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Unexpected reaction of chitosan with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline

Evgeniya A. Stepnova, Vladimir E. Tikhonov,* Tatyana A. Babushkina, Zinaida S. Klemenkova and Igor A. Yamskov

A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 119991 Moscow, Russian Federation. Fax: +7 495 135 5085; e-mail: tikhon@ineos.ac.ru

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The direct reaction of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) with chitosan amino groups, which resulted in the formation of *N*-(ethoxycarbonyl)chitosan, was found and confirmed chemically and by IR and ^{1}H NMR spectroscopy.

Chitosan is a partially or fully N-deacetylated natural polysaccharide chitin and is considered to be a copolymer of D-glucosamine and *N*-acetyl-D-glucosamine. Chitosan is of considerable interest since it is a biodegradable, nontoxic cationic polymer. During the last decades, chitosan has found numerous applications in medicine, cosmetics and food technology.[1] Chitosan has the apparent pK_a ~6.5 and is only soluble in acidic aqueous solutions with pH < 6.0, and under these conditions chitosan can react with carboxylic acids in the presence of a coupling reagent.[2] A widely used coupling reagent is 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ).[3] EEDQ is a stable, readily available reagent, which has been used for smooth preparation of amides and peptides in high yields,[4–6] as well as in neurochemical and pharmaceutical researches[7,8] and affinity sorbent preparations.[9] In organic solvents, EEDQ has been used for protecting the hydroxyl groups of 2-hydroxycarboxylic acids since the O-ethoxycarbonyl groups can be easily removed under weakly basic conditions.[4] For these reasons, EEDQ is a very attractive coupling reagent, especially because it fails to react with amines under conditions of peptide synthesis.[6,10]

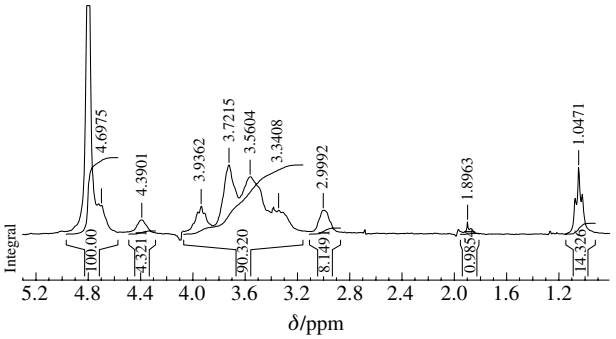

Figure 1 ^{1}H NMR spectrum of *N*-(ethoxycarbonyl)chitosan (1% DCl/D_2O).

Seeking for the preparation of *N*-acylchitosans, we have carried out a series of reactions of chitosan with aliphatic and aromatic carboxylic acids in the presence of 1–3 molar equivalents of EEDQ in aqueous methanol (50 vol%) at pH 5.5±0.5 and 25 °C. After the isolation of these chitosan derivatives as lyophilised solids, they were analysed by ^{1}H NMR and IR spectroscopy. Among others an intense triplet signal in the high field (1.05 ppm) was detected in the ^{1}H NMR spectrum of each sample while no signals assignable to quinoline ring protons were found. Two strong absorption bands at 1698 and 1542 cm^{-1} (one of the most prominent bands) characteristic of the urethane carbonyl group were found in the IR spectrum of each chitosan derivative. The data allowed us to suppose the formation of chitosan derivatives bearing *N*-ethoxycarbonyl groups bound to chitosan amino groups in an amount dependent on a chitosan/EEDQ ratio. The formation of the *N*-ethoxycarbonyl moieties in chitosan macromolecules was attributed to a possible reaction of chitosan amino groups with a mixed anhydride RC(=O)OC(=O)OEt **1** formed from EEDQ and carboxylic acid.[6]

To prove this attribution, we carried out a direct reaction between a low-molecular-weight chitosan (degree of N-acetylation, 4 mol%; M_w, 4.6 kDa; and polydispersity index, 1.6) and EEDQ taken in an amount of 3 molar equivalents towards amino groups: chitosan (0.5 g, 3 mmol) was dissolved in 0.1 M HCl (20 ml), and pH 5.5±0.2 in this solution was adjusted with 0.2 M NaOH. After diluting by MeOH (20 ml), EEDQ (9 mmol) was added and the resultant solution was kept at 25 °C for 12 h. Then, methanol was evaporated and the residue was acidified with hydrochloric acid (pH 2–3). The solution was dialysed against water for five days using a Dialysis tube (D2272, Sigma). The solution was lyophilised, and 0.4 g of the product was obtained.

The structure of the product obtained was investigated by IR and ^{1}H NMR spectroscopy, and the product was additionally subjected to several chemical treatments.

The ^{1}H NMR spectrum of the initial chitosan was identical to the one previously published.[11] Its resonance signal, which appeared in a high field at 1.89–2.05 ppm, was assigned to methyl protons of the acetyl group of *N*-acetamidoglucose residues. The ^{1}H NMR spectrum of the separated chitosan derivative revealed the weak signal of Me protons of acetyl-group residues and an intense high field triplet signal (δ 1.05 ppm), while there were found no signals characteristic of resonance absorbance of quinoline ring protons (Figure 1). This triplet and the corresponding multiplet (δ 3.94 ppm) were assigned to the *N*-ethoxycarbonyl group. The concentration of *N*-ethoxycarbonyl residues in chitosan was evaluated from a correlation of the intensity of absorbance signals of methyl protons of the ethoxycarbonyl group and that one of the multiplet (δ 3.00 ppm) of the C-5 protons of unsubstituted glucosamine residues of the chitosan polymer chain, and it was found to be about 37 per one hundred of monosaccharide units.

The IR spectrum of the product revealed the presence of two strong bands at 1698 (C=O) and 1542 cm^{-1} (N–H) assigned to amide I and amide II of ethoxycarbamoyl group,[12] respectively, which were not observed in the spectrum of the parent chitosan (Figure 2, curves *1*, *2*). To confirm the presence of carbamate groups in the chitosan polymer chain chemically, chitosan ($ChitNH_2$) was subjected to several consecutive conversions as follows:

$$ChitNH_2 \rightarrow \underset{\mathbf{2}}{ChitNHC(=O)OEt} \rightarrow \underset{\mathbf{3}}{ChitNHC(=O)ONa} \rightarrow$$

$$ChitNHCOOH \rightarrow ChitNH_2$$
$$\mathbf{4}$$

N-(ethoxycarbonyl)chitosan **2** was incubated in a 2 M NaOH solution in a boiling-water bath for 5 h. These conditions were required owing to the known high resistance of urethane (*O*-ethylcarbamate) to acidic and basic hydrolysis[13,14] and the resistance of *N*-(ethoxycarbonyl)chitosan towards basic hydrolysis by 0.1 M NaOH at 80 °C for 1 h found in preliminary experiments. After the incubation, the IR spectrum of the product **3** showed obvious alteration especially in the region of characteristic absorbance of the urethane carbonyl group. The IR spectrum of **3** showed the complete disappearance of bands at 1698 and 1542 cm^{-1} whilst the new low-intensity bands at 1645 and 1598 cm^{-1} (Figure 2, curve *3*) assigned to the chitosan

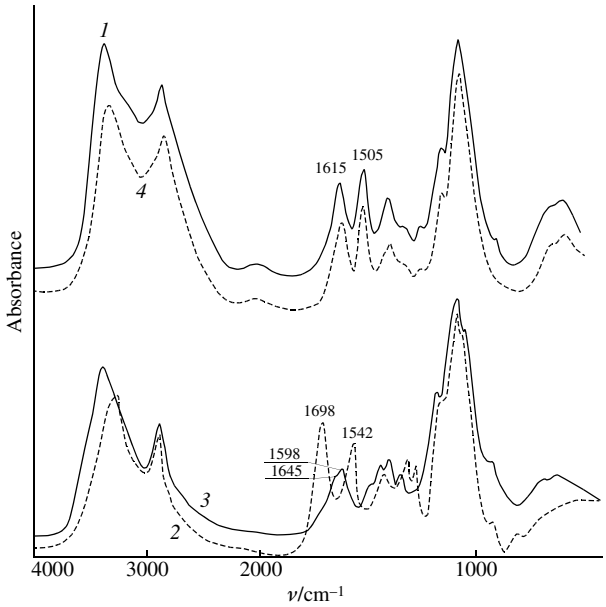

Figure 2 IR spectra of (*1*) $ChitNH_2$·HCl, (*2*) ChitNHC(=O)OEt, (*3*) ChitNHC(=O)ONa and (*4*) $ChitNH_2$·HCl.

carbamate sodium salt[15] were observed. Acidic treatment of the chitosan carbamate sodium salt with 0.1 M hydrochloric acid at room temperature (the conditions which cause fast acid-catalysed decarboxylation of N-substituted carbamate salts[16]) resulted in the decarboxylation of the intermediate *N*-carboxychitosan **4** and complete regeneration of chitosan. The recovered chitosan exhibited the ^{1}H NMR and IR spectral data completely identical to those of the parent chitosan (Figure 2, curves *1*, *4*).

To the best of our knowledge, the formation of *N*-(ethoxycarbonyl)chitosan represents the first evidence that EEDQ can react with an amine. The possibility of the reaction has been neglected in previous publications. In our opinion, this should be taken into account in case when EEDQ is going to be used for glucosamine-containing oligosaccharides, glycoproteins and enzymes modification and affinity sorbents preparation, at least in weak acidic aqueous solutions.

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