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Studies on Chitin

11. Graft Copolymerization of γ-Methyl L-Glutamate NCA onto Water-Soluble Chitin

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SUMMARY

Graft copolymerization of γ-methyl L-glutamate NCA onto water-soluble chitin with 50% deacetylation was carried out in an aqueous medium to give a novel chitin derivative having polypeptide side chains of different lengths. The grafting efficiency was fairly high on account of the homogeneous reaction conditions which became feasible by taking advantage of the water-soluble nature of the chitin. Alkaline hydrolysis of the graft copolymer converted the ester groups in the side chains to carboxyl groups.

INTRODUCTION

Chitin usually occurs in complexed forms with proteins in nature, and some of the amino groups of chitin were shown to be covalently linked to protein chains (1,2). These complexed forms, not chitin alone, actually play important roles in biological activities of chitin-containing organisms. Our continuing interest in the chemical modification of chitin has been directed to preparing chitin derivatives having polypeptide side chains as models for naturally occurring chitin. They are anticipated to shed light on the nature of natural chitin including structural and biological functions.

Although the chemical modification of chitin generally proceeds rather reluctantly owing to the lack of solubility in ordinary solvents, we have explored some reactions on water-soluble chitin with about 50% deacetylation in aqueous solutions or in pyridine (3-5). The utilization of water-soluble chitin thus proved to have a high potential of providing a general method for preparing derivatives from the intractable chitin. The results of a series of reactions suggested a possibility of graft copolymerization of an α-amino acid NCA at the amino groups under homogeneous conditions. In this communication, we report some preliminary results of the graft copolymerization of γ-methyl L-glutamate NCA onto water-soluble chitin to afford a derivative with polypeptide side chains attached at the free amino groups.

EXPERIMENTAL

Graft Copolymerization

γ-Methyl L-glutamate NCA, 0.50 g (1.9 molar equivalents to the amino groups of water-soluble chitin to be used), was dissolved in 10 ml of ethyl acetate by heating at 64°C. A trace amount of insoluble part was filtered, and the filtrate was added to a solution of 0.50 g of water-soluble chitin in 50 ml of water kept at 0°C in an ice bath with stirring. The mixture was stirred at 0°C for 2 hr to give a white cloudy suspension. It was poured into 650 ml of dimethylformamide (DMF) to precipitate the graft copolymer, and then 60 ml of water was added to dissolve γ-methyl

L-glutamate formed by hydrolysis of the NCA. After stirring the mixture for 5 min, the precipitate was filtered, washed thoroughly with acetone, and dried. The yield of a white powdery material was 0.81 g.

The graft copolymerization with 8.6 molar equivalents of NCA was carried out in the same manner, but the reaction mixture became gelatinous immediately.

Hydrolysis of the Copolymer

A part of the sample, 52 mg, of the graft copolymer obtained above was added to 40 ml of 1M aqueous sodium carbonate, and the mixture was heated at 70°C for 1 hr with stirring. The resulting clear solution was dialyzed against deionized water for 44 hr. The solution was concentrated and freeze-dried to give 34 mg of a white solid.

RESULTS AND DISCUSSION

Graft Copolymerization

γ-Methyl L-glutamate NCA was chosen for the reaction with water-soluble chitin since it would afford a chitin derivative having poly(γ-methyl L-glutamate) side chains which, on hydrolysis, give rise to the formation of carboxyl groups to render the derivative water-soluble. The reaction was carried out in a water/ethyl acetate medium as suggested by a report on the reaction of casein and the NCA (6).

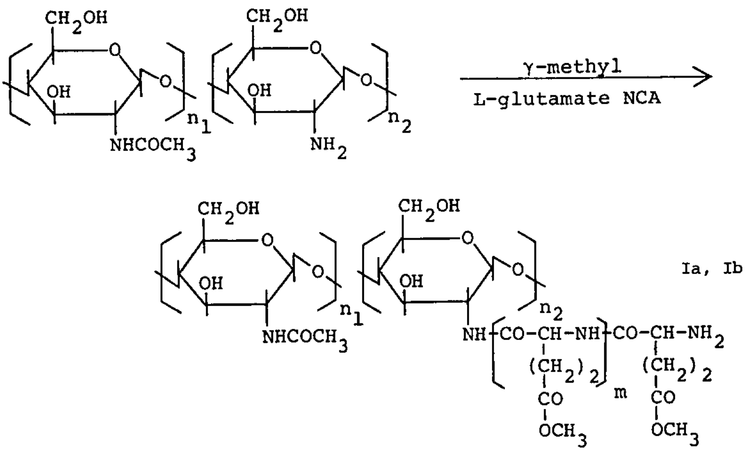
An aqueous solution of water-soluble chitin was treated with the NCA dissolved in ethyl acetate. The graft copolymer was isolated by pouring the mixture into DMF. Some side reactions may be possible, but the results of control reactions in the absence of water-soluble chitin confirmed rather simple nature of this reaction system; the hydrolysis of the NCA to the amino acid was by far the most predominant as a side reaction, and no homopolymerization was observed under the conditions used. Water was thus added to the precipitation mixture to make the DMF/water ratio 6:1 for complete dissolution of the amino acid. The homopolymer, if formed, should have been removed by the precipitation process in DMF.

The yield of the copolymer, hence the average degree of graft polymerization, increased with an increase in the ratio of the amount of NCA to that of free amino groups in the chitin. The degrees were calculated on the basis of the weight to be 1.6 and 6.4 for the copolymer (Ia) prepared at the ratio of 1.9 and that (Ib) at the ratio of 8.6, respectively, indicating that fairly high grafting efficiency was achieved under these homogeneous conditions. The results are summarized in Table 1. Copolymers Ia and Ib were obtained as white powdery materials. They are not soluble in water any more and swells in m-cresol.

Table 1. Graft copolymerization of γ-methyl L-glutamate NCA onto water-soluble chitin

Water-soluble chitin, g	γ-Methyl L-glutamate NCA, g	$\frac{\text{NCA, mol}}{\text{NH}_2\text{, mol}}$	Yield, g	DP of polypeptide chains[a]
0.50	0.50	1.9	0.81	1.6
0.51	2.23	8.6	1.80	6.4

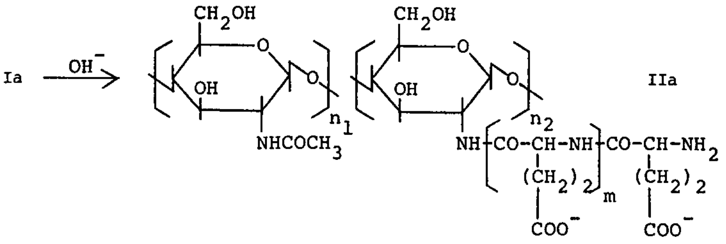
[a] Average degree of polymerization determined on the basis of the assumption that every free amino group of the chitin has a polypeptide chain.



Ia, Ib

Hydrolysis of the Ester Groups

The ester groups in the side chains of the resulting graft copolymer were then hydrolyzed to give a carboxyl-containing chitin derivative. A suspension of Ia in 1M sodium carbonate became a clear solution in about 0.5 hr at 70°C, and 1 hr hydrolysis was satisfactory judging from the change in the IR spectrum. The hydrolyzed copolymer (IIa) was isolated as a white solid. It shows good solubility in water and m-cresol.



IIa

Characterization

In the IR spectra of copolymers Ia and Ib, an ester band appeared at 1730 cm^{-1}, while it disappeared completely by the alkaline treatment. The ester and amide (1650 and 1550 cm^{-1}) bands were stronger with copolymer Ib than with copolymer Ia as expected from the difference in the length of the side chain polypeptides. It is noteworthy that in the spectra of copolymers Ia, Ib, and IIa, complex bands due to pyranose rings of chitin (1000-1150 cm^{-1}) were weaker and amide bands were stronger than those of the original water-soluble chitin. This is interpreted in terms of a decrease in the relative amount of pyranose rings and an increase in the peptide bonds as a result of graft copolymerization. These IR spectral data support the presence of different amounts of the peptide component along with the chitin component.

The possibility that the polymers obtained here are simply blends of two kinds of polymers can be ruled out from their solubility data. The remarkable solubility of copolymer IIa in water and m-cresol at any temperature is especially a good indication of the occurrence of graft copolymerization, since water-soluble chitin is soluble only in water under particular conditions using ice.

Consequently, the graft copolymerization of γ-methyl L-glutamate NCA onto water-soluble chitin was confirmed to be achieved successfully in water/ethyl acetate. The grafting efficiency was high owing to the homogeneous conditions, although the NCA is extremely susceptible to hydrolysis. Other NCA's may probably be applicable to prepare various chitin-protein complexes under similar conditions. The length of the polypeptide side chains can be regulated easily, and this implies a wide range of applications of the products.

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