenoenzymes. On the basis of their reaction and inhibition kinetics and substrate specificity, iodothyronine deiodinases are classified as three types, i.e. type I (DI), type II, and type III (4). DI is expressed mainly in liver, kidney, and thyroid and is a selenoprotein of relative molecular mass ~27 kDa with a selenocysteine (Sec) in the substrate-binding active site for optimum enzyme activity (5). The enzyme can carry out both 5’- and 5’-deiodination of T₄, to produce active T₃ and inactive 3,3’,5’-triiodothyronine (reverse T₃), and has an apparent Kₘ for T₄ in the micromolar concentration range. The 5’-deiodinative reaction proceeds in a two-substrate ping-pong mechanism with reduced dithiols as cosubstrates. The catalysis of DI is sensitively inhibited by gold thioglucose, which can form a very stable complex with the Sec residue in the active site of this enzyme, or by PTU, which can interact with an oxidized enzyme-selenenyl iodide intermediate to form a dead-end enzyme-Se-S-PTU adduct (4–7). DI is thought to be responsible for the major part of peripheral T₃ production, as well as for the clearance of plasma reverse T₃. Thus, the enzyme plays an important role in the metabolism and the physiological function of T₃. Deficiency of DI will result in some serious thyroid diseases.

Because of the low abundance of DI (5–20 pmol of DI/mg of liver or kidney microsomal protein) and the instability of the catalytic activity in detergents, etc., attempts to isolate and purify DI by conventional techniques of protein chemistry, e.g. affinity and immunoaffinity purification, have not been successful (8–10). For this reason the studies of biochemical properties and structure-function of the enzyme have been impeded. Obviating the concern is the preparation of a catalytic antibody with deiodinase activity for mechanistic studies and therapeutic applications. In addition, the antibody-catalyzed selective deiodination (or dechlorination) also will have broad prospects for organic synthesis, etc.

Most of the catalytic antibodies reported to date (11–14) have been obtained by producing monoclonal antibodies that have been elicited against transition state analogs. However, the generation of antitransition state analogs cannot be directly used to generate the catalytic antibodies containing in its binding pocket an inorganic cofactor or metal ion. Furthermore, the reaction transition state structure of T₄ and the detailed catalytic mechanism of DI are not clear, so it is very difficult to prepare the antibodies with deiodinase activity targeted to transition state analogs. Based on the initial mechanism of DI and the fact that a Sec in the active site of DI is indispensable to the optimum enzyme activity, we described the preparation of the first catalytic antibody with DI activity produced by chemical modification of the antibody. The study broadened the scope of antibody-catalyzed reactions and lays a foundation for further study on DI mimics.

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§ The abbreviations used are: T₃, 3,5,3’-triiodothyronine; BSA, bovine serum albumin; DI, type I iodothyronine deiodinase; DTT, dithiothreitol; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; RIA, radioimmunoassay; Sec, selenocysteine; T₄, thyroxine; PTU, 6-propyl-2-thiouracil.

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EXPERIMENTAL PROCEDURES

Materials—The following reagents were purchased from Sigma: thyroxine sodium, trioiodothyronine sodium, phenylmethanesulfonfyl fluoride, DTT, ebselen, 3,3',5,5'-tetramethybenzidine, bovine serum albumin (BSA), and ovalbumin. Hypoxanthine aminopterin and thymidine solution, hypoxanthine thymidine solution, and Iseove's modified Dulbeco's medium were purchased from Life Technologies, Inc. Balb/c mice and myeloma cells (NS-1) were supplied by Changchun University of Agriculture and Animal Husbandry Sciences. A free T3, 125I RIA kit was supplied by Benthone Medical University. Sephadex G-10 and G-25, SDS-polyacrylamide gel, and HiTrap protein A were purchased from Amersham Pharmacia Biotech. Tissue culture plates (96- and 24-well) were obtained from Nunc, and polyethylene glycol ~4000 was from Merck. All other chemicals were of analytical grade.

Preparation and Properties of DI Mimic

Preparation of Antigen—5.0 mg of 3,5-t-thyroxine was dissolved in 1.0 ml of the solution of sodium-sodium carbonate (1:1; pH 9.5) at ambient temperature. Then the solution was added to 1.0 ml of a 50 mM phosphate buffer (pH 7.0) containing 5.0 mg of BSA (or ovalbumin). 200 µl of 0.02% solution of glutaraldehyde in phosphate buffer was added dropwise to the mixture solution over 30 min, and the solution was stirred overnight at room temperature. The reaction mixture was purified by G-25 column chromatography to give the antigen, either T4-39 or T4-40. A 0.02% solution of glutaraldehyde in phosphate buffer was added dropwise to the mixture solution over 30 min, and the solution was stirred overnight at room temperature. The reaction mixture was purified by G-25 column chromatography to give the antigen, either T4-39 or T4-40. One screening with an enzyme-linked immunosorbent assay (ELISA). Because the hapten had a maximum absorbance at 325 nm, hapten density was determined by measuring A405.

Preparation and Purification of Monoclonal Antibody (mAb) against T4—mAbs were raised against BSA-T4 conjugate by using hybridoma methodology (15, 16). In short, five Balb/c mice were bled to obtain control serum and then injected intraperitoneally with a 0.2 ml suspension of a 1 mg/ml solution of the antigen mixed with an equal volume of Freund's complete adjuvant for the initial injection or Freund's incomplete adjuvant for subsequent injection. The mice were immunized with the same batch of T4-BSA conjugate at 2-week intervals for 4 orders and then hyperimmunized with the antigen in the absence of adjuvant 3 days after the last injection. Hybridoma was produced by fusion of mouse spleen cell with myeloma cells (NS-1) with ethylene glycol ~4000. Two lines of positive hybridomas were detected by ELISA, cloned three times, and propagated in vitro and in vivo. Asciteses were gathered after intraperitoneal injection with the hybridoma, and the antibody titers were measured by ELISA. The monoclonal antibody 4C5 from higher titer ascites was purified by 45% ammonium sulfate fractionation (4°C; pH 7.2), anion exchange chromatography on DEAE-52 (5 mM Tris, pH 8.0, 20–400 mM NaCl, the antibody eluted in the 100 mM NaCl, pH 7.5), and affinity purification on a HiTrap protein A column. The subtype of the monoclonal antibody 4C5 was determined with the mouse monoclonal antibody isotyping reagent kit (from Sigma). The amount of protein was determined by the method of Lowery et al. (17) in which BSA was used as standard.

Preparation of Selenium-containing Catalytic Antibody (Se-4C5)—Antibody structure analysis has shown that many antibodies contain 3–4 serine residues in the variable region of light chain of antibody (18). These serine residues can be converted into serylthio by chemical modification (19, 20). Here, Se-4C5 was prepared by the method as described by Bell et al. (20). 5.0 mg of mAb 4C5 was dissolved in 1.0 ml of 50 mM, pH 7.0, phosphate buffer containing 0.15 M NaCl and allowed to react with phenylmethanesulfonfyl fluoride (20 µl of a 21 M solution in acetonitrile) at 20 °C for 1 h. The resulting sulfonylated mAb 4C5 was isolated by gel filtration on Sephadex G-25 using 50 mM, pH 7.0, phosphate buffer as eluent. The protein fraction was concentrated, mixed with 200 µl of 1 M sodium hydrogen selenide solution prepared according to the procedure of Klayman and Griffin (21) and incubated at 37 °C for 1 h under an inert atmosphere. The protein was separated from the reaction mixture on a Sephadex G-25 column eluting with 50 mM, pH 7.0, phosphate buffer and further purified by affinity chromatography on thiopropyl-Sepharose 6B.

The selenium content of the catalytic antibody was determined by titrating the sodium borohydride-reduced protein with 5,5'-dithiobis(2-nitrobenzoic acid) (22) or by inductively coupled plasma mass spectrometry (23). 3D (2D, 2D) electron signals were studied by x-ray photoelectron spectroscopy with A405 as a target (24, 25). The purities of monoclonal antibody 4C5 and Se-4C5 were determined by SDS-polyacrylamide discontinuous gel electrophoresis. The dissociation constants of 4C5-T4 and Se-4C5-T4 complexes were measured by a competitive ELISA method (26).

T3 Deiodination Assay and RIA of T3—Se-4C5 was assayed for deiodination of T3 using a modification of the method of Visser et al. (27). 0.1 pmol of 4C5 was preincubated in a total volume of 0.9 ml of 50 mM phosphate buffer (pH 7.0) containing 1.0 mM DTT at 37 °C for 10 min. The T3 5′-deiodination was initiated by the addition of 0.1 ml of a 10 mM solution of T3 in 50 mM phosphate buffer, pH 7.0, containing 5% BSA and terminated by extracting with 3 volumes of 95% ice-cold ethanol. The extracted samples were centrifuged for 20 min at 3000 x g, and the supernatants were taken out and diluted 100-fold by the addition of 50 mM sodium phosphate buffer (pH 7.0) to both minimize the influences of ethanol on measurements of T3 and dilute the concentration of T3 into the range of measurement of the RIA. The amount of T3 generated was measured by a competitive RIA using a free 125I-T3 RIA kit (from Instar corporation) following the manufacture instructions. Both the T3 deiodinative reaction and the radioimmunossay were performed in duplicate. All reaction time was generally chosen so that no more than 10% of the substrate T3 was consumed in the reaction. To subtract the non enzymatic degradation of T3 in the reaction course, the control experiments were performed by substituting 50 mM phosphate buffer (pH 7.0) for the solution of Se-4C5 in the same reaction conditions as described above. The kinetics of the Se-4C5-catalyzed reaction was investigated by measuring the intensity of the initial reaction rate at several concentrations of one substrate while keeping the concentration of the other constant. For determination of Km and Vm values, the straight lines of double-reciprocal plots were drawn by the least-squares method. To investigate the effect of PTU on the conversion of T3 to T3, 100 µl of phosphate buffer (pH 7.0) in the incubation mixture was replaced by an equal volume of a solution containing the desired amount of the PTU. For the studies of the thermostability of Se-4C5, the Se-4C5-catalyzed 5′-deiodination of 1.0 mM T3 by 1.0 mM DTT was performed in 50 mM phosphate buffer (pH 7.0, containing 0.5% BSA) at 50 and 80 °C. The unit of initial velocity was expressed as pmol of T3 product/milligram protein/min in accordance with that of natural deiodinase activity.

RESULTS

Hapten Density of Antigen and the Character of 4C5—Each antigen molecule contained, on average, 24 haptons for BSA-conjugated T4 and 17 haptons for Ovalbumin-conjugated T4 as determined by the absorbance at 325 nm. The BSA-T4 complex was used to immunize Balb/c mice for the production of monoclonal antibodies. Two hybridoma cell lines, 2E7 and 4C5, stably secreted monoclonal antibodies against T4. Because of the higher antibody titer of 4C5 ascites fluid as determined by ELISA, hybridoma cell 4C5 was propagated and injected into mice for the production of ascites fluid, and mAb 4C5 was used for the preparation of catalytic antibody. After multiple-step purification, mAb 4C5 showed only a single band of light chain (26 kDa) and one heavy chain band (56 kDa) on SDS-polyacrylamide gel electrophoresis pattern (Fig. 1), manifesting that 4C5 had already been homogenous. The results of antibody-subtype analysis showed 4C5 was the subtype of IgG2b. The dissociation constant of antibody-T3 complex was
5.4 × 10^{-8} \text{M}, and the antibody cross-reactivity with T_{3} was less than 0.5%.

The Preparation of Se-4C5 and Se-4C5-catalyzed Deiodination of T_{4}—Although the mAb 4C5 had the same hydrophobic binding pocket as DI, there was no catalytic group such as Sec in its binding pocket. Therefore, introduction of the catalytic group into the hydrophobic pocket of mAb 4C5 might exhibit catalytic activity. The serines of variable regions of 4C5 were activated with phenylmethanesulfonyl fluoride to produce sulfonylester of the serine hydroxyl group and then sulfonate was replaced with selenium to generate Se-4C5. To expiate the probable oxidation of sodium hydrogen selenide by air, the amount of sodium hydrogen selenide used in the reaction was in great excess over that of phenylmethanesulfonyl fluoride used. The modification reaction was carried out at 37 °C rather than 40 °C reported (20) to minimize the denaturation of antibody. A mouse mAb Hp4 raised against glutathione was also chemically modified in the same way as mAb 4C5. The selenium contents of the two protein were determined, following anaerobic reduction of the samples with sodium borohydrdride, by titration with 5,5'-dithiobis(2-nitrobenzoic acid), and 1.90 equivalents of selenol were incorporated into each antibody Se-4C5 molecule, and 2.0 equivalents were incorporated into each molecule of Se-Hp4. The selenium 3d electron signals of Se-4C5 and Se-Hp4 determined by x-ray photoelectron spectroscopy were essentially identical, each emerging in 54.4 ± 0.3 eV region assigned to the selenium of Sec, suggesting that the selenium in Se-4C5 and Se-Hp4 was not bonded to oxygen but existed in the form of Sec like that of natural enzyme. SDS-polyacrylamide gel electrophoresis showed Se-4C5 also had two bands representing a single light and heavy chain that exhibited the same mobility as these of 4C5 (Fig. 1). The dissociation constant of Se-4C5-T_{4} complex was 3.2 × 10^{-7} \text{M}, slightly lower than that of 4C5.

The catalytic efficiencies of ascites, 4C5, Se-4C5, Se-Hp4, and ebselen were investigated at pH 7.0 and 37 °C. Their initial rates of the reaction (corrected for spontaneous degradation of T_{3} in the absence of catalyst) are shown in Table I. Among them, Se-4C5 (0.1 μM) exhibited the fastest reaction rate generating 1130 pmol/liter/min at 1.0 μM T_{4} and 1.0 mM DTT, whereas Se-Hp4 (1.0 μM) demonstrated a reaction rate of only 17 pmol/liter/min. Ebselen, ascites, and 4C5 all did not exhibit catalytic activity. Assuming that the initial rate had a first-order dependence on T_{4} concentration, these data indicated that Se-4C5 was at least 66-fold more efficient than Se-Hp4. The background rate was also determined in the same conditions. Results showed that T_{3} hardly reacted with DTT, generating T_{3} formation. Thus, the background rate was not of consequence on the catalyzed rate of T_{3} generation. The studies of thermostability of Se-4C5 manifested that Se-4C5 had a higher deiodinase activity at 50 °C than at 37 °C, but higher temperature (80 °C) inactivated Se-4C5.

Kinetic Characteristics of Se-4C5-catalyzed T_{4} 5'-Deiodination—To probe the mechanism by which Se-4C5 promotes the 5'-deiodinative reaction, detailed kinetic studies were undertaken. The initial rates for the T_{4} 5'-deiodination by DTT were determined as a function of substrate concentration at 37 °C and pH 7.0 by RIA, varying one substrate concentration while the other was fixed. As seen in Fig. 2, at each concentration of DTT, product T_{3} formation showed saturation kinetics with respect to T_{4}, and double-reciprocal plots of initial velocity versus T_{4} concentration yielded a family of parallel lines, consistent with a ping-pong mechanism involving at least one covalent enzyme intermediate. A replot of the intercepts of these parallel lines at the y axis (Fig. 2) versus the reciprocal of DTT concentration yielded the apparent maximal Michaelis constant for DTT and the V_{m} of the reaction (Table II). Michaelis-Menten kinetics for T_{4} and DTT were also observed under all the conditions investigated. The apparent kinetic parameters obtained at several DTT concentrations were summarized in Table III. In the light of mechanism of ping-pong reaction, the relevant steady-state equation for this system is represented in Equation 1.

![Double-reciprocal plots of deiodination rate versus T_{4} concentration at varying DTT concentrations](https://example.com/figure2.png)
where \( k_{\text{max}} \) is a pseudo first-order rate constant, and \( K_{T_4} \) and \( K_{\text{DTT}} \) are the Michaelis constants for \( T_4 \) and the reductant DTT, respectively. From the data in Table II and Table III, \( k_{\text{max}} = 4.05 \times 10^{-2} \text{ min}^{-1} \), \( K_{T_4} = 0.8 \mu\text{M} \), and \( K_{\text{DTT}} = 1.8 \mu\text{M} \). Thus \( k_{\text{max}}/K_{T_4} = 5.06 \times 10^{2} \text{ M}^{-1} \) \( \text{min}^{-1} \), and \( k_{\text{max}}/K_{\text{DTT}} = 22.5 \text{ M}^{-1} \) \( \text{min}^{-1} \). \( V_0 \) stands for the initial velocity of the reaction, and \([E]\) stands for the concentration of the mimetic enzyme. These data were reminiscent of those obtained for native 5'-deiodinase (28) and revealed that the Se-4C5-catalyzed \( T_4 \) 5'-deiodination was a bisubstrate reaction of a ping-pong type. Comparison of the apparent kinetic parameters for natural enzyme (28) and Se-4C5 revealed that the \( K_m \) of native enzyme for \( T_4 \) substrate was 6 times greater than that of Se-4C5, indicating that the affinity of Se-4C5 for \( T_4 \) was higher than that of natural enzyme, but the thiol requirement for Se-4C5 was 3.6 times that for the liver homogenate (source of 5'-deiodinase) (28).

**PTU Inhibition of Se-4C5-catalyzed \( T_4 \) 5'-Deiodination**—Previous studies of PTU inhibition of \( T_4 \) 5'-deiodinase have shown that exposure of liver or kidney homogenates to PTU lead to the persistent inhibition of the enzyme, and the degree of inhibition is increased if the exposure to PTU is performed under conditions favoring catalysis (i.e., in the presence of substrate and 37°C) (6). Additional studies suggest that the mechanism of persistent PTU inhibition involves the formation of an enzyme/PTU mixture by reaction of E-SeI intermediate with PTU. Because of the kinetic similarity of Se-4C5 and natural enzyme, the effect of PTU on \( T_4 \) deiodination by Se-4C5 was investigated. Se-4C5 was incubated under \( \text{N}_2 \) with several fixed PTU concentrations, 2.0 \( \mu\text{M} \), and increasing DTT concentration at 37°C. A family of straight lines with a point of intersection at \( y \) axial was obtained in double-reciprocal plots of \( T_3 \) deiodination rate versus DTT concentration at varying PTU concentrations (Fig. 3), indicating that PTU was a strong competitive inhibitor of DTT, with a \( K_i \) value of approximately 120 \( \mu\text{M} \). Thus, we suggested that PTU could also form stable Se-4C5-PTU adduct with reactive intermediate (Se-4C5-SeI), similar to that of type I deiodinase.
DISCUSSION

The Sec residue and T₄-binding pocket are both critical factors for the catalytic activity of DI. For example, the substitution of Sec by Leu results in the enzymatic inactivation of DI. In this report, we here utilized both essential factors for the preparation of a DI mimic. The association of the mAb 4C5, elicited against T₄, with the Sec residue has led to the formation of the tailor-made catalytic antibody Se-4C5. To confirm the catalytic activity of Se-4C5, the relative efficiencies of Se-4C5, 4C5, as well as the model compound ebselen were investigated at pH 7.0 and 37 °C. As seen from the initial rates of reaction measured by RIA in Table I, neither ebselen fluid nor 4C5 exhibited catalytic activities when background rate was deducted. This suggests that the hapten-binding site of 4C5 was insufficient to catalyze the deiodination of T₄ and that no natural deiodinase was present in ebselen fluid and 4C5. Se-4C5 generated by modifying 4C5 exhibited deiodinase activity, suggesting that the catalytic group Sec also played a pivotal role in enzymatic catalysis. In addition, the hapten-binding pocket of antibody also exhibited an important effect on its catalytic efficiency. Although Se-Hp₄ and the model compound ebselen posed such a catalytic group, reduced selenium, like that of Se-4C5, they showed very poor catalytic efficiencies, which could be mainly attributed to the fact that they all did not have the special T₄-binding pocket. Therefore, the hapten-binding pocket coupled with the catalytic group Sec bestowed the enzymatic activity on Se-4C5. The facts that the rate for deiodination of T₄ was linearly proportional to Se-4C5 concentration and that its activity could be destroyed by heat denaturation of the protein further demonstrated that Se-4C5 was an active zymoprotein.

As seen from the calculated dissociation constants, antibodies Se-4C5 and 4C5 exhibited high affinities for T₄, reflecting that there existed strong interactions between the antibodies and the hapten. This may contribute to the decrease of activation energy of the reaction (deiodination of T₄) in catalysis. The similarity of the dissociation constants of Se-4C5 and 4C5 for the hapten indicates that the three-dimensional structure of the modified protein was likely unaltered by the modification reaction.

We further examined the kinetic property and the PTU inhibition of T₄ 5'-deiodination by Se-4C5. It could be determined from the results that the deiodinative reaction displayed a typical enzymatic feature (see Fig. 2) and that the relationship between reactive velocity and substrate concentration was subject to the Michaelis-Menten equation. The characteristic parallel Dalziel plots revealed that the Se-4C5-catalyzed reaction mechanism was a of ping-pong type, which was identical to that of type I deiodinase (7), Thus, the Se-4C5 reacted with one of the substrates (T₄) to form a Michaelis complex that was followed by the dissociation to the first product T₃ leaving a modified enzyme form (E-Se-I) containing a moiety (I) from the first substrate. The second substrate, DTT, interacted with the modified enzyme (E-Se-I) only after dissociation of the first product and reduced the oxidized enzyme with release of iodide. The whole course of deiodination of T₄ was shown in Fig. 4. An important characteristic used to distinguish DI from the other deiodinases is its marked sensitivity to inhibition by PTU, which is thought to be highly dependent on the selenium atom. The results in Fig. 3 showed that PTU competitively inhibited the deiodination activity of Se-4C5 in a similar manner to the competitive inhibition of DI. Thus, the kinetic characteristics of Se-4C5 and the PTU inhibition of Se-4C5 define the catalytic antibody Se-4C5 as a mimic of DI.

At the present time, pure natural deiodinase is not available. Hence, we only compared the kinetic parameters for Se-4C5 with those reported (28) for rat liver homogenate containing natural enzyme with the exception of the apparent Vₘ, which could not be compared, because the quantity of natural deiodinase was uncertain in rat liver homogenate. The comparison of Se-4C5 with that of DI present in homogenates revealed that the specificity of Se-4C5 for T₄ was higher than that of DI, and the thiol requirement of Se-4C5 was very different from that of the liver homogenate. The differences indicate a difference in structure between Se-4C5 and DI. Further studies for Se-4C5 will contribute to the understanding of both the structure and the reaction mechanism of deiodinases.

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