PHOSPHONYLATION OF L-DOPA WITH SODIUM DIPHOSPHONATE IN AQUEOUS SOLUTION

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Phosphonylation of 3',4'-dihydroxy-L-phenylalanine (L-DOPA) has been achieved using inorganic diphosphonate (DP) in aqueous solution. The optimum condition for the phosphonylation of L-DOPA with DP is L-DOPA : DP = 1 : 10, pH 6 and 25 ºC. 3'-Hydroxy-4'-phosphonyl-L-phenylalanine and 3'-phosphonyl-4'-hydroxy-L-phenylalanine were synthesized with the total yield of more than 62 %. The phosphonylated products of L-DOPA were stable in neutral and acidic solution. The reaction mechanism of L-DOPA with DP was discussed.

(INTRODUCTION)

Perkinson’s disease is a progressive disabling neurodegenerative disorder. 3',4'-Dihydroxy-L-phenylalanine (L-DOPA) has been applied to Perkinson’s disease, but a poor solubility and a strong side effect are serious problem. By the introduction of phosphoryl group L-DOPA is expected to possess higher solubility and reduce a dose of L-DOPA. So sustained release of L-DOPA would be expected from phosphorylated L-DOPA, and then amount of dose and a side effect would be reduced.

![Structure of L-DOPA](image)

Sodium cyclo-triphosphate (P₃m), Na₃P₃O₉, which is simple inorganic condensed phosphate having a six-membered ring, is a very attractive phosphorylation reagent for synthesizing biologically important compounds in one step in aqueous solution. The phosphorylation with P₃m proceeded under alkaline condition such as pH 12. However, L-DOPA is unstable and changes to black at pH 12. Therefore, L-DOPA should be reacted under weak acidic condition.

Disodium diphosphonate (DP), Na₂P₂H₂O₅, is efficient phosphonylating agent to react with several nucleophiles. One of the authors reported the phosphorylation of nucleoside 5'-monophosphate and nucleoside 5'-diphosphate by DP. According to Scheme 1, the phosphonyl transfer reaction was performed at 5'-monophosphate of the \(\beta\)-D-ribofuranosyl unit to form an H-phosphonate analogue of 5'-ADP. The phosphorylation with DP proceeded under weak acidic condition of pH 4 - 6. It would be possible to apply this phosphonyl transfer reaction to L-DOPA.

![Scheme 1](image)
RESULTS AND DISCUSSION

Reaction of 3',4'-dihydroxy-L-phenylalanine (L-DOPA) with DP

The phosphonylation of L-DOPA with DP was performed in aqueous solution. Figure 2 show the HPLC profiles for the reaction solution of L-DOPA (0.1 M) and DP (1.0 M) incubated at pH 6 at 25 ºC. The peak of the product appeared at a retention time of 3.0 min. Although the HPLC profiles showed a single peak attributable to the reaction product, $^{31}$P NMR spectra (Fig. 4) showed two phosphonate esters, 1 and 2, which could not be separated by HPLC.

The total yield of products increased gradually with the increase of reaction time to reach the maximum of 62 % after 2 d and then decreased gradually.

![FIGURE 2 HPLC profiles of the reaction solution of L-DOPA (0.1 M) with DP (1.0 M) at pH 6 and 25 ºC](image)

Table 1 summarizes the total yields of products 1 and 2 under various reaction conditions. At molar ratios of L-DOPA : DP = 1 : 10 (0.1 M : 1.0 M), 1 : 5 (0.1 M : 0.5 M), 1 : 3 (0.1 M : 0.3 M), 1 : 2 (0.1 M : 0.2 M), and 1 : 1 (0.1 M : 0.1 M), the total yields of products 1 and 2 were 62, 49, 33, 28, and 15 %, respectively. Therefore, a molar ratio of L-DOPA : DP = 1 : 10 is preferable. One of the authors already reported the reaction of adenosine 5'-monophosphate and DP, suggesting that the most suitable molar ratio is 5'-AMP : DP = 0.1 M : 1.5 M. DP excess condition is suitable for the phosphonylation by DP.

<table>
<thead>
<tr>
<th>Conc. (M)</th>
<th>pH</th>
<th>Temp. (ºC)</th>
<th>Time (d)</th>
<th>Total Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-DOPA</td>
<td>DP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>1.0</td>
<td>6</td>
<td>10</td>
<td>12</td>
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<tr>
<td></td>
<td></td>
<td>6</td>
<td>25</td>
<td>62</td>
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<tr>
<td></td>
<td></td>
<td>6</td>
<td>40</td>
<td>44</td>
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<td></td>
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<td>5</td>
<td>25</td>
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<td>4</td>
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<tr>
<td>0.1</td>
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<tr>
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<td>0.1</td>
<td>0.1</td>
<td>6</td>
<td>25</td>
<td>27</td>
</tr>
</tbody>
</table>

At a molar ratio of 1 : 10 and 25 ºC, the total yields of products 1 and 2 were 10 % at pH 4, 37 % at pH 5, and 62 % at pH 6. On the other hand, at a molar ratio of 1 : 10 and 25 ºC, the total yield and the time to attain products 1 and 2 were 74 % and 8 hours at pH 7, 69 % and 1 hour at pH 8, 63 % and 30 minutes at pH 9. However, in basic solution (pH 7 – 9) the phosphonylated products is very unstable as shown in Fig. 3 and the reaction solution changes to black. Therefore, pH 6 is suitable pH.

![FIGURE 3 The effect of pH on the total yield of phosphonylated products in the reaction of L-DOPA (0.1 M) with DP (1.0 M) at 25 ºC](image)

At a molar ratio of 1 : 10 and pH 6, the total yields of products 1 and 2 were 51 % at 10 ºC, 62 % at 25 ºC, and 44 % at 40 ºC. The yield at 25 ºC remained constant after 7 d without hydrolysis of phosphonate (P III) and L-DOPA. Therefore, preferable temperature is 25 ºC. Consequently, the optimum condition for the phosphonylation of L-DOPA with DP is L-DOPA : DP = 1 : 10, pH 6 and 25 ºC.

To identify the reaction product in the phosphonylation of L-DOPA with DP, $^{31}$P and $^1$H NMR spectra were measured. A proton-decoupled spectrum of the phosphonylated product of L-DOPA (Fig. 4b) shows four singlets. Two singlets are assigned to P III (4.02 ppm) and DP (-4.10 ppm) on the basis of the chemical shifts for the authentic samples. Two unknown singlets (4.48 and 4.58 ppm) are phosphonylated products (1 and 2), although the HPLC profiles showed one peak due to the reaction product. As shown in Fig. 4a, a singlet at 4.48 and 4.58 ppm in the $^1$H decoupling spectrum became doublets with large $J$ values on $^1$H non-decoupling spectrum, which is characteristic for P-H bond. The doublets (1 and 2) show a splitting of 660 Hz for the hydrogen atom attached directly to a phosphorus atom. A previous work indicated that the phosphonylation product of adenosine 5'-monophosphate with DP is P-H phosphonate esters with a P-H bond. These products showed a characteristic splitting of 665.5 Hz for hydrogen atom.
attached directly to a phosphorus atom.

In order to assign the site of phosphonylation of L-DOPA, \(^{31}\)P-\(^1\)H heteronuclear multiple bond correlation (HMBC) 2D-NMR spectrum was measured.

![Diagram of phosphonylation of L-DOPA](image)

**FIGURE 4** \(^{31}\)P NMR spectra of reaction solution of L-DOPA (0.1 M) with DP (0.3 M) at pH 6, 25 °C, 9 d (a) \(^1\)H non-decoupling spectrum, (b) \(^1\)H decoupling spectrum

Figure 5 shows the \(^{31}\)P-\(^1\)H HMBC 2D-NMR spectrum of products \(1\) and \(2\). The peak at 4.58 ppm of the \(^{31}\)P NMR spectrum was assigned to product \(1\). A correlation between \(^{31}\)P signal at 4.58 ppm of product \(1\) and \(^1\)H signal at 7.00 ppm was observed. The doublet at 7.00 ppm could be assigned to H-5’ of product \(1\). The down-field shift from 6.60 ppm due to L-DOPA itself to 7.00 ppm indicates the phosphonylation of L-DOPA with DP. This assignment was confirmed by \(^1\)H-\(^1\)H COSY NMR spectrum. The \(^{31}\)J_P,H value was 665 Hz, which is obtained from the \(^{31}\)P NMR data. Therefore, product \(1\) was verified to be 3’-phosphonyl-4’-hydroxy-L-phenylalanine.

**Stability of phosphonylated L-DOPA**

Figure 6 shows the time dependence of the amount of L-DOPA and its phosphonylated products. The yields of the products \(1\) and \(2\) reached maximum after 2 day (62 %) and then the phosphorylated products gradually hydrolyzed to L-DOPA and phosphonate (P(VII)).

![Graph of changes in concentrations](image)

**FIGURE 6** Changes of the amounts of \(1\) and \(2\) in the reaction of L-DOPA (0.1 M) with DP (1.0 M) at pH 6 and 25 °C

◆ : product (\(1 + 2\)), ● : L-DOPA

Figure 7a shows the stability of phosphorylated products at 10, 25, and 40 °C. The products were synthesized under the reaction condition of pH 9, 40 °C, and a molar ratio of L-DOPA : P3m = 0.1 M : 1.0 M for 30 min, and then pH was adjusted to 3. At 40 °C, phosphorylated products decomposed gradually and decreased to 5 % after 6 days. At 25 °C, phosphorylated products were stable after 4 days and then decomposed gradually. On the other hand, phosphorylated products were stable at 10 °C. Therefore, phosphorylated products of L-DOPA were stable at 10 °C.

Figure 7b shows the stability of phosphorylated products at pH 3, 5, 7, and 9. The products were synthesized under the reaction condition of pH 9, 40 °C, and a molar ratio of L-DOPA : DP = 0.1 M : 1.0 M for 30 min, and then temperature was adjusted to 10 °C. At pH 9, phosphorylated products were decomposed remarkably and decreased to 8 % after 6 days. On the other hand, phosphorylated products were stable at pH 3 – 7. Therefore, phosphorylated
products of L-DOPA were stable in neutral and acidic solution. Although the phosphonylated products undergo hydrolysis to L-DOPA and P^III with the passage of time, they are stable at 10 °C and pH 3 – 7.

![Figure 7](image_url)

**FIGURE 7** Stability of phosphonylated L-DOPA synthesized under the condition of L-DOPA : DP = 0.1 M : 1.0 M, pH 9, 40 °C, and 30 min (a) : pH 3 and 10 °C, ▲ : pH 3 and 25 °C, ■ : pH 3 and 40 °C, (b) : pH 3 and 10 °C, ■ : pH 5 and 10 °C, ▲ : pH 7 and 10 °C, ● : pH 9 and 10 °C

**Reaction mechanism of L-DOPA with DP**

The reaction of L-DOPA with DP may be explained by the following mechanism. At pH 6, phosphonyl group is easily transferred from DP to the hydroxyl group on L-DOPA (Scheme 2). The 3'-OH and 4'-OH of L-DOPA reacts with DP to form 3'-hydroxyl-4'-phosphonyl-L-phenylalanine (1) and 3'-phosphonyl-4'-hydroxyl-L-phenylalanine (2). Therefore, we concluded that the side chain on L-DOPA did not react.

![Scheme 2](image_url)

**SCHEME 2** Reaction mechanism of L-DOPA with DP

**Conclusion**

In the reaction of L-DOPA with DP, 3'-hydroxyl-4'-phosphonyl-L-phenylalanine (1) and 3'-phosphonyl-4'-hydroxyl-L-phenylalanine (2) were synthesized in the total yield of 62 %. These results suggest that the synthesis of novel anionic molecules of L-DOPA containing phosphonyl group is a promising area for the application to prodrug with enhanced solubility.

**EXPERIMENTAL**

**Materials and methods**

Disodium diphosphonate Na_2P_2H_2O_5, (DP), was prepared according to the previous paper. L-DOPA and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). Other reagents were purchased from Wako Chemicals (Osaka, Japan).

³¹P NMR spectra with and without broad band ¹H decoupling and ³¹P-¹H 2D HMBC spectra were obtained with a Varian INOVA-500 spectrometer using 85 % H_3PO_4 as an external standard.

HPLC analysis was carried out with a JASCO HPLC system consisting of a PU-2080 Plus pump, a UV-2075 Plus detector (JASCO, Japan). An CAPCELLPACK column (250 × 10.0 mm I.D., 5 µm, Shiseido, Japan) was used and the column temperature was maintained at 25 °C. The amount of sample injection was 100 µL. An isocratic elution technique using 10 % methanol-0.025 M phosphate solution was employed. The flow rate of the eluent was 1.0 mL min⁻¹. The UV absorbance of the effluent was monitored continuously at 280 nm. The system control, data collection, and data analysis were carried by JASCO- BORWIN system.

LC-MS measurement was performed by using an Exactive (Thermo Fisher Scientific).
The procedure for the syntheses of products 1 and 2

The reaction of L-DOPA (0.1 M, 2.5 mL) with DP (1.0 M, 2.5 mL) was carried out at pH 6 by adding 6 M sodium hydroxide aqueous solution at 25 °C. The yields and the structures of the products were determined by HPLC, NMR and LC-MS measurement.

3'-hydroxyl-4'-phosphonyl-L-phenylalanine (product 1) : \(^1\)H NMR (D\(_2\)O) \(\delta\) 6.85 (1H, s, H-2'), 7.00 (1H, d, \(J_{\text{H5',H6'}} = 8.5\) Hz, H-5'), 6.76 (1H, dd, \(J_{\text{H2',H6'}} = 2.0\) Hz, \(J_{\text{H5',H6'}} = 8.5\) Hz, H-6'), 3.81 (1H, m, H-2), 2.85 (1H, dd, \(J_{\text{H3A,H3B}} = 14.5\) Hz, \(J_{\text{H2,H3A}} = 5\) Hz, H-3\(\alpha\)), 3.02 (1H, dd, \(J_{\text{H3A,H3B}} = 14.5\) Hz, \(J_{\text{H2,H3B}} = 5\) Hz, H-3\(\beta\)), 6.96 (1H, d, \(J_{\text{P,H}} = 665\) Hz, P-H). \(^{31}\)P NMR (D\(_2\)O) \(\delta\) 4.58 (1P, d, \(J_{\text{P,H}} = 665\) Hz, P-H).

LC-MS \(m/z\) : Calced \([\text{C}_9\text{H}_{12}\text{NO}_6\text{P}+\text{H}]^+\) 262.04 Found : 262.04.

3'-phosphonyl-4'-hydroxy-L-phenylalanine (product 2) : \(^1\)H NMR (D\(_2\)O) \(\delta\) 6.95 (1H, s, H-2'), 6.76 (1H, d, \(J_{\text{H5',H6'}} = 8.5\) Hz, H-5'), 6.76 (1H, dd, \(J_{\text{H2',H6'}} = 1.5\) Hz, \(J_{\text{H5',H6'}} = 8.5\) Hz, H-6'), 3.84 (1H, m, H-2), 2.91 (1H, dd, \(J_{\text{H3A,H3B'}} = 14.5\) Hz, \(J_{\text{H2,H3A}} = 5\) Hz, H-3\(\alpha\)), 3.17 (1H, dd, \(J_{\text{H3A,H3B'}} = 14.5\) Hz, \(J_{\text{H2,H3B'}} = 5\) Hz, H-3\(\beta\)), 6.94 (1H, d, \(J_{\text{P,H}} = 664\) Hz, P-H). \(^{31}\)P NMR (D\(_2\)O) \(\delta\) 4.48 (1P, d, \(J_{\text{P,H}} = 664\) Hz, P-H).

LC-MS \(m/z\) : Calced \([\text{C}_9\text{H}_{12}\text{NO}_6\text{P}+\text{H}]^+\) 262.04 Found : 262.04.

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REFERENCES