SYNTHESIS AND CHARACTERIZATION OF PHOSPHORYLATED CHITOSAN BY NEW METHOD

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Abstract

Chitosan, a natural based polymer it is non-toxic, biocompatible and biodegradable. Chemical modification of chitosan to generate new bifunctional materials and finally would bring new properties depending on the nature of the group introduced. Several techniques to obtain phosphate derivatives of chiosan have been proposed due to the interesting biological and chemical properties of such compounds. In our present study, we prepared phosphorylated chitosan (P-chitosan) by the $H_3PO_4/P_2O5/Et_3O_4$/hexanol method. Previously, the P-chitosan was carried out by P_2O_5/CH_3SO_3H or H_3PO_4/Urea/DMF method. The demerit of this method, the molecular weight was drastically decreased due the using of strong methane sulphonic acid. From our present method, we got high yield, high molecular weight and high degree of substitution (DS) of P-chitosan (up to 1.67). The structure of P-chitosan was confirmed by FT-IR, ^{13}C-NMR, ^{31}P-NMR, elemental, XRD, TGA and SEM studies. The molecular weight and crystallinity of P-chitosan was drastically decreased due to the phosphorylation. The P-chitosan was less thermal stability than chitosan due to the phosphorylation. The SEM studies of P-chitosan showed very porous and rough morphology due to the phosphorylation. X-ray diffraction studies showed that the P-chitosan has a poor crystallinity comparing to original chitosan. The relatively high levels of phosphorous incorporation attainable with this method may be used orthopedic and tissue engineering applications.

Introduction

Chitosan, the fully or partially deacetylated form of chitin, the principal component of living organisms such as fungi and crustaceans, contains 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose groups. This polymer is known to be nontoxic as well as being enzymatically biodegradable. Much attention has been paid to its biomedical, ecological, and industrial application in the past decades. Chitosan and its derivatives have been reported to be useful biomedical applications such as wound healing and dressings, drug delivery agents, anti-cholesterolemic agents, blood anti-coagulants, anti-tumor agents, and immunoadjuvants [1].Chemical modification of chitosan to generate new bifunctional materials is of prime interest because the modification would not change the fundamental skeleton of chitosan, would keep the original physicochemical and biochemical properties and finally would bring new properties depending on the nature of the group introduced. Several techniques to obtain phosphate derivatives of chitosan have been proposed due to interesting biological and chemical properties of such compounds. Among other, it could exhibit bactericidal [2] and metal chelating properties [3]. Introduction of groups such as phosphonic acid or phosphanate onto chitosan by reaction of phosphorylating agent onto the amino groups are known to increase the chelating properties [4-6] of chitosan and could modify its solubility properties. Phosphorylation of hydroxyl functions of

chitosan to give phosphonate has been studied according two main ways. In one hand, the reaction is carried out between chitosan hydroxyl functions and phosphorous pentoxide functions in the presence of methane sulphonic acid [7-10]. On the other hand, the chitosan, the chitosan hydroxyl functions are reacted with phosphoric acid in the presence of urea [11]. The use of such derivatives concerns mainly biomedical [9, 11] or metal chelating fields [7-10]. Phosphate derivatives of chitosan may also be obtained by interpolymer linkage of chitosan with tripolyphosphate or polyphosphate [12, 13]. Few work deals with the introduction of α-aminomethylphosphonic acid functions onto chitosan using the Kabachnik-Fields reactions [14, 15] in the spite of the interest of such groups [4-6].

In our present study, we prepared P-chitosan by the $H_3PO_4/P_2O_5/Et_3O_4$/hexanol method. This method was first proposed by [16] for the hemi synthesis of a water soluble and non-degraded flame-resistant textile material was described. The choice of this method was based on the possibility of obtaining products free of biologically hazardous chemical compounds present in products obtained by other available methods [17, 18]. In this work, the synthesis of P- chitosan was optimized in terms of reaction parameters. And also, the P-chitosan was characterized by FTIR, ^{31}P- and ^{13}C-NMR, solubility, elemental analysis, scanning electron microscopy (SEM), thermogravimetric (TGA) & differential thermal analysis and X-ray diffraction studies (XRD) have been reported.

Material and Methods

Materials

Chitosan (M_W-48,000, degree of deacetylation 85%) was received from Fujibo Company Ltd. Ortho phosphoric acid (85%), triethyl phosphate (97%), hexanol and phosphorous pentoxide were received from Wako Chemicals. All other materials used were of analytical grade.

Synthesis of P-chitosan

3.0 g of chitosan powder was added to the P_2O_5 (10 ml), H_3PO_4 (5 ml), Et_3PO_4 (5 ml) and hexanol (5 ml) mixture in a 100 ml flask. The reaction mixture was stirred continuously for 72 h at 35 ºC. After 72 h the reaction mixture was poured into excess methanol for precipitation. The precipitate was filtered and washed with methanol. The precipitate was dried in vacuum oven at 37 ºC for 24 h. The yield of the product was 78 %. The degree of substitution was found to be 1.27. The synthesis of P-chitosan was shown in Figure 1.

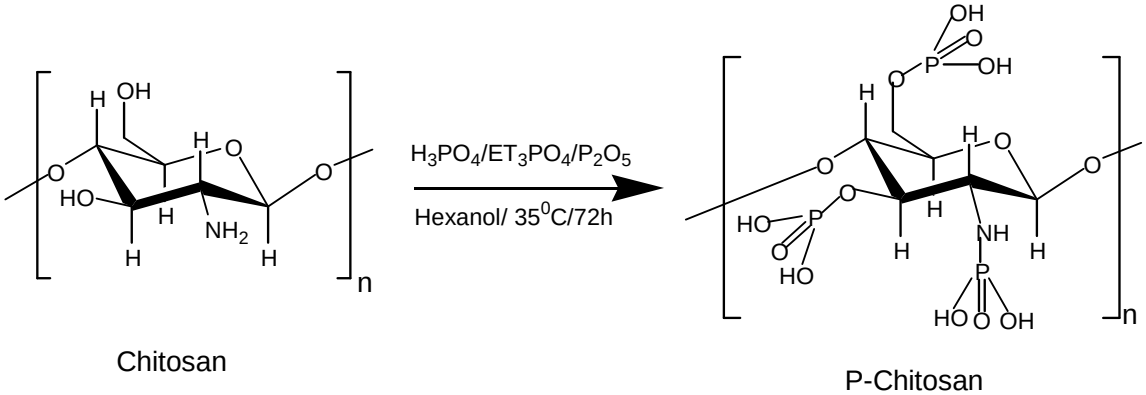


Figure 1: Synthesis of P-chitosan

Measurements

The IR spectra of the polymers were recorded in a Perkin Elmer FT-IR 2000 series spectrophotometer at room temperature with the KBr pellet method. The ^{13}C-NMR spectra of the polymers were recorded with a JEOL JMN-GSX-400 MHz spectrometer in D_2O using tetramethylsilane (TMS) as an internal standard. ^{31}P-NMR spectra of the polymers were recorded with a JEOL JMN-GSX-400 MHz spectrometer in D_2O using 85 % H_3PO_4 as reference standard. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA)

were performed with a SII TG-DTA 6200 thermal analyzer using 2 mg of the sample at a heating rate of 10 ºC/min in nitrogen. The surface morphology of samples was analyzed by scanning electron microscopy (SEM) by using JEOL JSM-6700 microscope. X-ray diffractograms were recorded according to a powder method with a Mac Science M_3X (model no. 1030) diffractometer using CuKα radiation. The molecular weight of the P-chitosan was determined by using GPC Hittachi L-7490 chromatography. A Perkin-Elmer 2400 carbon-hydrogen analyzer was used for elemental analysis. The solubility of the polymers was tested in various polar and non polar solvents by taking 10 mg of polymers in 2 mL of different solvents in a closed test tube and set aside for one day. The solubility of the polymers was noted after 24 h. The phosphorous content was determined spectrometrically by the Kjeldhal method [19]. The degree of substitution was calculated as previously reported [20].

Results and Discussion

Synthesis of P-chitosan

The phosphorylation reaction of chitosan was carried out by using $H_3PO_4/P_2O_5/ET_3PO_4$/ hexanol method. Previously, the phosphorylation of chitosan was carried out and reported by P_2O_5/CH_3SO_3H or H_3PO_4/Urea/DMF method [11, 12]. By using of strong methanesulphonic acid, the molecular weight of the polymer was drastically decreased. And also using urea in the phosphorylation reaction, it is very difficult to purify the polymer. From our method, we got high yield and high degree of substitution of P-chitosan (up to 1.67). And also the purification of P-chitosan is very simple. The DS of the P-chitosan was calculated by comparing the C and N molar ratio obtained from the elemental analysis in each derivative (Table 1). The DS of P-chitosan increased, the C and N content was decreased at the same time the H and P content was increased. This confirms the phosphorylation reaction was occurring in chitosan. The molecular weight of the P-chitosan was around 1.3 x 10^4 (DS-1.27), lower than the original chitosan. The solubility data of P-chitosan in different solvents were shown in Table 2. The phosphorylation of chitosan was increased the solubility properties in aqueous media and in organic solvents. These results suggested that phosphorylation enhanced water solubility. The P-chitosan showed good film forming ability as same as chitosan.

DS (P-chitosan)	Found (%)			
	H	C	N	P
Chitosan	6.93	39.48	7.29	-
1.27	7.01	34.16	5. 23	6.54
1.67	7. 46	35. 21	4.72	7.98

Table 1: Elemental analysis data of P-chitosan

Solvent	Chitosan	P-chitosan
H_2O	Insoluble	Soluble
NaOH (1%)	Insoluble	Soluble
HCl (1%)	Swelling	Soluble
Acetic acid (1%)	Soluble	Soluble
Dimethyl acetamide	Swelling	Insoluble
Dimethyl formamide	Swelling	Insoluble
Dimethyl sulfoxide	Insoluble	Insoluble
Pyridine	Swelling	Insoluble
Acetone	Insoluble	Insoluble
Ethanol	Insoluble	Insoluble

Table 2: Solubility data of P-chitosan

Characterizations

FT-IR Spectra

Figure 2 shows the FTIR spectrum of chitosan and P-chitosan. A broad peak at 3500 cm^{-1} was due to P-OH group. The peak at 1380 cm^{-1} can be attributed to P=O stretching. The peaks at 1050 and 500 cm^{-1} was due to P-OH group. The FTIR spectra confirmed the structure of phosphorylated chitosan.

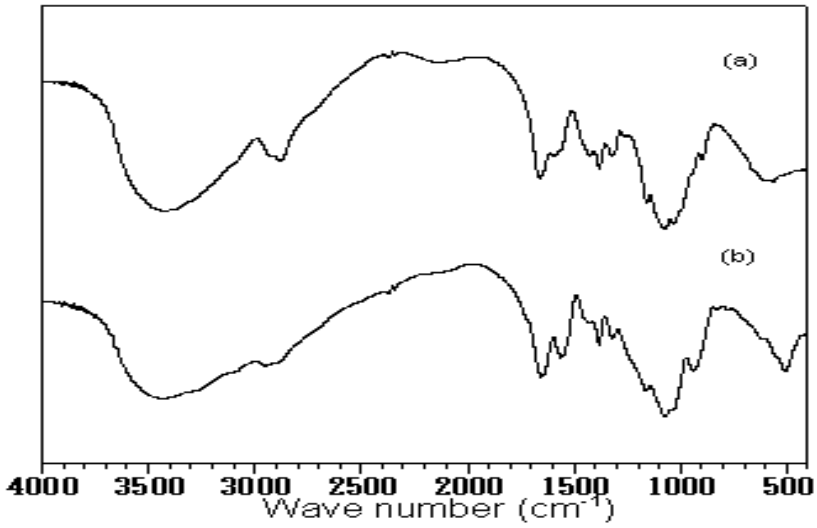


Figure 2: FTIR Spectra of (a) Chitosan and (b) P-chitosan

Solid State ^{13}C-NMR and ^{31}P-NMR spectra

Figure 3 show the ^{13}C-NMR spectra of P-chitosan. The spectra showed that all the signals from each carbon were well separated from each other, and the carbons attached to the substituted hydroxyl group are clearly distinguished from the non-substituted one. The analysis of the spectrum (Fig.3) shows the peaks at 52.85, 56.52, 71.3, 73.80 and 76.2 ppm are attributed to C_2, C_6, C_3, C_5 and C_4 respectively of the pyranose cycle [21, 22] and the peaks located at 98.44, 97.4 and 94.35 ppm are attributed anomeric carbons. Chemical shift for C_6 moves from 57.39 ppm to 57.63 ppm and for C_3 from 72.01 ppm to 70.5 ppm by substitution with phosphate group. It can be also seen that the chemical shift for C_4 also undergo slight change from 75.82 ppm to 76.59 ppm, perhaps by the effect of substitution at the neighboring C_3 position. The peaks at 52.85 ppm (C_2) was shifted to 54.27 ppm, due to the substitution of P-OH group is C_2 position. These results confirmed that phosphorylation reaction was occurring in chitosan.

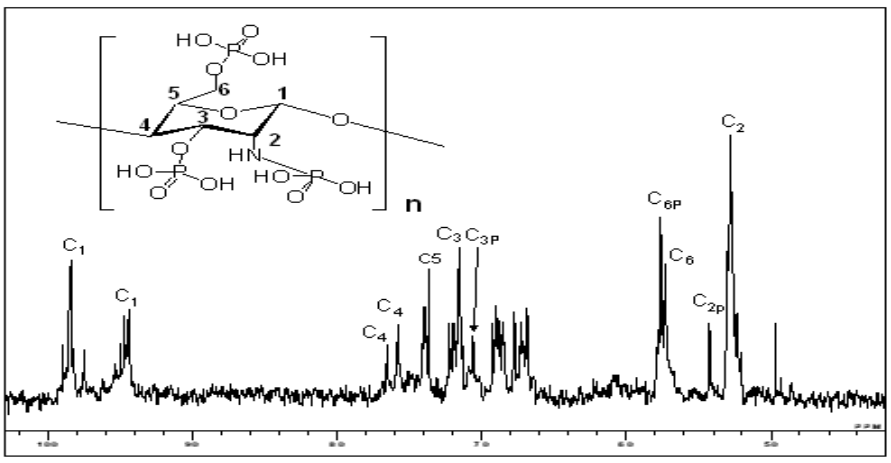


Figure 3: ^{13}C-NMR spectra of P-chitosan

Figure 4 shows the ^{31}P-NMR of the P-chitosan. ^{31}P-NMR spectra exhibited a signal at 0.53 ppm. These peaks are generally expected for ^{31}P functionalities [23, 24]. The ^{31}P-NMR studies confirmed that phosphate groups are chemically bonded to the material.

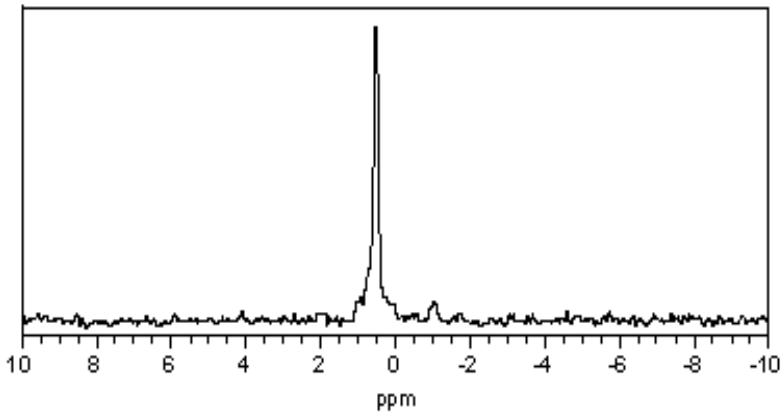


Figure 4: ^{31}P-NMR spectra of P-chitosan

Thermal Properties

Figure 5 show the thermogram of chitosan and P-chitosan. The phosphorylation was also supported by TGA analysis. TGA of chitosan (Fig. 5a) shows a weight loss in two stages. The first stage ranges between 10 and 100 °C. This may correspond to the loss of adsorbed and bound water. The second stage of weight loss starts at 210 °C and continues up to 360 °C during which there was 44% weight loss due to the degradation of chitosan. However, the P-chitosan shows different weight loss. The P-chitosan (Fig.5b) has showing three stages of weight loss. The first stage of weight loss starts at 10 °C and continues up to 180 °C. The second stage from 180 to 220 °C and the third stage from 220 to 360 °C may contribute to the decomposition of different structure of the P-chitosan. The results demonstrate the loss of the thermal stability for P-chitosan more than the original chitosan. Introduction of phosphorours group into polysaccharide structure should disrupt the crystalline structure of chitosan, especially through the loss of the hydrogen bonding.

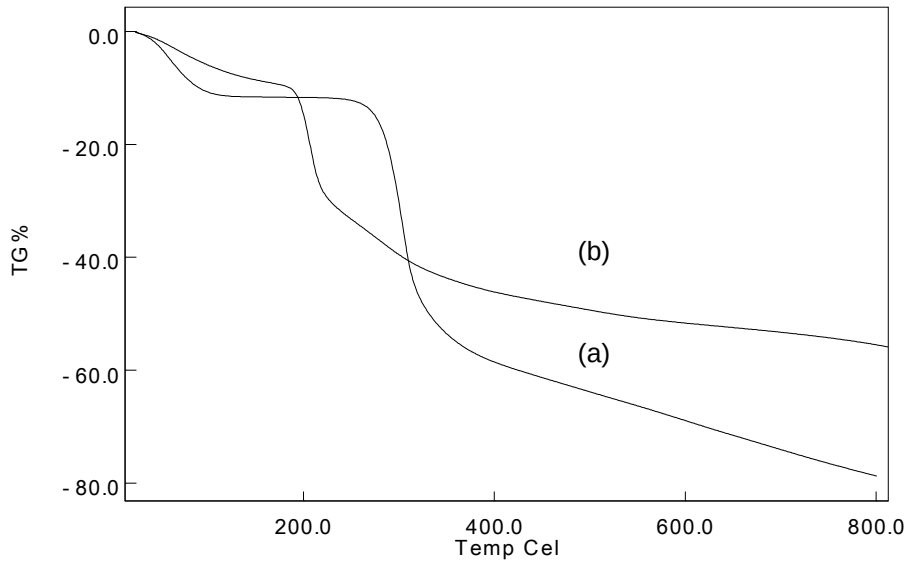


Figure 5: TGA of (a) Chitosan and (b) P-chitosan

Figure 6 shows the differential thermal analysis of chitosan and P-chitosan. The DTA of chitosan shows a broad endothermic peak around 96 °C and sharp exothermic peak around at 320 °C. The

former endothermic peak may be due to the water evaporation. While the latter, may be attributed to the decomposition of chitosan. The endothermic peak of P-chitosan around 98 °C may be due to the loss of water and moisture content in the polysaccharide. The broad exothermic peak at 320-400 °C corresponds to chitosan thermal decomposition. The results indicated that the structure of chitosan chains has been changed due to the introduction of phosphorous group and the reduced ability of crystallization.

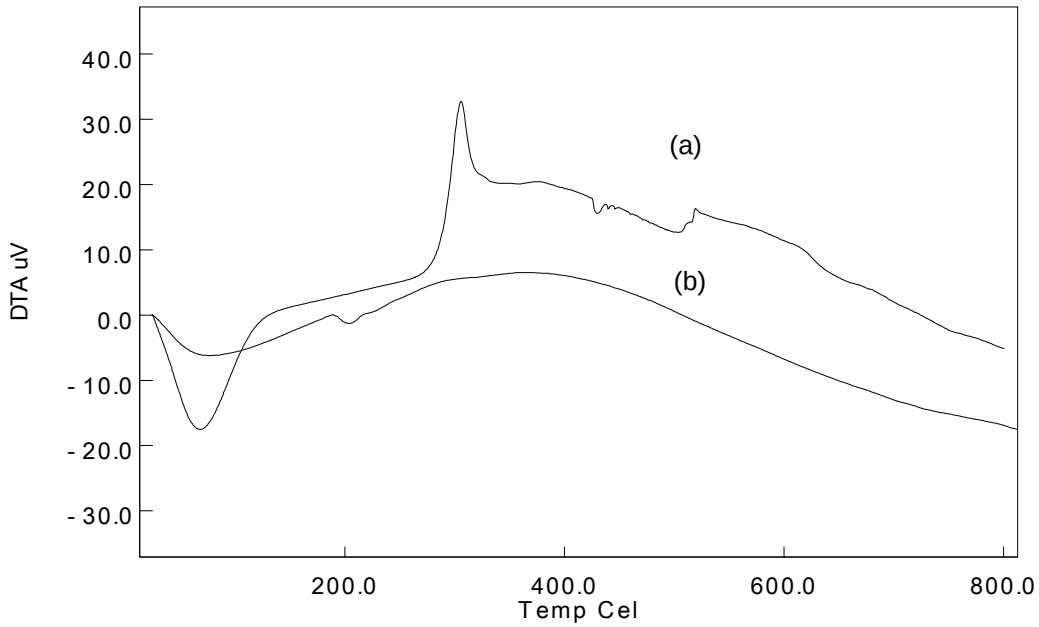
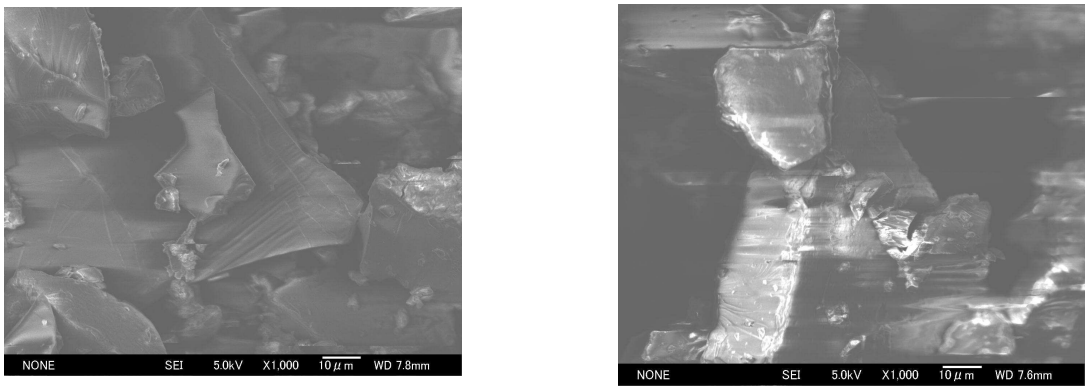


Figure 6: DTA of (a) Chitosan and (b) P-chitosan

SEM studies

Figure 7 show the surface morphology of chitosan and P-chitosan. The chitosan seems to have a smooth surface morphology. But the P-chitosan showed very porous and rough morphology. This may be due to the phosphorylation of chitosan. It shows a relatively homogeneous aspect with a tightly packed structure. The surface morphology studies also confirmed the phosphorylation reaction.



(a) (b)

Figure 7: SEM of (a) Chitosan and (b) P-chitosan

XRD studies

Figure 8 shows the XRD patterns of chitosan and P-chitosan. The incorporation of phosphorous group into chitosan sharply decreased the crystalline nature of the polymer. Chitosan shows diffraction peaks around at 20 2θ values, which corresponds to crystal forms. But P-chitosan does not showing any sharp diffraction peaks it is due to the presence of phosphorous group in the polymer backbone. Introduction of substituents into polysaccharide structures should disrupt the crystalline structure of chitosan, especially by the loss of the hydrogen bonding [9, 25]. After phosphorylation onto chitosan, the original crystallinity of chitosan was destroyed.

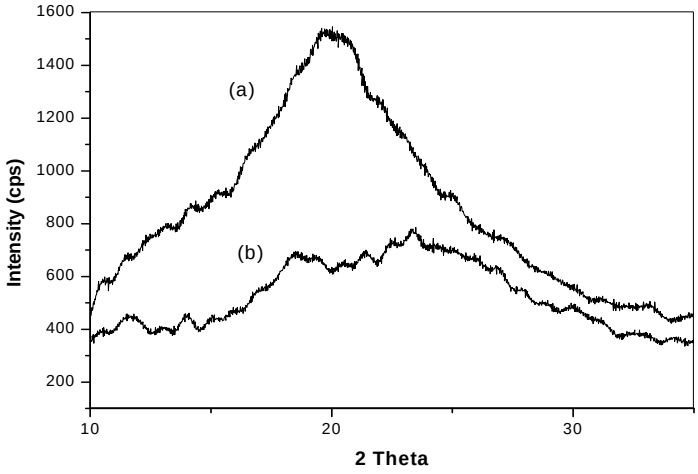


Figure 8: XRD patterns of (a) chitosan and (b) P-chitosan

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