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Chemical Modification of Ribonucleosides with *N*-Acetyl-3,5-di[^{125}I]iodotyrosyl Hydrazide

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Several 3,5-diiodotryrosyl derivatives have been synthesized by both sodium iodide–iodine and the sodium iodide–iodic acid methods. Conditions optimizing yield and purity of the product have been established for the latter reaction. Under those conditions, treatment of *N*-acetyl-tyrosyl ethyl ester with sodium [^{125}I]iodide and iodic acid gave *N*-acetyl-3,5-di[^{125}I]iodotyrosyl ethyl ester (ADITEE) with high specific activity. Hydrazination of [^{125}I]ADITEE produces *N*-acetyl-3,5-di[^{125}I]iodotyrosyl hydrazide. This hydrazide has been successfully used to modify four different ribonucleoside dialdehydes.

Postlabeling of ribonucleic acids with radioactive ^{125}I is a new method for sequencing or mapping RNA developed in the past few years (*1–6*). Recently, Randerath reported a new radioiodination reagent for ultrasensitive detection and determination of periodate-oxidized nucleoside derivatives (*7*). ^{125}I is preferable to ^{32}P because of its higher specific activity and longer half-life. We have designed and synthesized several 3,5-diiodotyrosyl derivatives. *N*-acety-tyrosyl ethyl ester (ATEE) and the sodium iodide–iodic acid method were chosen for ^{125}I labeling to optimize both yield and purity of the product. The principles for synthesis of acetyl-di[^{125}I]iodotyrosyl hydrazide (ADITH) are as follows: Treatment of ATEE with sodium [^{125}I]iodide and iodic acid gives rise to [^{125}I]ADITEE. Hydrazination of [^{125}I]ADITEE produces [^{125}I]ADITH. The four different ribonucleosides were oxidized with sodium periodate to the corresponding ribonucleoside dialdehydes and then modified with [^{125}I]ADITH.

MATERIALS AND METHODS

L-tyrosine, ATEE, and polyamide thin-layer sheets were obtained from East Wind Biochemicals of Shanghai. *N*-carbobenzoxyl-tyrosyl ethyl ester (ZTEE)

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was a gift of Dr. Du. Ascending polyamide thin-layer chromatography for separation of ADITEE, ADITH, and the ^{125}I-labeled ribonucleosides were carried out at room temperature using the solvents A: toluene : n-hexane : acetic acid, 2 : 1 : 1 (v/v); and B: isopropanol : dioxane : 1 N NH_4OH, 20 : 5 : 5 (v/v).

In 50% isopropanol, the absorbance maximum for ATEE and ZTEE is at 278 nm whereas that for ADITEE and ZDITEE is at 290 nm and excess sulfite did not interfere with the measurements. Therefore such measurements were carried out in 50% isopropanol, using the ratio A_{290}/A_{278} to indicate the iodination of ATEE and ZTEE in the reaction mixture. The ratio $A_{310.7}/A_{292}$ was used to indicate the iodination of tyrosine as described previously (*8*).

Synthesis of 3,5-Diiodotyrosyl Derivatives

(I) NaI–I_2 Method

N-Acetyl-3,5-diiodotyrosyl ethyl ester. A 10-ml aqueous solution containing 2.02 g (7.96 mmol) iodine and 2.5 g (13.48 mmol) sodium iodide was added slowly to a solution of 1 g (3.98 mmol) ATEE in 8 ml of isopropanol and 2 ml of ethylamine (65–70%), with constant shaking at room temperature over a period of 30 min. After incubation for another 30 min, solid sodium metabisulfite was added to the mixture until the solution became colorless. Upon adjustment of pH to 5.0 with acetic acid, a white precipitate appeared immediately. The precipitate was collected by suction filtration, washed three times with distilled water, and dried *in vacuo* over P_2O_5 to give 1.7 g (84.9% yield) of the crude product. This was dissolved in 30 ml of hot (about 70°C) 95% ethanol, and 15 ml of hot distilled water and 10 drops of 1 N HCl were added. When the temperature gradually decreased, white crystals appeared. After they were kept at 4°C overnight, the crystals were collected by suction filtration, washed three times with distilled water, and dried *in vacuo* over P_2O_5 to give 1.3 g (80% yield) of crystalline ADITEE with a melting point of 154–156°C.

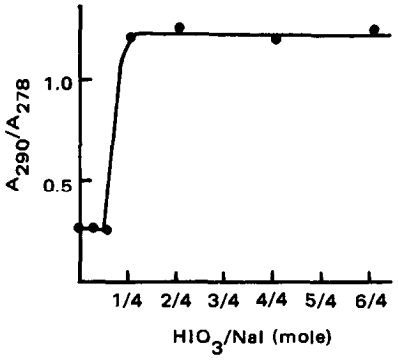

FIG. 1. Effect of molar ratio of iodic acid to sodium iodide on the yield of ZDITEE. The reaction mixtures contained 0.025 mmol ZTEE, 0.1 mmol sodium iodide, 0.25 ml acetic acid, and 0.05 ml carbon tetrachloride. The indicated amounts of iodic acid in 0.05 ml distilled water were added to a final volume of 0.35 ml. The reaction was run with magnetic stirring at 50°C for 30 min and stopped by the addition of 0.7 ml (1.05 mmol) sodium sulfite. The reaction mixture was then diluted 340-fold with 50% isopropanol and the ratio A_{290}/A_{278} obtained.

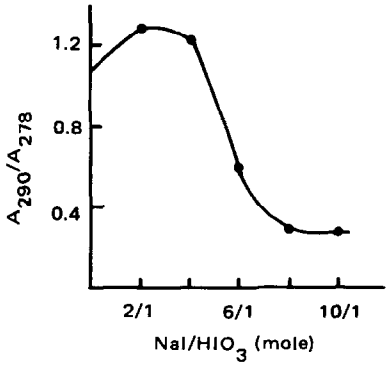

FIG. 2. Effect of molar ratio of sodium iodide to iodic acid on the yield of ZDITEE. The reaction mixtures contained 0.05 mmol ZTEE, 0.05 mmol iodic acid, 0.45 ml acetic acid, and 0.1 ml carbon tetrachloride. The indicated amounts of sodium iodide in 0.1 ml water were added to a final volume of 0.65 ml. The reaction was run with magnetic stirring at 50°C for 30 min and stopped by the addition of 0.5 ml (0.75 mmol) sodium sulfite. The mixture was then diluted 550-fold with 50% isopropanol and the ratio A_{290}/A_{278} obtained.

Anal. Calcd for $C_{13}H_{15}O_4N_1I_2$: N, 2.80; I, 50.50. Found: N, 2.69; I, 49.60.

N-Acetyl-3,5-diiodotyrosyl hydrazide. To 1.0 g (1.99 mmol) of ADITEE in 22 ml of 95% ethanol was added 4 ml of 85% hydrazine (82.30 mmol). The mixture was kept at room temperature for 24 h, and at 4°C for a further 24 h. After evaporation under reduced pressure to a small volume and adjustment of the pH to 6 with acetic acid, the white precipitate formed was collected by suction filtration, washed three times with distilled water, and dried *in vacuo* over P_2O_5. Its melting point was 208–210°C. This crude product was dissolved in a hot mixture of 10 ml 95% ethanol and 10 ml 1 N NH_4OH. The pH value was adjusted to 6–7 with acetic acid, and the temperature allowed to decrease gradually. The white crystalline product formed was collected by suction filtration, washed three times with distilled water, and dried *in vacuo* over P_2O_5 to give 756 mg (77.7% yield) of ADITH with a melting point of 210–212°C.

Anal. Calcd for $C_{11}H_{13}O_3N_3I_2$: N, 8.59; I, 51.94. Found: N, 8.09; I, 52.22.

(II) NaI–HIO$_3$ Method

Optimal reaction conditions. 3,5-diiodotyrosine and its derivatives can be synthesized by means of oxidation of iodide with iodic acid solution. The reaction can be expressed as follows:

$$10\ I^- + 2\ IO_3^- + 12\ H^+ \rightarrow 6\ I_2 + 6\ H_2O$$

$$6\ I_2 + 3\ \text{tyrosine} \rightarrow 3\ \text{DIT} + 6\ \text{HI}.$$

The molar ratios of the reaction components are theoretically tyrosine : iodic acid : sodium iodine, 3 : 2 : 10. Experimentally iodination of one mol tryosine was found to require 1 mol iodic acid and 4 mol sodium iodide (Fig. 1). The yield of 3,5-diiodotyrosine decreased sharply if the amount of sodium iodide was over 4 mol (Fig. 2). These results indicate that the optimal molar ratio for the iodination

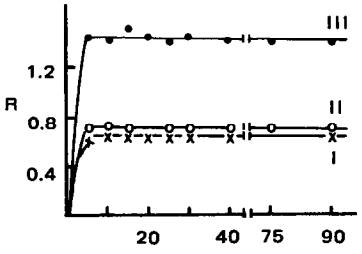

FIG. 3. Time course of iodination of tryosine and its derivatives. I, ATEE: The 3.25-ml reaction mixture contained 0.25 mmol ATEE, 1 mmol sodium iodide, 0.25 mmol iodic acid, 2.25 ml acetic acid, 0.5 ml carbon tetrachloride, and 0.5 ml distilled water. It was incubated at 50°C with magnetic stirring, and 0.04-ml aliquots were withdrawn at intervals. Each aliquot was mixed with 0.5 ml 1.5 M sodium sulfite to stop the reaction. The ratio A_{290}/A_{278} was determined after dilution of the sample with 4 ml of 50% isopropanol. II, ZTEE: The experimental procedures were the same as described in I, but ZTEE was the reactant. III, Tryosine: Tyrosine was the reactant. Each aliquot was mixed with 0.4 ml (0.6 mmol) sodium sulfite in order to stop the reaction. The ratio $A_{310.7}/A_{292}$ was determined after dilution of the sample with 5 ml 0.1 N NaOH. The other procedures were the same as described in I.

reaction is tyrosine : iodic acid : sodium iodine, 1 : 1 : 4. Thus more iodic acid and sodium iodide were required experimentally than expected theoretically. Under these optimal conditions, the iodination of tyrosine and its derivatives proceeded within 15–20 min (Fig. 3).

Synthesis of 3,5-diiodotyrosine and its derivatives. Employing optimal reaction conditions, 3,5-diiodotryosine and two of its derivatives have been synthesized as follows:

(a) 3,5-diiodotyrosine (DIT):

To a solution containing 181 mg (1 mmol) tyrosine and 744 mg (4 mmol) sodium iodide in 9 ml acetic acid and 2 ml carbon tetrachloride was added 176 mg (1 mmol) iodic acid in 2 ml distilled water at 55°C with magnetic stirring. The reaction mixture was stirred at 55°C for 90 min before 15 ml of 1.5 M sodium sulfite (2.25 mmol) was added to stop the reaction. After evaporation under reduced pressure to a small volume, adjustment of the pH to 4–5 with acetic acid, and then standing at 4°C for 6 h, the crystals formed were collected by centrifugation, washed twice with dilute acetic acid (pH 4–5), and dried *in vacuo* over P_2O_5 to give 199 mg (0.46 mmol, 46% yield) of DIT with mp 198–199°C. Another 23.4 mg (0.054 mmol, 6.5% yield) was recovered from the mother liquor. Its absorbance maximum at 285–286 nm, pH 2, and R_f value on paper chromatogram (n-butanol saturated with 2 N acetic acid) were the same as those reported (*8*). No monoiodotyrosine was found.

(b) *N*-Acetyl-3,5-diiodotyrosyl ethyl ester:

To a solution containing 62.8 mg (0.25 mmol) ATEE and 186 mg (1 mmol) NaI · $2H_2O$ dissolved in 2.25 ml acetic acid and 0.5 ml carbon tetrachloride was added 44 mg (0.25 mmol) iodic acid in 0.5 ml distilled water at 50°C with magnetic stirring. The reaction mixture was stirred at 50°C for 1 h, before 4 ml 1.5 M sodium sulfite (6 mmol) was added to stop the reaction. After standing at 4°C overnight, the crystals formed were collected by centrifugation, rinsed three times with dilute acetic acid (pH 4–5) and ether, and dried *in vacuo* over P_2O_5 to give 59.6 mg (0.16 mmol, 48% yield) crystalline ADITEE with a melting point of 154°C.

The absorbance maximum at 290 nm in 50% isopropanol and the R_f value on paper chromatography (in tertiary butanol : 3% NH_4OH = 3 : 1) were identical with those of the product prepared by the sodium iodide and iodine method.

(c) *N*-Carbobenzoxyl-3,5-diiodotyrosyl ethyl ester: (ZDITEE):

Twenty-two milligrams (0.125 mmol) iodic acid dissolved in 0.25 ml distilled water was added to a solution containing 43 mg (0.125 mmol) *N*-carbonbenzoxyl-tyrosyl ethyl ester and 93 mg (0.5 mmol) of NaI · $2H_2O$ in 1.3 ml acetic acid and 0.25 ml carbon tetrachloride. The reaction mixture was magnetically stirred at 50°C for 30 min before 2 ml 1.5 M sodium sulfite (3 mmol) was added to stop the reaction. Upon evaporation of the mixture to a small volume, the white precipitate formed was collected by centrifugation, rinsed five times with distilled water, dried *in vacuo* over P_2O_5 overnight, and finally ground into powder form. It was dissolved in 2 ml ethyl acetate, and 30 ml petroleum ether was added. After standing at 4°C overnight, the crystals formed were collected by centrifugation, rinsed three times with dilute acetic acid (pH 4–5), and dried *in vacuo* over P_2O_5 to give 41.2 mg (0.069 mmol, 55% yield) ZDITEE, mp 119–121°C. Another 8 mg (0.013 mmol, 10% yield) product was recovered from the mother liquor; its melting point was 119–120°C.

Absorbance maximum of the product in 50% isopropanol was observed at 289–291 nm. Its R_f value on paper chromatography (in tertiary butanol : 3% NH_4OH = 3 : 1, v/v) is 0.93.

Synthesis of ^{125}I-labeled tyrosine derivatives. ^{125}I-Labeled ethyl ester and hydrazide derivatives of ATEE were synthesized by means of oxidation of sodium [^{125}I]iodide with iodic acid.

(a) ADITTEE:

ATEE (62.8 mg, 0.25 mmol) was dissolved in 1.25 ml acetic acid and 0.5 ml carbon tetrachloride, to which were added 0.25 ml sodium [^{125}I]iodide (19.3 mCi/ml) and 5 μl 0.5 M iodic acid (0.025 mmol) in water, the reaction mixture was magnetically stirred at 50°C for 5 min, and another 186 mg (1 mmol) sodium iodide in 1 ml acetic acid and 0.5 ml 0.5 M iodic acid were added. After the reaction mixture was heated at 50°C with stirring for 60 min, 4 ml 1.5 M sodium sulfite was added to stop the reaction. The crystals, appearing after the reaction mixture had been kept at 4°C overnight, were collected by centrifugation, rinsed three times with distilled water, and dried *in vacuo* over P_2O_5. The product contained about 28–37% of the initial radioactivity.

Recrystallization was carried out by dissolving the crude ADITEE in concentrated NH_4OH in an ice bath, adjusting the pH value immediately to 5–6 with acetic acid, and leaving the mixture at 4°C overnight. Crystals were collected by centrifugation, washed three times with distilled water, and dried *in vacuo* over P_2O_5 to give 72.23 mg (0.14 mmol, 56% yield) of [^{125}I]ADITEE containing 9.73×10^9 cpm.

(b) ADITH:

Hydrazine (0.4 ml, 85%, 8.2 mmol) was added to a solution containing 12.05 mg (0.02 mmol) [^{125}I]ADITEE in 0.3 ml 95% ethanol in an ice bath. The mixture was kept at 0°C for 4.5 h, and then diluted with 2 ml distilled water. After the pH value was adjusted to 5–6 with acetic acid, the crystals that appeared were

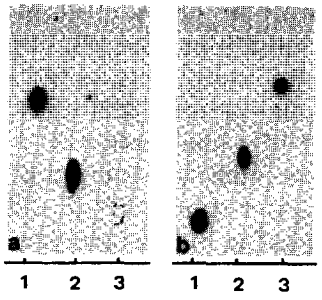

FIG. 4. Polyamide thin-layer chromatography of ^{125}I-labeled tyrosine derivatives using solvent A. (a) Radioactive derivatives: (1) [^{125}I]ADITEE; (2) [^{125}I]ADITH; (3) *N*-acetyl tyrosine (under uv). (b) Nonradioactive derivatives (under uv): (1) *N*-acetyl tyrosine; (2) ADITH; (3) ADITEE.

collected by centrifugation, rinsed three times with distilled water, and dried in a desiccator over P_2O_5 to give 8.75 mg (0.018 mmol, 90% yield) product.

As indicated in Fig. 4, both radioactive and nonradioactive ADITEE and ADITH chromatographed on polyamide thin-layer sheet as a single spot.

Synthesis of ^{125}I-ribonucleosides. Three milligrams of adenosine, guanosine, cytidine, or uridine in 0.2 ml 0.2 M acetate buffer, pH 5.0, was oxidized with 0.2 ml 0.1 M sodium periodate in the dark at room temperature for 2.5 h. Afterwards the pH was adjusted to 7 with diluted NH_4OH, and excess IO_4^- was removed by passing the reaction mixture through a column of anionic exchange resin (acetate form, 0.5 × 1 cm, 200 mesh). The ribonucleoside dialdehydes were eluted with distilled water and stored at −20°C. To 0.1 ml of ribonucleoside dialdehydes solution (2.5 mg/ml) was added 50 μg of [^{125}I]ADITH in 50 μl dimethyl sulfoxide. The reaction mixture was left at room temperature for 24 h, and the modified ribonucleosides were analyzed by chromatography on a polyamide thin-layer sheet (Fig. 5).

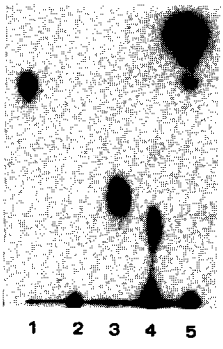

FIG. 5. Polyamide thin-layer chromatography of ^{125}I-labeled nucleosides using solvent B. (1) adenosine; (2) cytidine; (3) uridine; (4) guanosine; (5) [^{125}I]ADITH.

DISCUSSION

Postlabeling of ribonucleic acids with radioactive isotopes is a powerful approach for sequencing or mapping RNA developed in the past few years (*1–6*). ^{125}I is preferable to ^{32}P because of its higher specific activity and longer half-life. In this paper, we have reported the chemical modification of ribonucleosides with a radioactive carbonyl reagent, [^{125}I]ADITH. For this purpose, we designed and synthesized several 3,5-diiodotyrosine derivatives by different methods. ATEE and the sodium iodide–iodic acid method were chosen for ^{125}I labeling to optimize both yield and purity of the products. The synthesis of the ^{125}I-labeling carbonyl reagent is as follows: Treatment of ATEE with sodium [^{125}I]iodide and iodic acid gives rise to [^{125}I]ADITEE. Hydrazination of [^{125}I]ADITEE produces [^{125}I]ADITH. The four different ribonucleoside were oxidized with sodium periodate to the corresponding ribonucleoside dialdehydes and then labeled with [^{125}I]ADITH.

This carbonyl reagent, [^{125}I]ADITH, can be used for labeling of 3′-terminus-oxidized RNA and other ketone or aldehyde groups.

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REFERENCES

1. BRUCE, A. G., AND UHLENBECK, O. (1978) *Nucl. Acids Res.* **5,** 3665–3677.
2. TROPP, J., AND SIGLER, P. B. (1979) *Biochemistry* **18,** 5489–5495.
3. ROBERTSON, H. D., DICKSON, E., MODEL, P. AND PRENSKY, W. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3260–3264.
4. HEWLETT, M. J., AND FLORKIEWICZ, R. Z. (1980). *Proc. Natl. Acad. Sci. USA* **77,** 303–307.
5. DUBE, S. K. (1973) *Nature* (*London*) **246,** 483.
6. DICKSON, E., PAPE, L. K., AND ROBERTSON, H. D. (1979) *Nucl. Acids Res.* **6,** 91–109.
7. RANDERATH, K. (1981) *Anal. Biochem.* **115,** 391–397.
8. LIU, W., ZHOU, J., AND WONG, J. T. (1982) *Anal. Biochem.* **120,** 204–207.