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European Polymer Journal 39 (2003) 1629–1634
EUROPEAN POLYMER JOURNAL
www.elsevier.com/locate/europolj

Synthesis and characterization of water-soluble O-succinyl-chitosan

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Received 18 December 2002; received in revised form 16 March 2003; accepted 18 March 2003

Abstract

The efficient procedure to prepare novel water-soluble O-succinyl-chitosan was established by using one three-step reaction. Phthaloyl group was firstly chosen as the protection group for the amino group of chitosan, and O-succinylation was then completed. Protection group was removed finally by using hydrazine hydrate. The chemical structure of the modified chitosan was characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis. Some physical properties were analyzed by X-ray diffraction, differential scanning calorimetry (DSC), thermogravimetry (TG) and solubility test. It indicates that after O-succinylation, the modified chitosan shows much better solubility in water. The study of enzymatic degradation showed that the O-succinyl-chitosan was of low susceptibility to lysozyme. O-succinylation-chitosan is a useful intermediate, which permits further chemical modification for amino group and may be have potential applications in biomedical system.

Keywords: Chitosan; O-Succinyl-chitosan; Water-soluble; Characterization

1. Introduction

Chitosan is a non-toxic, biocompatible and biodegradable polymer that has been prepared from *N*-deacetylation of chitin. Chitosan consists of 2-amino-2-deoxy- ($1\rightarrow\beta$) residues (D-glucosamine units) and contains no or small amount of *N*-acetyl-D-glucosamine units (A-units). It has become of great interest not only as an underutilized resource, but also as a new functional material of high potentials in various fields, which could solve numerous problems in environmental and biomedical engineering.

Chitosan is insoluble in either water or most of organic solvent, although it is soluble in aqueous diluted acids; the poor solubility of chitosan becomes the major limiting factor in its utilization, such as the application of chitosan in biology, in which many enzyme assays are performed at neutral pH. If water-soluble chitosan could be prepared in a simple manner, it is expected that the biological and physiological potentials of chitosan would be developed dramatically.

A special emphasis has been placed on the chemical modifications to prepare several chitosan derivatives with higher solubility in water, such as O-, *N*-carboxymethyl-chitosan [1], *N*-carboxymethyl-chitosan [2], O-carboxymethyl-chitosan [3,4], *N*-sulfate-chitosan [5], O-sulfate chitosan [6], O-butyryl-chitosan [7], *N*-methylene phosphonic chitosan [8], hydroxypropyl chitosan [9], *N*-trimethyl chitosan [10], *N*-succinyl-chitosan [11] and so on.

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doi:10.1016/S0014-3057(03)00068-5

Chitosan is a versatile carrier for biologically active species and drugs due to the presence of free amino groups as well as its low toxicity [12]. *N*-succinyl-chitosan synthesized via introduction of succinyl groups at the *N*-position of the glucosamine units is water soluble, low toxic [13,14] and fewer biodegradables in the body [15]. Therefore, it is expected to serve as a useful macromolecular drug carrier showing long-term retention in the body [11,16]. In this paper, a novel water-soluble chitosan derivative, O-succinyl-chitosan was synthesized. Due to succinyl group introduced into the hydroxyl group of chitosan, the water solubility has been greatly enhanced while the free amino group of O-succinylation-chitosan can act as useful intermediate, which permits other chemical modifications for further adjustment of solubility and biodegradable behavior. O-succinyl-chitosan may also be directly applied in biomedical system. The chemical structure and physical properties of the modified chitosan were characterized by elemental analysis, FTIR, ^{1}H-NMR, ^{13}C-NMR, X-ray diffraction, differential scanning calorimetry (DSC) and thermogravimetry (TG). The study of enzymatic degradation showed that the O-succinyl-chitosan was of low susceptibility to lysozyme.

2. Experiment

2.1. Materials

Chitosan was provided by Nantong Shuanglin Biochemical Co. Ltd (China), which has a deacetylation degree of 91.5% and viscosity average molecular weight of 25 000 D. All commercially available solvents and reagents were used without further purification.

2.2. Synthesis of chitosan derivative

2.2.1. Synthesis of phthalimide chitosan

Chitosan (0.5 g) was added into a solution of 1.4 g of phthalic anhydride in 10 ml DMF [17–19], and this mixture was heated to 130 °C in N_2 atmosphere with stirring. After 8 h of reaction, the solution was filtered and the filtrate solution was poured into ice water. The precipitate was collected, simultaneously washed with alcohol and ethyl ether, and then dried under vacuum at 60 °C overnight to give 0.4 g of the product.

2.2.2. O-succinoylation of chitosan

Phthalimide chitosan (3 g) was dissolved in DMF (60 ml) with stirring. Succinic anhydride (10 g) was added; pyridine (30 ml) was subsequently dropped into it. The reaction was maintained at room temperature for 24 h. The viscous precipitate was obtained and washed with acetone and ethyl ether respectively, and finally dried.

2.2.3. Deprotection of chitosan derivative

The above solid was dissolved in DMF (50 ml), hydrazine monohydrate (20 ml) and water (40 ml) were added. The mixture was heated to 100 °C under N_2 atmosphere with stirring. After 15 h for reaction, the suspension was filtered; 50 ml of water was added into the filtrate and then dried in vacuum with a rotary evaporator. The residue was dissolved in water, dialyzed (MWCO 10 000) against distilled water for 4 days, then lyophilized and 1.5 g of the brown product was obtained.

2.3. Characterization of chitosan derivatives and measurement of physical properties

^{1}H-NMR and ^{13}C-NMR spectra were performed on a Bruker (AVACE) AV-500 spectrometer, chitosan was dissolved in the mixed solvent D_2O and F_3CCOOD. Phthalimide chitosan and O-succinyl-chitosan were dissolved in DMSO-d_6 and D_2O, respectively. Zgpr pulse for suppressing of water was used in ^{1}H-NMR.

Elemental analysis was carried on Element Vario EL III analyzer.

IR spectra were recorded on Fourier-transform infrared spectrometer (Nicolet 2000) in KBr discs.

X-ray diffraction spectrometry was obtained by using XD-3A powder diffraction meter with CuKα radiation in the range of 5–40° (2θ) at 40 kV and 30 mA.

The results of DSC and TG were obtained with NETZSCH DSC 204 and NETZSCH TG 209 equipment respectively. The temperature range is 30–550 °C and heating rate of 20 °C/min.

2.4. Solubility test

The solubility of different concentrations of O-succinyl-chitosan was evaluated in aqueous media at a wide range of pH. The results are summarized in Table 1.

2.5. Enzymatic degradation

Pulverized chitosan and O-succinyl-chitosan were treated with lysozyme in pH 4.5 acetate buffer solution at 37 °C. The amount of the resulting reducing ends formed by degradation was determined using ferricyanide as reported previously [20]. Briefly, pulverized samples 25 mg, buffer solution 50 ml and the lysozyme solution 25 ml containing 0.8 mg lysozyme were added to 100 ml flask kept at 37 °C. 3 ml of above mixture solution was mixed with potassium ferricyanide solution 4 ml (0.5 mg/ml, 0.5 M Na_2CO_3, freshly prepared). Then the solution was heated at 100 °C for 15 min, cooled, and the absorbance at 420 nm was measured with an Agilent 8453 UV–VIS spectrometer. All samples were prepared in triplicate.

Table 1
Observation of the water solubility of different concentrations of O-succinyl-chitosan

Concentration (%, w/v)	Water	Acetic acid (1%)	NaOH (1%)
4	Soluble (immediate)	Soluble (immediate)	Soluble (immediate)
4.5	Soluble (immediate)	Low viscosity gel	Soluble (immediate)
5	Soluble (immediate)	Low viscosity gel	Low viscosity gel
6	Soluble (immediate)	Low viscosity gel	Low viscosity gel
7	Soluble (immediate)	Low viscosity gel	Low viscosity gel
8	Low viscosity gel	Low viscosity gel	Low viscosity gel

3. Results and discussion

3.1. Synthesis of O-succinyl-chitosan

Since the amino group has stronger nucleophilicity than hydroxyl group, phthaloyl group was chosen as the protective group for the amino group of chitosan. Then O-succylation was carried out and *N*-phthalimido group was removed efficiently from the derivative (Scheme 1).

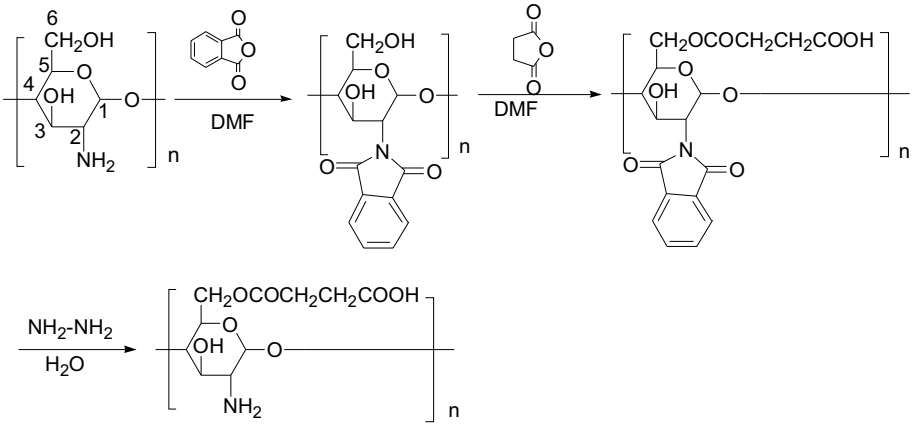
3.2. Characterization

The infrared spectra of chitosan, *N*-phthaloyl-chitosan and O-succinyl-chitosan are showed in Fig. 1. From the chitosan spectrum, it was found that distinctive absorption bands appear at 1662 cm^{-1} (Amide I), 1598 cm^{-1} ($-NH_2$ bending) and 1380 cm^{-1} (Amide III). The absorption bands at 1156 cm^{-1} (asymmetric stretching of the C–O–C bridge), 1075 and 1033 cm^{-1} (skeletal vibration involving the C–O stretching) are characteristics of its saccharine structure [21]. Compared with that of chitosan, the IR spectrum of *N*-phthaloyl-chitosan showed new signals at 1770 and 1710 cm^{-1} should be assigned to the phthalimido groups and the peak of amino group at 1590 cm^{-1} disappeared. It was confirmed that phthalimido group substituted the amino group of chitosan, completely.

From the IR spectrum of O-succinyl-chitosan, the new absorption band at 1731 cm^{-1} was attributed to carbonyl group of ester group [C═O of O (COR)] and the peak at 1662 cm^{-1} was assigned to the carbonyl group of acylamino group. It was found the signal of the phthalimide group at 1770, 1710 cm^{-1} disappeared. These results indicated that the protection group, the phthalimido group, was removed and the acyl group was introduced into the hydroxyl group.

The ^{1}H-NMR spectra of the original chitosan and the modified chitosan were given in Fig. 2. The ^{1}H-NMR assignments of chitosan was as follows [22–24]: ^{1}H-NMR (D_2O/F_3CCOOD) $\delta = 4.76$(H1), $\delta = 3.09$(H2), $\delta = 3.43$–3.81 (H3, H4, H5, H6), $\delta = 1.96$(NOCOCH3). For the ^{1}H-NMR (DMSO-d_6) spectra of *N*-phthalimide-chitosan, the chemical shift at $\delta = 7.46$–7.95 (m) were assigned to the aromatic proton. Compared with that of chitosan, the ^{1}H-NMR (D_2O) of O-succinyl-chitosan shows that the new signals at $\delta = 3.3$–3.9 ppm were attributed to methylene groups.

Fig. 3 shows the ^{13}C-NMR spectra of chitosan and the modified chitosan. Similarly, the assignments and chemical shifts of ^{13}C-NMR (D_2O/F_3CCOOD) of chitosan were $\delta = 97.5$ (C1), $\delta = 76.5$ (C4), $\delta = 75$ (C5), $\delta = 70$ (C3), $\delta = 60$ (C6), $\delta = 55.6$ (C2). For the ^{13}C-NMR (DMSO-d_6) of *N*-phthalimide-chitosan, the new peak at $\delta = 168.2$ ppm was assigned to carbonyl carbon and the



Scheme 1. Synthesis of O-succinyl-chitosan.

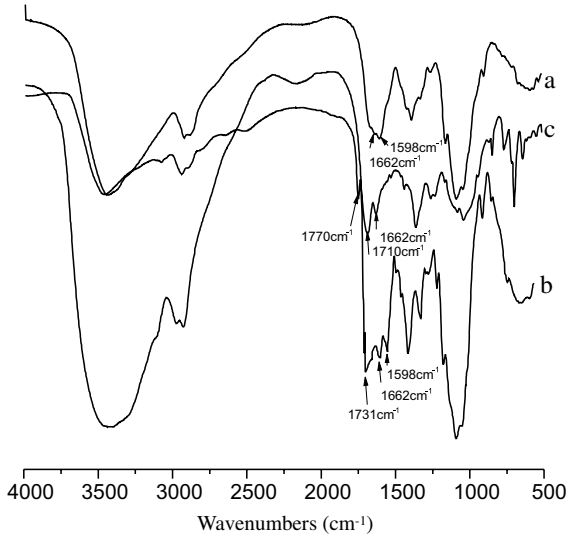


Fig. 1. IR spectra of (a) chitosan, (b) O-succinyl-chitosan and (c) *N*-phthalimide-chitosan.

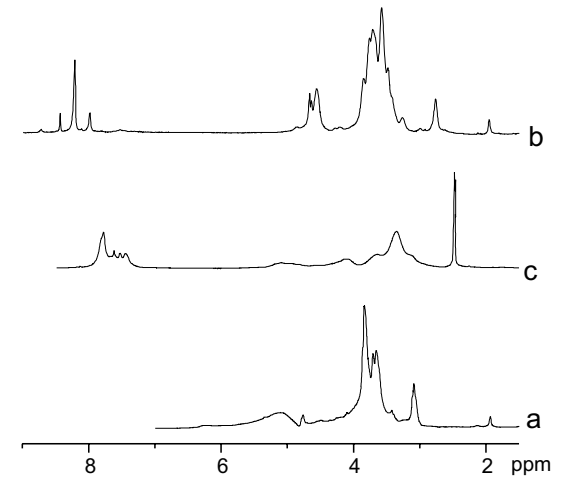


Fig. 2. ^{1}H-NMR spectra of (a) chitosan, (b) O-succinyl-chitosan and (c) *N*-phthalimide-chitosan.

new peaks at $\delta = 123.4$, 128.7, 133.0, 134.8 ppm were attributed to aromatic carbon. These results indicated that the phthaloyl reaction was completed on amino group. The ^{13}C-NMR (D_2O) spectrum of O-succinyl-chitosan shows that the chemical shifts at $\delta = 168.2$ and 164.7 appeared in two type of carbon signal of carbonyl groups, carbonyl group of ester group and carbonyl group of carboxyl group. The new peaks at $\delta = 53.8$ and 54.1 ppm were assigned to methylene carbon. The results supported the claimed existence of succinyl group in the derivative.

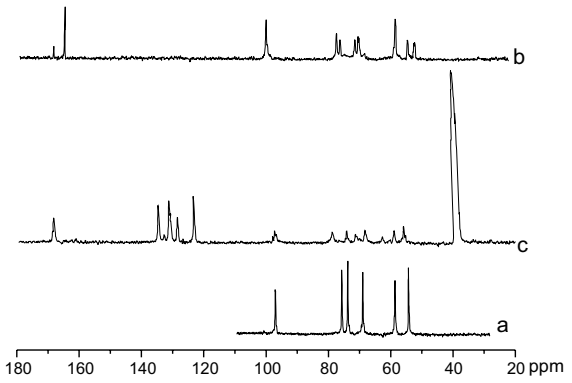


Fig. 3. ^{13}C-NMR spectra of (a) chitosan, (b) O-succinyl-chitosan and (c) *N*-phthalimide-chitosan.

In addition to these results, elemental analysis on O-succinyl-chitosan confirmed that the degrees of acetylation and succinyl substitution were 8.5% and 30% [25], respectively. Anal. Calcd for $[C_6H_8O_4(C_2H_3O)0.085\text{-}(C_4H_5O_3)_{0.3}NH_{2.615}]n$: C,45.45; N, 7.19; H, 6.4. Found: C, 46.92; N, 7.31; H, 7.11.

According to the results of elemental analysis, FTIR, ^{1}H-NMR and ^{13}C-NMR, the suggested chemical structure of O-succinyl-chitosan was confirmed.

3.3. Physical properties of modified chitosan

X-ray diffraction spectra of chitosan and its derivative (Fig. 4) show that chitosan exhibits three reflection fall at $2\theta = 11°$, $2\theta = 20°$, $2\theta = 22°$. Samuels et al. reported that the reflection fall at $2\theta = 11°$ was assigned to crystal forms I and the strongest reflection appears at $2\theta = 20°$

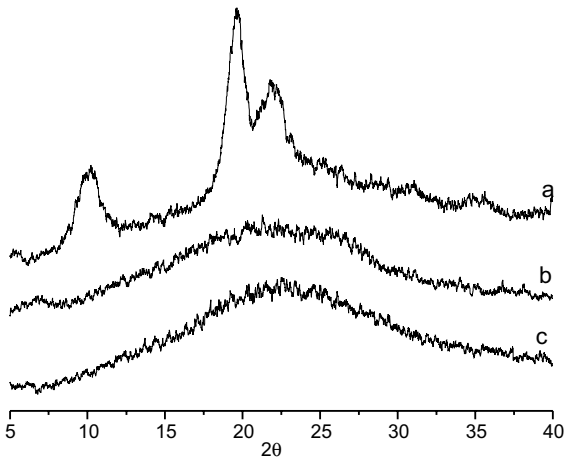


Fig. 4. WAXD patterns of (a) chitosan, (b) O-succinyl-chitosan and (c) *N*-phthalimide-chitosan.

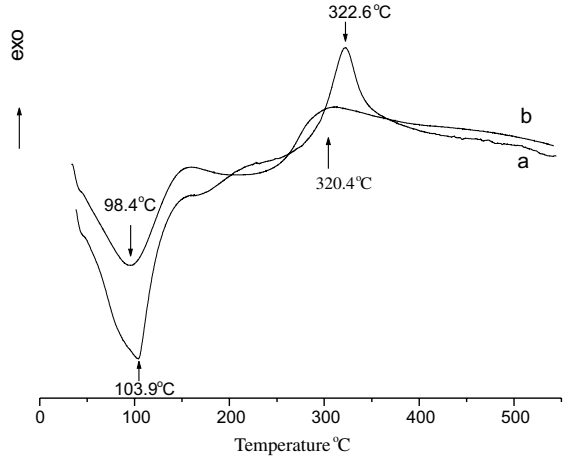


Fig. 5. DSC thermograms of (a) chitosan and (b) O-succinyl-chitosan.

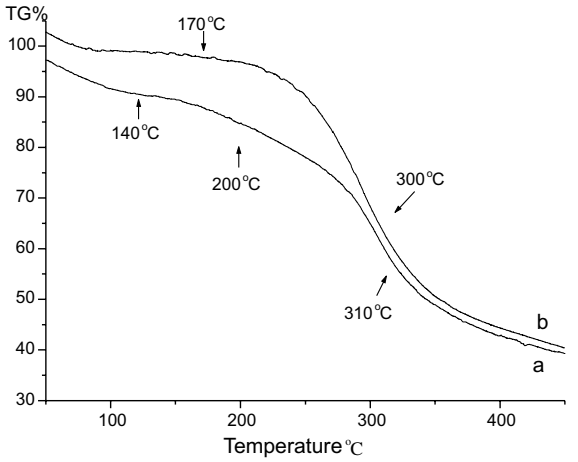


Fig. 6. Thermogravimetric curves of (a) chitosan and (b) O-succinyl-chitosan.

which corresponds to crystal forms II [26]. *N*-phthaloyl-chitosan and O-succinyl-chitosan show only one broad peak at around $2\theta = 20°$. It was indicated that its ability of forming hydrogen bond might be decreased after chemical modification and the chitosan derivatives could be amorphous.

DSC thermograms of chitosan and O-succinyl-chitosan are shown in Fig. 5. The spectra of chitosan shows a broad endothermic peak around 103.9 °C and sharp exothermic peak at 322.6 °C. The former endothermic peak may be due to the water vapor that the chitosan contains. While the latter may be attributed to the decomposition of chitosan. The endothermic peak of O-succinyl-chitosan around 98.4 °C may be due to the loss of water and moisture content in the polysaccharide. The broad exothermic peak at 320.4 °C corresponds to its thermal decomposition. The results indicated that the structure of chitosan chains has been changed due to the introduction of succinyl group and the reduced ability of crystalization.

Thermographs of the chitosan and O-succinyl-chitosan are shown in Fig. 6. The chitosan shows slow weight loss starting from 140 to 200 °C due to the decomposition of polymer with low molecular weight, followed by more obvious loss of weight starting from 200 to 310 °C, which could be attributed to a complex process including dehydration of the saccharide rings, depolymerization and decomposition of the acetylated and deacetylated units of the polymer [27]. A fast process of weight loss appears in TG curve for O-succinyl-chitosan decomposing from 170 to 300 °C, due to the removal of a part of the polymer with low molecular weight by dialysis. The results demonstrate the loss of the thermal stability for O-succinyl-chitosan to the original chitosan. Introduction of succinyl group into polysaccharide structure should disrupt the crystalline structure of chitosan, especially through the loss of the hydrogen bonding.

3.4. Aqueous solubility

Table 1 lists the solubility of O-succinyl-chitosan in aqueous acid and alkali solvents. It was found that the O-succinyl chitosan was dissolved in the aqueous medium, at least up to the concentration of 7% (w/v). On the other hand, it was dissolved in the acidic and alkaline media at the concentrations of 4% and 4.5% (w/v), respectively. Furthermore, the solubility of O-succinyl-chitosan is significantly enhanced, compared with that of chitosan.

3.5. Enzymatic degradation

The amount of reducing ends formed as a result of enzymatic degradation studies with lysozyme was determined using ferricyanide, and the differences in ferricyanide absorbance (ΔAbs) corresponding to the amount of the reducing ends were plotted as a function of time (Fig. 7). It indicated that the chitosan and O-succinyl chitosan were of low susceptibility to lysozyme. This is attributable to the low degrees of acetylation (8.5%) of chitosan and O-succinyl chitosan and decrease in the *N*-acetylglucosamine sequences crucial as a substrate to be recognized by lysozyme because lysozyme hydrolyses the $1 \rightarrow \beta$ linkages between A-unit in chitin [28]. Lysozyme also hydrolyses partially *N*-acetylated chitosans, and the specificity of lysozyme has been determined as a preferential hydrolysis towards only two of sixteen possible tetrad sequences, i.e. the sequences -AA-A-A- and -A-A-A-D- are preferentially cleaved at the middle glycosidic bond [29].

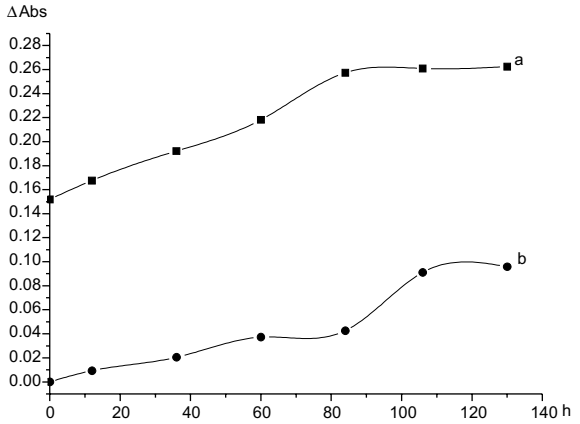


Fig. 7. Susceptibility of (a) chitosan and (b) O-succinyl-chitosan to lysozyme.

4. Conclusion

The new method was studied to introduce O-succinyl group into chitosan under the protection of amino group. Protection group was removed lastly by using hydrazine hydrate. The chemical structures and physical properties of O-succinyl-chitosan were characterized by elemental analysis, FTIR, ^{1}H-NMR, ^{13}C-NMR, X-ray diffraction, DSC and TG. O-succinyl-chitosan shows much higher solubility in water and the study of enzymatic degradation exhibited that the O-succinyl-chitosan was of low susceptibility to lysozyme.

Introducing succinyl group into the hydroxyl of chitosan in order to improve water solubility of chitosan marks the significance of this study. The change of chitosan structure decreases the hydrogen bonds form of inter-molecules and damages the formation of crystallization. The obtained product can be further chemically modified and may have potential biomedical applications.

Acknowledgements

The Special Funds for Major State Basic Research (30271554), Natural Science Foundation of Jiangsu, China (BK 2001077) and High a Projects of P.R.China (G1999064705), National Natural Science Foundation of China (30271554), Natural Science Foundation of Jiangsu, China (BK2001077) and High New-Tech Foundation of Jiangsu, China (BG2002020) supported the research work.

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