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Protonation behavior of dextran amino acid esters

Konrad HOTZEL[1], **Lidija FRAS ZEMLJIČ**[2], **Matej BRAČIČ**[2], **Thomas HEINZE**[1,*]

[1]Center of Excellence for Polysaccharide Research, Institute of Organic Chemistry and Macromolecular Chemistry, Friedrich Schiller University of Jena, Jena, Germany

[2]Laboratory for Characterization and Processing of Polymers, Faculty of Mechanical Engineering, University of Maribor, Maribor, Slovenia

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Abstract: Dextran esters of different amino acids (glycine, β-alanine, L-ornithine, L-lysine) are investigated regarding their pK_a values by potentiometric titration. The pK_a values of the dextran derivatives are generally dependent on the position of the amino group in relation to the ester group, i.e. the nearer they are located in the molecule, the lower the resulting pK_a values are, L-ornithine ester being the exception. An influence of the polymer backbone is ruled out. Stability against hydrolysis at different pH values and over longer periods at constant pH value is measured by potentiometric and polyelectrolyte titration. The β-alanine ester shows slowest hydrolysis at alkaline pH values, starting at a pH value of 8. The esters investigated are polycations at physiological pH values; thus, the charging properties are essential for using these esters as nonviral vectors in gene delivery.

Key words: Dextran amino acid esters, hydrolysis, pK_a, potentiometric titration, polyelectrolyte titration

1. Introduction

Because of their potential usefulness, polycations bearing a polysaccharide backbone have gained great interest. Apart from naturally occurring polycations like chitosan that may be applied in various fields (e.g., in wastewater treatment [1] and as coantibiotics [2]), interest lies in chemically modified polysaccharides, like cationic cellulose [3], starch [4], and dextran derivatives. Polycationic dextran derivatives have gained great interest in the field of gene delivery [5,6] due to the low toxicity and high biodegradability of dextran [7]. For gene delivery, polymers have to bear protonable amino groups due to the necessity of polycationic moieties that play an important role in various steps of gene delivery [8]. Polycations, suited for gene delivery, must form stable polyplexes with the polyanionic biopolymer DNA. The polyplex possesses a positive overall charge at the surface, which enables it to penetrate and enter the cell by endocytosis. After entering, the polyplex is taken into the endosomal compartment, where it is exposed to a decreasing pH value [9]. Although the endosomal escape of the polyplex is still not fully understood, it is believed that the so-called proton sponge effect causes the endosome to disrupt and release its content into the cell. The polyplex dissolves and the DNA can enter the cell nucleus, where transfection takes places. The polycation unloaded should be nontoxic and easily digested by the cell.

It is well known that a connection between high transfection rates and high cytotoxicity exists, e.g., the synthetic polycation poly(ethylenimine) is widely investigated as a gene delivery vector because of its high transfection rates. However, the high toxicity is a problem that has to be overcome, on one hand [10]. On the

*Correspondence: thomas.heinze@uni-jena.de

other hand, vectors based on poly-L-lysine are nontoxic but generally show low transfection [11]. Recently, the synthesis of dextran esters of different amino acids and their application as nonviral vectors in gene delivery was studied [12]. The esters of β-alanine show no toxicity and efficient transfection properties. In this work, dextran esters of glycine, β-alanine, L-lysine, and L-ornithine were investigated regarding their stability against hydrolysis in alkaline, neutral, and acidic media. Moreover, overall protonation behavior and their long-term stability have been investigated. The main aim of this study was to provide physicochemical details, which contribute to understanding the role of polycations in the process of gene delivery.

2. Materials and methods

2.1. Materials

Dextran of leuconostoc mesenteroides (M_r ~ 60,000 g/mol) and 1,1'-carbonyldiimidazole (CDI) purchased from Sigma-Aldrich were used. The amino acids were purchased as Boc-protected (glycine, β-alanine) or unprotected forms (L-lysine, L-ornithine), which subsequently were protected with the tert-butyloxycarbonyl (Boc)-group according to a procedure described in the literature [13], from Sigma-Aldrich. Polyvinylsulfonic acid (sodium salt) in aqueous solution (PVS, 25%, w/v), technical grade, was purchased from Sigma-Aldrich, diluted with ultrapure water to 10×10^{-3} M, and used in polyelectrolyte titrations.

2.2. Synthesis

2.2.1. Synthesis of dextran amino acid esters

In a typical example, to 12.96 g (74.0 mmol) of Boc-protected glycine in 100 mL of DMSO, 13.01 g (80.3 mmol) of CDI was added. After 60 min, the gas formation was finished and 3.99 g (24.7 mmol) of dextran was added. The mixture was allowed to react for 16 h at 80 °C. The product was isolated by precipitation in 400 mL of water, filtrated, and dried. Half of the resulting Boc-protected dextran amino acid ester was dissolved in 150 mL of dioxane. Concentrated sulfuric acid (15 mL) was added and the mixture was allowed to react under constant stirring for 60 min. The product was precipitated in 300 mL of diethyl ether and filtrated, washed with ethyl acetate, dissolved in 50 of mL water, dialyzed, and lyophilized.

Dextran glycine ester (DGE)

^{1}H NMR (250 MHz, D_2O, δ): 5.18 (1H, H2s), 5.08 (1H, $H1_{C2s}$), 4.90 (1H, H1), 4.00–3.32 (7H, AGU H2–H6, CH_2)

^{13}C NMR (62.5 MHz, D_2O, δ): 168.03 (C=O), 97.64 (C1), 95.19 ($C1_{C2s}$), 77.61 (C2s), 73.35 (C3), 71.26 (C2), 70.13 (C4), 69.41 (C5), 65.50 (C6), 40.11 (C_α)

Anal. found: C 29.86, H 5.62, N 5.99, S 7.05 (DS = 1.29)

Dextran β-alanine ester (DAE)

^{1}H NMR (250 MHz, D_2O, δ): 5.29 (1H, H2s), 5.13 (1H, $H1_{C2s}$), 4.96 (1H, H1), 4.3–3.36 (5H, AGU H2–H6), 3.25 (2H, H_β), 2.89 (2H, H_α)

^{13}C NMR (62.5 MHz, D_2O, δ): 171.96 (C=O), 97.54 (C1), 95.16 ($C1_{C2s}$), 76.18 (C2s), 73.35 (C3), 71.12 (C2), 70.01 (C4), 69.51 (C5), 65.80 (C6), 34.95 (C_β), 31.16 (C_α)

Anal. found: C 32.07, H 6.1, N 6.58, S 7.71 (DS = 1.79)

Dextran L-lysine ester (DLE)

^{1}H NMR (250 MHz, D_2O, δ): 5.15 (1H, H2s), 5.07 (1H, $H1_{C2s}$), 4.87 (1H, H1), 4.39–3.32 (6H, AGU H2–H6, H_α), 2.94 (2H, H_ε), 1.95 (2H, H_δ), 1.66 (2H, H_β), 1.47 (2H, H_γ)
^{13}C NMR (62.5 MHz, D_2O, δ): 170.16 (C=O), 97.64 (C1), 95.17 ($C1_{C2s}$), 77.48 (C2s), 73.37 (C3), 71.36 (C2), 70.11 (C4), 69.36 (C5), 65.27 (C6), 52.8 (C_α), 39.98 (C_ε), 29.21 (C_δ), 26.16 (C_β), 21.26 (C_γ)
Anal. found: C 32.61, H 6.8, N 7.77, S 8.84 (DS = 1.20)
Dextran L-ornithine ester (DOE)
^{1}H NMR (250 MHz, D_2O, δ): 5.17 (1H, H2s), 5.08 (1H, $H1_{C2s}$), 4.89 (1H, H1), 4.31–3.32 (6H, AGU H2–H6, H_α), 2.99 (2H, H_δ), 2.02 (2H, H_γ), 1.81 (2H, H_β)
^{13}C NMR (62.5 MHz, D_2O, δ): 169.98 (C=O), 97.72 (C1), 94.88 ($C1_{C2s}$), 77.63 (C2s), 73.33 (C3), 71.43 (C2), 70.22 (C4), 69.53 (C5), 65.59 (C6), 52.52 (C_α), 38.70 (C_δ), 26.80 (C_β), 22.51 (C_γ)
Anal. found: C 34.72, H 6.40, N 5.53, S 6.34 (DS = 0.55)

2.3. Characterization

2.3.1. Potentiometric titration

Solutions were prepared with ultrapure water containing very low carbonate amount. The carbonate was removed by boiling and cooling of deionized water under nitrogen atmosphere. Ionic strength of the solutions was adjusted with KCl to 0.1 M. A double-burette instrument (Mettler Toledo) equipped with a combined glass electrode (Mettler Toledo DG 117) was used to conduct the titration experiments. Aqueous solutions of 0.1 M HCl and 0.1 M KOH were used as titrants.

Around 20 mg of dextran amino acid esters was dissolved (DGE, DAE, DLE, DOE), respectively, in 10 mL of water. The samples were titrated in forward (acidic to alkaline) and backward (alkaline to acidic) runs between pH 3.0 and pH 11.5. The titrant was added at varied intervals of 0.001–0.25 mL. The stability criterion during the analysis after each titrant addition was set at dE/dt = 0.1 mV/50 s, and 50 s was the minimum time for reaching equilibrium conditions between two additions of the titrant. A blank HCl–KOH titration was performed under the same conditions to eliminate any error due to the presence of water contaminants such as dissolved CO_2. The experiments were performed at room temperature. The titrant volume was normalized to the mass of the titrated dextran amino acid esters and expressed as charges per mass (in mmol g^{-1}) versus pH curve. The charging isotherms, in terms of mmol of charged ionic species as a function of pH, were calculated from the potentiometric titration curves as difference between the H-acidities of the analyzed sample and the blank, which was described in detail in previous works [14,15].

2.3.2. Polyelectrolyte titration

For a standard experiment, solutions of amino acid dextran esters were prepared in water with a concentration of 2 mg/mL at different pH values. The pH values were adjusted to 3.0, 7.4, 8.4, and 10.0 (by 0.1 M HCl or 0.1 M NaOH). For the experiment, 0.5 mL of the DGE or DAE samples as well as 0.25 mL of DLE or DOE samples, respectively, were added to 40 mL of water at the respective pH. The cationic indicator toluidine blue was added to determine the equivalence point photometrically. The polyelectrolyte titrant was 0.001 M aqueous solution of polyvinylsulfonic acid, sodium salt (PVS). The titration was carried out with a Mettler Toledo DL 53 titrator adding PVS with increments of 0.1 mL. The absorbance was measured as potential change in mV, using a Mettler Toledo Phototrode DP660 at 660 nm. To determine the amount of protonated amino groups the absorbance was plotted against the volume of added PVS. A detailed description of the procedure is given in the literature [14,16].

2.3.3. Attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy

To check the general behavior under light alkaline conditions, a sample of the DAE was solved at pH 8.5. The pH value was kept constant during periods of 1 h and 24 h. For comparison, another DAE sample was dissolved in water at pH 12.5 as a reference for hydrolysis. The pH value was decreased with aqueous HCl to about 3.5 after the time passed, and the samples were dialyzed and dried by lyophilization and further submitted to ATR-FTIR spectroscopy.

Spectra (16 scans at 4 cm^{-1} resolution) were recorded at room temperature and ratioed to the appropriate background spectrum. A PerkinElmer Spectrum GX spectrometer with ATR accessory (supplied by Specac Ltd., UK) was used.

2.3.4. Elemental analysis

Elemental analysis was performed with a CHNS 932 Analyzer (Leco).

3. Results and discussion

3.1. Synthesis

The reaction of tert-butyloxycarbonyl (Boc) protected amino acids after in situ activation with CDI with dextran yields esters DGE, DAE, DOE, and DLE in two steps (esterification, deprotection), as described before [12,17]. The synthesis will be discussed in detail in future contributions. The resulting esters have DS values of 1.29 (DGE), 1.79 (DAE), 1.20 (DLE), and 0.55 (DOE). A reaction scheme and the structures of the resulting dextran amino acid esters are shown in Figure 1.

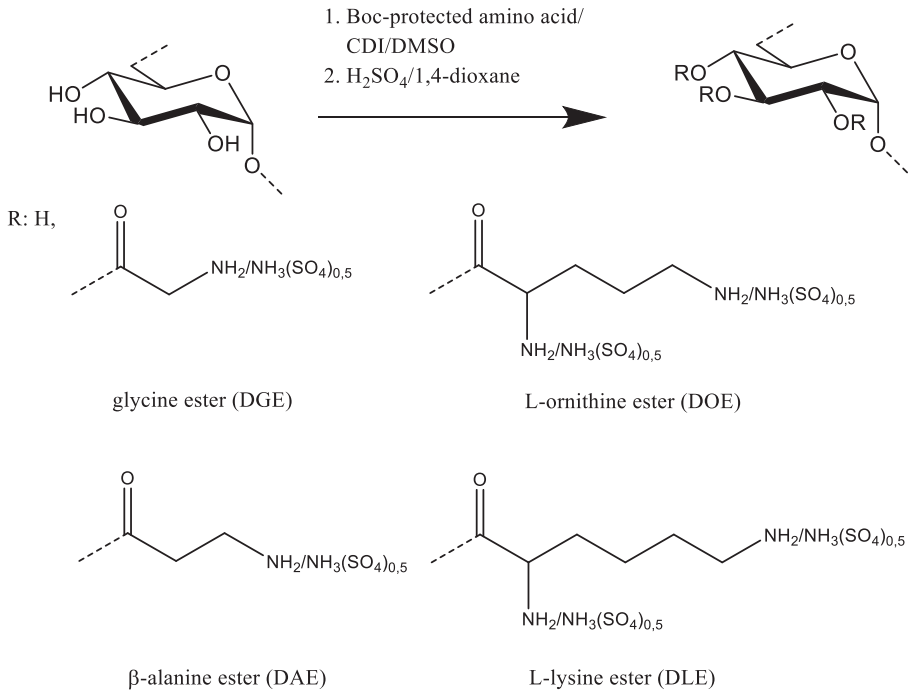

Figure 1. Reaction scheme and structure of resulting dextran amino acid esters.

3.2. Charging behavior

In order to establish that the charges in the titration system arise from the charged moieties of the sample and to check that the normalized values are independent of the initial titrated mass of sample, two different concentrations (c $\approx$ 0.3 mg/mL and c $\approx$ 0.6 mg/mL) of DGE, DAE, DLE, and DOE were titrated. The mass-independent charging isotherms do not vary with mass, as shown in Figure 2 for DGE. The same result is obtained for DAE, DLE, and DOE.

For DGE and DAE, the mass-independent charging isotherm shows one protonation step as expected for amino acid esters containing one amino group (Figure 3). This step takes place roughly between 5 < pH < 9.5 for DGE and 7 < pH < 11 for DAE. Isotherms for DOE and DLE possess two different deprotonation steps between 5.5 < pH < 7 and 7.5 < pH < 10 for DOE and 5 < pH < 8 and 8.5 < pH < 11 for DLE. The resulting pK_a values compared to those of amino acid methyl esters and amino acids from the literature are summarized in Table 1. There is a notable change of the pK_a value of the α-amino groups for the esters compared to the amino acid. Due to the electronic environment caused by the ester bond, the amino groups become more acidic, since the substituent of the ester is not able to provide electron density for the carboxylic group, like the proton of the acid does. This acidification of amino groups is dependent on the chain length of the amino acid. The β-amino group of β-alanine does not change its pK_a value (1.3 units) as much as the α-amino groups of glycine (1.9 units), ornithine (2.3 units), and lysine (2.2 units). The ε-amino groups of lysine are almost not affected by the change of the electronic environment; only the δ-amino group of ornithine, albeit with a certain distance to the carboxylic group, is very heavily influenced with a drop of 2.4 units, which is in accordance with the literature as was shown for the methyl ester [18]. Moreover, methyl and dextran esters of the amino acids do not show great differences of pK_a values (Table 1). Thus, an influence of the polymer backbone of dextran can be negated. DLE and DOE, each having two protonated amino groups at a pH value of 3, are first deprotonated at the α-amino group and subsequently at the δ- and ε-amino groups, respectively. Complete deprotonation is achieved at a pH value of 10 for DGE and DOE as well as at a pH value of 11 for DAE. DLE is not completely deprotonated in the range of 3 < pH < 11.

To quantify the degree of protonation for the amino acid esters of dextran, polyelectrolyte titration was

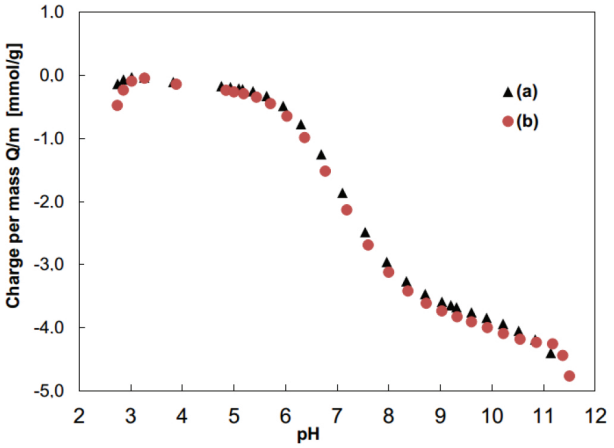

Figure 2. Charging isotherm of dextran glycine ester, concentration (a) c = 0.3 mg/mL and (b) c = 0.7 mg/mL.

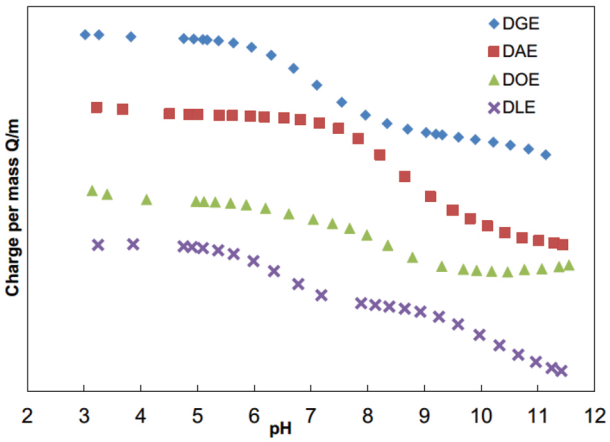

Figure 3. Charging isotherms of dextran glycine ester (DGE), dextran β-alanine ester (DAE), dextran L-ornithine ester (DOE), and dextran L-lysine (DLE) between 3.0 < pH < 11.5 for determination of pK_a values.

Table 1. pK_a values of amino groups of glycine, β-alanine, ornithine, lysine, and their methyl and dextran esters.

Amino acid	pK_a (-NH_2) of amino acid [20]	pK_a (-NH_2) of amino acid methyl ester	pK_a (-NH_2) of amino acid dextran ester
Glycine	9.6	7.7 [21]	7.7
$\beta-$Alanine	10.19	9.25 [22]	8.9
L-Ornithine	8.69/10.76	6.50/8.32 [18]	6.4/8.4
L-Lysine	8.95/10.53	6.96/10.25 [18]	6.75/9.9

carried out. Polyelectrolyte titration measurements were performed under acidic (pH 3), physiological (pH 7.4), and alkaline (pH 8.5, 10.0) conditions. Since charging isotherms obtained by potentiometric titration show complete protonation at a pH value of 3, this was defined as 100% protonation degree. By going from acidic to alkaline medium, the amount of detected amino groups decreases. DOE is already completely deprotonated at the pH value of 8.5, while DGE and DAE still show a degree of protonation of 73.3% and 77.2%, respectively. For the pH value of 10.0, DLE shows a certain degree of protonation only (17.1%). These percentages are in good accordance with the estimation that resulted from the isotherms obtained by potentiometric titration and are easily explained by the estimated pK_a values of the esters. The values are summarized in Table 2.

Table 2. Degree of protonation of dextran amino acid esters in dependence on pH value.

pH value	Degree of protonation in %			
	DGE[a)]	DAE[b)]	DOE[c)]	DLE[d)]
3.0	100	100	100	100
7.4	82.0	92.7	55.1	80.0
8.5	73.3	77.2	0	41.4
10.0	0	0	0	17.1

[a)]Dextran glycine ester; [b)]dextran β-alanine ester;
[c)]dextran L-ornithine ester; [d)]dextran L-lysine ester.

3.3. Stability of samples

As is known, esters hydrolyze in alkaline medium rather fast [19]. To preliminarily test the stability of amino acid dextran esters in alkaline medium, the protonation behavior was analyzed by potentiometric titration of the samples in the range of pH values from 3 to 11 ("Forth"), respectively, and back to pH value of 3 ("Back") twice. The titration isotherms are shown in Figure 4. Having no change of the isotherms for increasing and decreasing of the pH value would mean that no chemical change in the solution takes place, whereas a change of isotherms would indicate possible chemical changes of the polymer, e.g., hydrolysis and/or structural changes (agglomeration). The samples of DGE and DLE show no change of isotherms of "Forth" and "Back" titration in the titration range from 3 to 7 pH, i.e. they are stable in this interval. DOE already starts to hydrolyze in the range of $3 < pH < 7$ and DAE shows stability up to a pH value of 8. Increasing the pH value, samples start to degrade, albeit slowly, when an alkaline pH value is reached. The titrations took a time of about 10 min for one "half cycle", which is not enough for the sample to hydrolyze completely. The fourth half cycle

("Back" curve) shows a more pronounced change of the protonation isotherm (higher hysteresis of curve) than the isotherm of the second half cycle ("Back" curve). This is due to the longer time of the sample being exposed to alkaline conditions compared to the first "Forth" curve. Since hydrolysis is a process and does not happen instantly, these changes are to be expected [19].

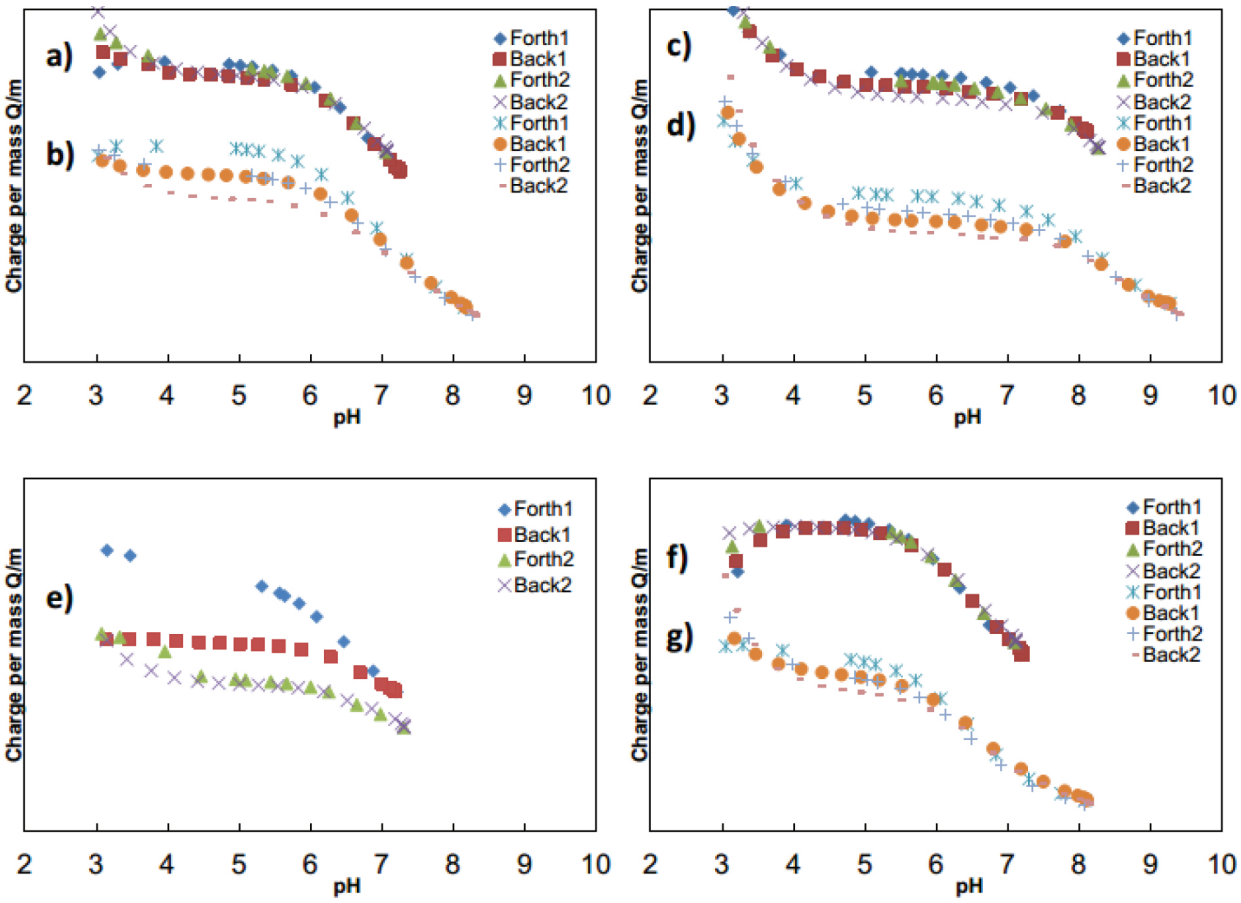

Figure 4. Charging isotherms for titration of dextran glycine ester in the range of a) pH value of 3–7, b) pH value of 3–8; of dextran β-alanine ester sample in the range of c) pH value of 3–8, d) pH value of 3–9; of dextran L-ornithine ester sample in the range of e) pH value of 3–7; and of dextran L-lysine ester sample in the range of f) pH value of 3–7 and g) pH value of 3–8 ("Forth" and "Back" indicate increase and decrease of pH value).

FTIR spectroscopy was used to prove hydrolysis and examine sample stability. For this purpose, DAE was dissolved in water at a pH value of 8.5. The pH value was kept constant during periods of 1 h and 24 h. For comparison, another DAE sample was dissolved in water at a pH value of 12.5 for the same period of time (reference for hydrolysis). Subsequently, the pH value was decreased with aqueous HCl to about 3.5 after the respective time had passed and the samples were dialyzed and dried by lyophilization to remove low-molecular-weight components, especially free amino acids. Remaining amino acids may add to the peak of the C-O-group, for example, which would render the FTIR spectrum senseless.

FTIR spectra of the pure DAE sample are shown in Figure 5. Deesterification, due to hydrolysis, is witnessed in many bands of the spectra. For comparison, the starting dextran ester is shown (see Figure 5a), where ν_{OH} (~ 3600–3100 cm^{-1}), ν_{CH} (~ 3000–2750 cm^{-1}), and ν_{NH} (~ 3500–3000 cm^{-1}) form a common

band. The signals of ν_{OH} and ν_{CH} are separated after deesterification due to vanishing ν_{NH}. The very strong $\nu_{C=O}$ (1739 cm^{-1}) is witnessed to partly disappear depending on the harshness of the conditions of treatment. While this band is still relatively strong after treatment at a pH value of 8.5 after 1 h (Figure 5b), it nearly vanishes after 24 h at a pH value of 8.5 (Figure 5c). After treatment at a pH value of 12.5 (reference treatment, Figure 5d), it disappeared after 1 h. The δ_{NH3+} (~ 1500 cm^{-1}) disappeared for treatments longer than 1 h or at pH value of 12.5, too. Because of dialyzing before measuring samples by FTIR, no carboxylates, like in the literature [14], were found in FTIR spectra.

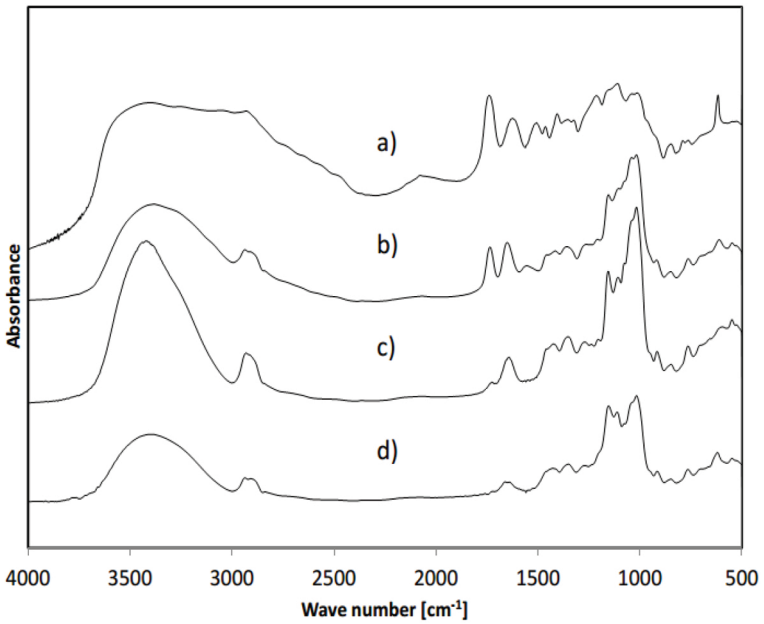

Figure 5. FTIR spectra of dextran-β-alanine ester (DS = 1.79) a) and after storing b) for 1 h at pH value of 8.5, c) for 24 h at pH value of 8.5, and d) for 1 h at pH value of 12.5.

The loss of ester moieties and hence amino groups (due to hydrolysis for DAE) is also witnessed by the decreasing nitrogen content found by elemental analysis because amino groups of free amino acids had been removed by dialyzing previously. The starting sample has a DS of 1.79, which is reduced depending on the pH value and time; at a pH value of 8.5, the DS is still 0.44 after 1 h, and after one day, the ester moieties are almost removed (DS = 0.07). At a pH value of 12.5, already after 1 h only a little amount of β-alanine is still bound to the polymer backbone (DS = 0.03, Table 3).

To analyze the stability kinetics at different pH values, the dextran amino acid esters were stored at pH values of 3.0, 7.4, and 10.0 for one week. Stability at a pH value of 7.4 is interesting for physiological applications, i.e. in gene delivery. These samples were subjected to potentiometric and polyelectrolyte titration during this period in order to estimate their protonation behavior. If hydrolysis takes place, the amount of amino groups bound to the polymer backbone will be reduced, resulting in lower values of amino groups in the polyelectrolyte titration. To obtain full protonation of samples the measurements were carried out at a pH value of 3.0 regardless of the storage pH value.

By storing the samples at a pH value of 3.0, the amino acid esters of dextran lose some amino acid ester groups due to hydrolysis within 1 h, the loss being lower than 10% (see Table 4). After this period, the content of bound amino acid of the dextran esters appears to be constant within one week.

Table 3. Dependence of functionalization of dextran β-alanine ester on different pH value and time.

Hydrolysis conditions			
pH value	Time	N content[a)]	DS[b)]
	[h]	[%]	
-	-	6.58	1.79
8.5	1	2.99	0.44
8.5	24	0.55	0.07
12.5	1	0.27	0.03

[a)]Content of nitrogen determined by elemental analysis; [b)] degree of substitution.

Table 4. Results of polyelectrolyte titration of dextran glycine (DGE), dextran β-alanine (DAE), dextran L-ornithine (DOE), and dextran L-lysine esters (DLE) stored at pH 3 depending on time of storing.

pH value	Time	DGE	DAE	DOE	DLE
		c(AA[a)])	c(AA)	c(AA)	c(AA)
	[h]	[mmol/g]	[mmol/g]	[mmol/g]	[mmol/g]
3.0	0	5.37	5.16	2.78	3.28
	1	5.12	5.12	2.75	3.28
	3	4.70	-	2.59	3.06
	4	-	4.94	-	-
	24	4.84	4.97	-	3.06
	48	-	4.97	2.53	3.09
	120	4.59	-	2.37	-
	144	4.56	-	2.31	2.75
	168	-	5.12	2.37	2.65
		4.91 ± 0.31	5.05 ± 0.10	2.53 ± 0.19	3.03 ± 0.24

[a)]AA: Amino acid substituent.

After one week, amino acid groups are still present in the polymer by storing the samples at a pH value of 7.4, apart from DAE, which does not change its amino acid content over 1 week. DGE, DOE, and DLE show a trend of constantly losing functionality by hydrolysis of the ester bond, which is expressed in an increasing standard deviation (see Table 5).

The samples stored at different pH values also show a lower content of amino acid bound from the start; thus, the initial loss of functionality happens with a high reaction rate. For example, DAE shows at t = 0 a concentration of 5.16 mmol/g at pH value of 3.0 and 4.30 mmol/g at pH value of 7.4. For storage at a pH value of 10.0, all esters are completely hydrolyzed after 24 h, DOE not even showing bound amino acid at t = 0 and DAE showing still residues after 3 h that result from the absence of an α-amino moiety. It is known that β-amino groups of β-alanine do not possess strong interaction with the ester moiety, for example, and hence do not facilitate the hydrolysis of the ester compared to α-amino groups [18].

3.4. Conclusions

Different dextran amino acid esters (glycine, β-alanine, L-ornithine, and L-lysine) were investigated regarding their protonation behavior with potentiometric and polyelectrolyte titration. The pK_a values of the amino

Table 5. Results of polyelectrolyte titration of dextran glycine (DGE), dextran β-alanine (DAE), dextran L-ornithine (DOE), and dextran L-lysine esters (DLE) depending on pH value and time of storing.

pH value	Time	DGE	DAE	DOE	DLE
		c(AA[a)])	c(AA)	c(AA)	c(AA)
	[h]	[mmol/g]	[mmol/g]	[mmol/g]	[mmol/g]
7.4	0	3.47	4.31	1.72	2.71
	1	4.03	4.31	1.62	2.62
	3	3.93	4.31	1.59	2.59
	24	3.72	4.31	1.53	2.47
	72	3.40	4.31	1.41	2.25
	168	3.21	-	1.37	2.09
		3.63 ± 0.32	4.31 ± 0	1.54 ± 0.13	2.46 ± 0.24
10	0	1.06	3.28	0	1.84
	1	0	0.66	0	0.06
	3	0	0.19	0	0
	24	0	0	0	0

[a)] AA: Amino acid substituent.

groups of these dextran esters were determined. They appear to be independent of the polymer backbone, as comparison with methyl esters showed. At physiological pH values, the dextran esters are not fully protonated, the highest value being found for the dextran β-alanine ester. Consequently, the dextran β-alanine ester has shown the highest stability in alkaline medium by being not completely hydrolyzed at a pH value of 10 and possessing a constant degree of substitution (DS) within one week at physiological pH values, as proved by qualitative FTIR spectroscopy as well as quantitatively by elemental analysis and polyelectrolyte titration. The other dextran esters show a steady decrease in DS over the same period of time. Under acidic conditions, the decrease of functionalization is still notable, although not as strong. Because of their broad spectrum of properties, the dextran amino acid esters as nonviral vectors in gene delivery could be beneficial in different stages of the process.

In general, the dextran esters studied possess promising properties, especially sufficient pK_a values regarding their potential as nonviral vectors in gene delivery. While dextran esters with amino groups of high pK_a values are able to interact very well with the nucleic acids, forming stable polyplexes and presenting sufficient positive charges for penetration of the cell membrane, the β-alanine esters buffer at relevant physiological pH values, from which the endosomal release should benefit. Glycine esters show pK_a values that are too low for the physiological range, limiting their application as nonviral vectors. The stability of the esters regarding the pH values is a limiting factor, too. The results indicate that β-alanine esters would be most suited for application for long-term use, which is under investigation.

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