SHORT COMMUNICATION

**Convenient Preparation of Poly(L-histidine) by
the Direct Polymerization of L-Histidine
or *N*^{im}-Benzyl-L-histidine with
Diphenylphosphoryl Azide**

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Poly(L-histidine) is a very interesting poly(α-amino acid) as a synthetic functional polymer or as a model for the functional biopolymer such as enzymes. It is known, however, that poly(L-histidine) can not be prepared by the Fuchs–Farthing method¹ which is very popular for preparing poly(α-amino acid)s. A synthetic route with several steps involving the synthesis of NCA (α-amino acid *N*-carboxyanhydride) by the rather classical Leuchs method,² have been used for the preparation of poly(L-histidine) as shown in Scheme I-(A).³ Among the many successful attempts for the direct polycondensation of amino acids,^{4,5} that of Guilly *et al.*⁵ for the one-step preparation of poly(L-

histidine) by the polymerization of L-histidine with iodine-phosphonic acid esters was unsuccessful. The simplification of the synthetic route for poly(L-histidine) is still necessary.

Diphenylphosphoryl azide (DPPA) has been used as a convenient reagent for racemization-free peptide synthesis, since it was synthesized by Shioiri *et al.* in 1972.⁶ Recently, we reported that this reagent can also be used successfully for the polymerization of amino acids or peptides.^{7–10} A polypeptide can easily be prepared by simply stirring the solution or the suspension of amino acid or peptide in a suitable solvent with DPPA and a tertiary amine such as triethylamine. One of the merits of DPPA in peptide

Scheme I. Synthetic routes of poly(L-histidine) (A) with the NCA method proposed by Patchornik *et al.*,³ and (B) with DPPA method: Bzl, benzyl; Z, benzyloxycarbonyl.

synthesis is that the protection of imidazole group is not necessary for the preparation of histidine-containing peptide,¹¹ since the reaction proceeds *via* acyl azide¹² similarly as in the case of the well-known azide coupling method.

In the present study, the one-step preparation of poly(L-histidine) by the polymerization of L-histidine with DPPA was investigated to simplify the preparative method for poly(L-histidine). The polymerization of *N*^{im}-benzyl-L-histidine to give

Figure 1. Infrared absorption spectra of poly(L-histidine)s prepared by (a) DPPA method, and (b) NCA method.³

Figure 2. ¹³C NMR spectra of poly(L-histidine)s in aqueous solution (pD ≈ 3) measured at 15.04 MHz: repetition time, 2 s; (a) DPPA method; (b) NCA method.³

poly(*N*^{im}-benzyl-L-histidine) has also been reported. The synthetic routes proposed in the present study are shown in Scheme I-(B).

To stirred suspensions of L-histidine (500 mg) in DMSO or some other solvent were added DPPA and triethylamine or another base. These mixtures were stirred for 1 h at 5–8°C followed by stirring under various reaction conditions. Variation in the reactions was made by changing the amounts of solvent or reagent, reaction time and other reaction conditions. The polymers were precipitated by the addition of saturated aqueous sodium chloride (100 ml). The sirupy precipitates were collected and washed with water (× 3), 5% aqueous sodium bicarbonate (× 3), and with water (× 3), by decantation. The residues were treated with acetone to give the powders which were centrifuged and washed with acetone (× 3), methanol (× 3), and ether (× 1), and then dried *in vacuo*.

The IR and ¹³C NMR spectra of poly(L-histidine) thus obtained were found to show the same patterns as those of poly(L-histidine)³ prepared by the NCA method shown in Figures 1 and 2.

The reaction conditions, such as a reaction time of two days at room temperature or a reaction time of six days at 5–8°C using 1 ml of DMSO, 1.3 equiv mol of DPPA and 2.3 equiv mol of triethylamine to 500 mg of L-histidine, were considered to be most suitable. Under these reaction conditions, poly(L-histidine) of $[\eta] = 0.16$ (in DCA at 25°C) can be obtained in 24–25% yield directly from L-histidine. The cause of the low yield is considered due to the removal of very low molec-

ular weight peptides by the thin-layer chromatographies carried out on the mother liquor. However, this yield of poly(L-histidine) is no worse than the overall yield by the NCA method, since the synthetic route *via* NCA requires several reaction steps.

The polymerizations of *N*^{im}-benzyl-L-histidine were also investigated. The polymers were precipitated with 5% aqueous citric acid saturated with sodium chloride. The sirupy precipitates were collected and washed with 5% aqueous citric acid (× 3), water (× 3), warm 5% aqueous sodium bicarbonate (× 3), and then with water (× 3) by decantation. The residues were treated with ether to give the powders which were centrifuged, washed with ether and then dried *in vacuo*. The results were compared with each other as in the case of the polymerizations of L-histidine. Consequently, the optimum reaction conditions were determined as follow: Reaction time of one day at room temperature employing 0.6 ml of DMSO, 1.3 equiv mol of DPPA and 2.3 equiv mol of triethylamine to 300 mg of *N*^{im}-benzyl-L-histidine, followed by the reaction for another one day with the additional same amount of solvent and reagents, and final heating at 90°C for one hour at the end of the polymerization. Poly(*N*^{im}-benzyl-L-histidine), which shows an intrinsic viscosity ($[\eta]$) of 0.12 in DCA at 25°C, can be obtained in a 46% yield by these optimum conditions.

The IR spectrum of poly(*N*^{im}-benzyl-L-histidine) thus obtained showed the same pattern as that of poly(*N*^{im}-benzyl-L-histidine)³ prepared by NCA method as shown in Figure 3.

The amino acid analyses and the silica-gel thin-

Figure 3. Infrared absorption spectra of poly(*N*^{im}-benzyl-L-histidine)s prepared by (a) DPPA method, and (b) NCA method.³

layer chromatographies, carried out on two different solvent systems (upper layer of 1-butanol–acetic acid–water = 4:1:5 and 1-butanol–pyridine–acetic acid–water = 15:10:3:12), for the acid hydrolysates of poly(L-histidine) and poly(*N*^{im}-benzyl-L-histidine) showed only starting amino acids. This, along with the IR and NMR spectra, suggest the absence of side reactions induced by the Curtius rearrangement which sometimes accompanies azide coupling.

It was found that poly(L-histidine) and poly(*N*^{im}-benzyl-L-histidine) could be prepared directly from L-histidine and *N*^{im}-benzyl-L-histidine, respectively, by the DPPA method. The procedures for the polymerization reaction or working up are simple and easy. This novel method may be recommended as a convenient synthetic method for poly(L-histidine).

Further investigation will be made for obtaining poly(L-histidine) of higher molecular weight in higher yield. In particular, polymerization reactions using polymer matrices must be investigated in further detail.

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