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A Novel Synthesis of 3,5-Diiodotyrosine with Iodic Acid

WANGYI LIU,* JIANGUANG ZHOU,*,[1] AND J. TZE-FEI WONG†

**Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China, and †Department of Biochemistry, University of Toronto, Toronto M5S 1A8, Ontario, Canada*

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Based on Landolt's reaction between iodic acid and sulfite to produce iodine, a method has been developed for the high-yield iodination of tyrosine to form diiodotyrosine without significant formation of monoiodotyrosine. The use of sulfite as reductant for iodic acid cannot be effectively replaced by citric or oxalic acid under the reaction conditions.

The iodination of tyrosine plays an important role in the study of the structure and function of proteins and of thyroxine metabolism, and a variety of procedures have been described for iodination of free tyrosine and tyrosine residues in proteins. In earlier work, iodination was carried out with iodine (1), sodium iodide–iodine (2), sodium iodide–potassium iodate in alkaline solution (3), iodine monochloride (4,5) and electrolysis of potassium iodide (6,7). More recently, chloramine-T (8,9), thallium trichloride (10,11), and peroxidase (12,13) also have been employed as iodination agents. These various iodination methods, however, mainly give rise to a mixture of monoiodotyrosine (MIT) and diiodotyrosine (DIT), from which the purification of DIT is tedious and time-consuming.

In acid solution, iodic acid is reduced by sulfite to produce iodide ion, which is further oxidized by excess iodic acid to generate iodine. On the basis of this reaction, known as Landolt's reaction (14), we have developed a novel method for the synthesis of 3,5-diiodotyrosine essentially free from monoiodotyrosine.

MATERIALS AND METHODS

Ascending paper chromatography for separation of tyrosine and iodinated derivatives was performed at room temperature using a solvent consisting of 1-butanol saturated with 2 N acetic acid. Active charcoal for isolating these compounds was pretreated with 1 N HCl in a boiling water bath for 1 h, and washed with distilled water to pH 4–5. The iodination reaction was stopped by the addition of excess sodium sulfite. At pH 2, tyrosine and DIT exhibited maximal absorbance at 274.1 and 286.0 nm, respectively (15), but their measurements were seriously hindered by the high absorbance of excess sulfite. At pH 13, maximal absorbance by tyrosine and DIT occurred at 292.0 and 310.7 nm, respectively, under which condition excess sulfite did not interfere with their measurements. Therefore such measurments were carried out in 0.1 N NaOH solution, using the ratio $A_{310.7}/A_{292.0}$ to indicate the amount of DIT produced from tyrosine in the reaction mixture.

RESULTS AND DISCUSSION

Optimal conditions for iodination of tyrosine. In acid solution, the synthesis of DIT with iodic acid can be expressed by the consecutive reactions

$$10IO_3^- + 30SO_3^{2-} \rightarrow 10I^- + 30SO_4^{2-} \quad [1]$$

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

$$6I_2 + 3 \text{ tyrosine} \rightarrow 3 \text{ DIT} + 6HI \quad [3]$$

Accordingly, the molar ratios of tyrosine:

[1] Present address: Department of Biology, Beijing University, Beijing, China.

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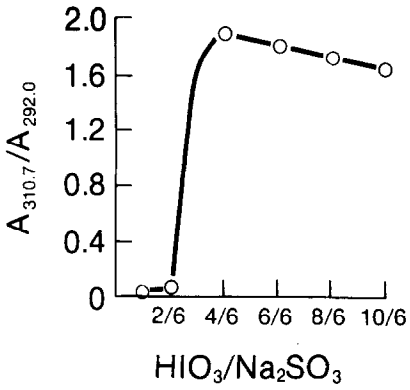

FIG. 1. Effect of molar ratio of iodic acid to sodium sulfite on the yield of DIT. Reaction mixture contained 0.25 ml acetic acid, 0.01 mmol tyrosine, and 0.06 mmol sodium sulfite. Indicated amount of iodic acid and distilled water were added to a final volume of 0.6 ml. The reaction was run with magnetic stirring at 55°C for 30 min and stopped by the addition of 0.75 ml of 1.5 M sodium sulfite. The reaction volume was then diluted 50-fold with 0.1 N NaOH, and the ratio $A_{310.7}/A_{292.0}$ obtained.

IO_3^-:SO_3^{2-} taking part in these reactions are 1:4:10.

The amount of DIT formed from tyrosine in the presence of sulfite varied with the quantity of iodic acid (Fig. 1). Optimal yield of DIT was obtained with molar ratios of tyrosine:IO_3^-:SO_3^{2-} at 1:4:6. When the molar ratio of iodic acid/sulfite was less than 2:6, the former was completey reduced by sulfite to iodide ion, which was ineffective in iodinating tyrosine. When the molar ratio of iodic acid/sulfite was greater than 4:6, the yield of DIT significantly declined, likely owing to the oxidation and destruction of tyrosine and DIT by excess acid. At the optimal molar ratios, the rate of iodination of tyrosine was very fast, such that maximal amount of DIT was formed within 15 to 30 min (Fig. 2). No formation of MIT was visible on the paper chromatogram, and elution of the MIT position gave no significant absorbance in excess of background (Fig. 3).

Procedure for synthesis of 3,5-diiodotyrosine. The optimal procedure for synthesis of DIT with high yield and purity is therefore as follows.

SOLUTION 1. 181 mg (1 mmol) of tyrosine suspended in 25 ml of acetic acid and 15 ml of distilled water is magnetically stirred at 55°C until the suspension is completely dissolved.

SOLUTION 2. 756 mg (6 mmol) of sodium sulfite is dissolved in 5 ml of distilled water.

SOLUTION 3. 704 mg (4 mmol) of iodic acid is dissolved in 5 ml of distilled water.

Solutions 2 and 3 are successively added to solution 1 at 55°C, and the mixture is incubated with continuous stirring for 25 min at that temperature. After the addition of 7.5 ml of 1.5 M sodium sulfite (11.25 mmol) to stop the reaction, the reaction mixture is passed through a charcoal column (10 × 1 cm). About 91% of the DIT is adsorbed to the column. The column is then washed with 150 ml of distilled water, and the DIT is eluted with a solution of 95% ethyl alcohol:H_2O:concentrated ammonium hydroxide (250:100:3.55, v/v). The eluate is evaporated *in vacuo* to the appearance of white crystals. After storage at 4°C overnight, the crystals are collected by centrifugation, and washed three times with dilute acetic acid (pH 4–5). They are dried *in vacuo* over P_2O_5. In a typical run 271 mg (0.63 mmol, or 63% yield) of DIT with mp

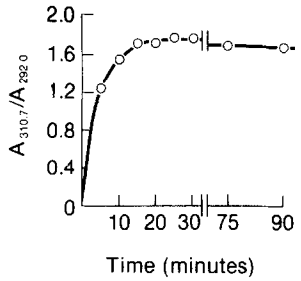

FIG. 2. Time course of iodination of tyrosine. Reaction mixture contained 2.5 ml of acetic acid, 0.1 mmol tyrosine, 0.6 mmol sodium sulfite, 0.4 mmol of iodic acid, and distilled water added to a final volume of 6 ml. It was incubated with magnetic stirring at 55°C, and 0.04-ml aliquots were withdrawn at intervals. Each aliquot was mixed with 0.4 ml (0.6 mmol) sodium sulfite in order to stop the reaction. The ratio $A_{310.7}/A_{292.0}$ was determined after dilution of the sample with 0.1 N NaOH.

198–199°C was obtained. Further evaporation of the mother liquor to a small volume under reduced pressure gave another 28.2 mg of DIT with mp 196–197°C. No formation of MIT was detected on the paper chromatogram in either the crystals or the mother liquor.

The maximal absorption wavelengths (286–287 nm at pH 2 and 310–311 nm at pH 13) of DIT obtained were practically identical with those recorded by Harrison and Garratt (15), and its ascending R_f value on paper chromatogram was the same as that prepared by the NaI–I_2 method (2).

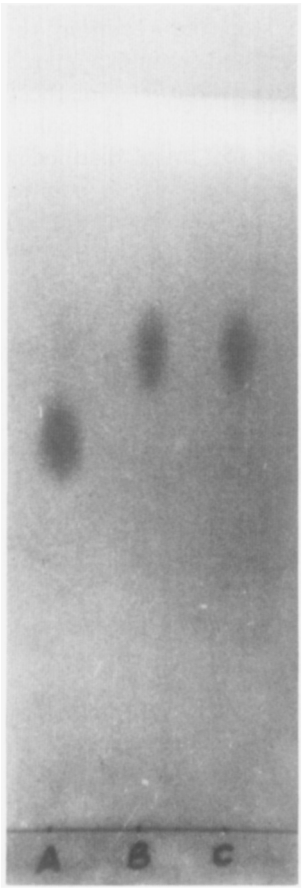

FIG. 3. Paper chromatography of iodinated tyrosines. (A) MIT marker obtained from East Wind Biochemicals of Shangai; (B) DIT crystals prepared by the present method; (C) DIT in the mother liquor after separation of the crystals.

TABLE 1

EFFECTS OF CITRIC ACID AND OXALIC ACID ON THE IODINATION OF TYROSINE WITH IODIC ACID

Reducing agent	$A_{310.7}/A_{292.0}$
None[a]	0.19
Citric acid (0.22 mmol)	0.52
Oxalic acid (0.22 mmol)	0.52
Sodium sulfite (0.22 mmol)	2.01
Sodium sulfite (0.22 mmol) + citric acid (0.22 mmol)	1.99
Sodium sulfite (0.22 mmol) + oxalic acid (0.22 mmol)	2.01

Note. Total volume of the reaction mixture was 1.9 ml, containing 0.5 ml acetic acid, 1.4 ml distilled water, 0.02 mmol tyrosine, 0.2 mmol iodic acid, and the indicated amounts of reducing agent(s). The mixture was incubated with magnetic stirring at 55°C for 20 min before 0.75 ml of 1.5 M sodium sulfite was added to stop the reaction. A 0.1-ml aliquot of the reaction mixture was withdrawn and mixed with 5 ml of 0.1 N NaOH. The ratio $A_{310.7}/A_{292.0}$ was determined.

[a] Tyrosine was added after iodic acid was reduced by sodium sulfite.

Effects of citric acid and oxalic acid on iodination reaction. Citric acid and oxalic acid had been observed to slowly reduce iodic acid (16,17). It was therefore of interest to determine whether citric or oxalic acid could replace sodium sulfite in the synthesis of DIT. The data presented in Table 1 show that tyrosine was only slightly iodinated by iodic acid in the presence of citric or oxalic acid, as compared with sulfite. Neither citric nor oxalic acid exerted a sizable effect on the reduction of iodic acid by sulfite. Sulfite therefore remains the preferred reductant for iodic acid in the present method.

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