Polynucleotide-Chitosan Complex, an Insoluble but Reactive Form of Polynucleotide[1)]

Hikoya HAYATSU,*,[a] Takashi KUBO,[a] Yuji TANAKA,[a] and Kazuo NEGISHI[b]

Faculty of Pharmaceutical Sciences[a] and Gene Research Center,[b] Okayama University, 1-1-1 Tsushima Naka, Okayama 700, Japan. Received March 10, 1997; accepted April 25, 1997

DNA formed an insoluble complex on mixing with chitosan (poly-D-glucosamine) in solution. The DNA content of the complex was about 50% and the DNA remained insoluble in aqueous media of pH 2—7; *e.g.*, on treatment of the DNA–chitosan complex with phosphate-buffered saline at pH 7 and 37 °C for 26 h, the DNA released into the aqueous phase was less than 0.05%. Obviously, DNA and chitosan formed a tight complex due to ionic interactions. The DNA can be solubilized by treatment with 0.1 N NaOH. RNA and other polynucleotides formed similar insoluble complexes with chitosan. The DNA attached to chitosan can be digested with a mixture of DNase I and phosphodiesterase. Cytosine residues in the DNA (denatured DNA) can be deaminated by treatment with sodium bisulfite, forming uracil DNA–chitosan. The uracil DNA–chitosan served as a substrate for uracil DNA glycosylase. Using polynucleotide–chitosan as an adsorbent, the affinities of reagents for polynucleotides can be determined directly. With this technique it was found that carcinogenic heterocyclic amines have an affinity for RNA as well as DNA. The results with homopolyribonucleotide–chitosans as adsorbents for 4 heterocyclic amines indicated that the binding occurs in a purine nucleotide-specific manner. These results suggest that the polynucleotides in the chitosan complex are accessible to enzymes and reagents.

This new derivative may be useful in chemical and biological studies of polynucleotides and substances interacting with polynucleotides.

Key words DNA; chitosan; complex; polynucleotide; heterocyclic amine

Recent work from our laboratory has shown that chitin (β-1,4-poly-*N*-acetyl-D-glucosamine) is a suitable solid support for a ligand.[2)] As an extension of this work, we have explored the possibility of using chitosan, an *N*-deacetylated derivative of chitin, as a support for certain dye ligands (unpublished work). It occurred to us that chitosan, being a polyamine, may form a complex with DNA. It is known that solid chitosan particles can adsorb DNA[3)] and that streptomycin, a poly-cationic compound, can form an insoluble complex with DNA.[4)] We report here that, by mixing chitosan and DNA solutions, a precipitate of the DNA–chitosan complex is formed in which the DNA content is as high as 50% and yet the complex is insoluble in aqueous media over a wide range of pH values. RNA and other polynucleotides also formed similar complexes. The DNA in the chitosan complex was accessible to reagents and enzymes. Thus, the DNA on chitosan can be chemically modified and agents such as ethidium bromide and carcinogenic heterocyclic amines can bind to polynucleotides in the chitosan complex. DNA attached to chitosan can be attacked by nucleases and uracil DNA glycosylase.

Materials and Methods

Reagents DNA (calf thymus and salmon testes), oligo d(pT) and poly G were obtained from Sigma (St. Louis, MO, U.S.A.), RNA (*E. coli* and yeast) from Calbiochem (La Jolla, CA, U.S.A.), tRNA (yeast) from Boehringer (Mannheim, Germany), and poly I·poly C, poly A, poly U and poly C from Yamasa (Choshi, Japan). The chitosan used throughout this work was chitosan-1000 from Wako Chemicals (Tokyo, Japan; from crab shell, with a viscosity 800—1500 cP for a 0.5% solution, having a degree of deacetylation of 75—90%); in some experiments, chitosan-500, -100 and -10, which have a viscosity lower than chitosan-1000, also from Wako, were used; in one experiment, chitosan from Aldrich (Milwaukee, WI, U.S.A.) was used. Heterocyclic amines and other reagents used were of reagent grade.

Preparation of DNA–Chitosan and Other Polynucleotide–Chitosans A phosphate-buffered saline solution (PBS; 10 mM Na phosphate, pH 7.2, 0.15 M NaCl) of DNA or other polynucleotides (10 mg in 10 ml) was cooled in ice and stirred mechanically. To it was added dropwise a cold solution of chitosan (30 mg in 10 ml; prepared by neutralizing slowly a solution of chitosan in 0.05 N HCl with 0.025 N NaOH to pH 5). A fibrous precipitate was immediately formed, and the mixture was allowed to stand in the ice for 0.5 h. The precipitate was collected by centrifugation and washed three times with 20 ml PBS, then with water (10 ml), ethanol and diethyl ether, and dried. From DNA, a 10—20 mg complex was obtained. As judged from the absorbance of the supernatants of the reaction mixture and the washings, about 95% of DNA used was precipitated (see Table 1). From heat-denatured DNA and other polynucleotides, similar precipitates were obtained (see Table 1).

This procedure can be scaled down to 1 A_{260} unit polynucleotide/0.1 ml, with a similar high efficiency of precipitation. We also scaled up the reactions to 50 mg DNA, obtaining 80 ± 15 mg DNA–chitosan precipitate, with a 2—5% loss of DNA into the supernatant.

Bisulfite-Mediated Deamination of Cytosine in DNA[6)] Either native DNA (calf thymus), denatured DNA, native DNA–chitosan, or heat denatured DNA–chitosan (each 1 mg/ml PBS) was mixed with an equal volume of 2 or 4 M sodium bisulfite (pH 5), and the mixture was allowed to stand at 37 °C for 20 h. For the reaction of DNA–chitosans, the supernatant was discarded and the precipitate was washed with PBS and then treated with 1 M NH_4Cl–NH_3, pH 9.0, at 37 °C for 5 h. For the reaction of DNA with bisulfite in the solution, DNA was precipitated after the reaction with 3 vol. cold ethanol, dissolved in water, and subjected to dialysis against water. The dialyzed solution was lyophilized and the DNA obtained was treated with 1 M NH_4Cl–NH_3, pH 9.

The treated DNA–chitosan was washed with PBS and subjected to enzymatic digestion. For the solution reactions, the DNA was precipitated with ethanol and subjected to enzymatic digestion. These treatments were carried out using duplicate samples.

Nuclease Digestion of Polynucleotides in Chitosan Complexes DNA in the DNA–chitosan complex (1 mg) or in solution (0.5 mg) was treated as follows.

To digest into nucleosides, the material was incubated in 400 μl 7.5 mM Tris–HCl, pH 7.4, containing 100 μg DNase I (Sigma), 1.5 μmol $MgCl_2$, and 0.004 μmol coformycin at 37 °C for 2 h with shaking. Next, 10 μl 1 M Tris–HCl, pH 8.9, 25 μg snake venom phosphodiesterase I (Funakoshi, Tokyo) and 10 μg alkaline phosphatase (Sigma) were added, and the mixture was further incubated at 37 °C for 2 h. The supernatant (or the solution for the free DNA-reaction) was mixed with 3 vol. ethanol and

* To whom correspondence should be addressed.

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the mixture was allowed to stand at $-20\,^{\circ}C$ overnight. After centrifugation to remove the precipitated proteins, HPLC analysis of the nucleosides was carried out.

To estimate the DNA content in DNA–chitosan, the insoluble complex was digested into mononucleotides by the procedure described above except that no alkaline phosphatase was used, and the UV absorption of the digest was determined.

For enzymatic digestion of ribopolynucleotides, RNA–chitosan (or poly U–chitosan) (1 mg) was treated with RNase A (Sigma) (1.5 mg) in 0.1 M Tris–HCl, pH 7.4 (1.5 ml), for 90 min at 37 °C, and the absorbance of the solution was recorded.

Digestion with Uracil DNA Glycosylase 2 M Bisulfite-treated denatured DNA–chitosan (1 mg; containing 140 nmol uracil residues) was digested with uracil DNA glycosylase (Gibco BRL, Gaithersberg, MD, U.S.A.) (20 units) in 500 μl 30 mM Tris–HCl (pH 8.3)–50 mM KCl–5 mM $MgCl_2$ at 37 °C for a desired period of time with shaking. After centrifugation, the supernatant was collected and mixed with 3 vol. ethanol to precipitate proteins. The soluble portion, after evaporation to remove ethanol, was subjected to HPLC analysis for uracil. UV spectra of this material were also recorded. The DNA–chitosan after treatment with uracil DNA glycosylase, was examined to determine its nucleoside composition by the procedures described above (nucleases and HPLC). The experiments were performed using duplicate samples.

HPLC The column used was an Inertsil C8 (250 mm × 4.6 mm i.d., GL Science, Tokyo). For analysis of nucleosides, elution was peformed with 70 mM sodium dihydrogen phosphate–6% methanol at a flow rate of 1.5 ml/min with a column temperature of 40 °C. The observed retention times for standard nucleosides were dCyd 3.3 min, dUrd 4.2 min, dGuo 5.6 min, dThd 7.6 min, and dAdo 12.0 min.

For analysis of uracil, elution was performed with a mixture of 20 mM sodium dihydrogen phosphate–methanol (92 : 8, v/v) at a flow rate of 0.8 ml/min without heating the column. The retention times for standard nucleobases were Cyt 4.5 min, Ura 5.6 min, Gua 6.8 min, Thy 9.1 min, and Ade 12.2 min.

Adsorption of Reagents to Polynucleotide–Chitosan A solution of reagent in PBS, 1 ml, was mixed with polynucleotide–chitosan (3 mg) and the mixture was swirled at 30 rpm at room temperature ($22\pm2\,^{\circ}C$) for 90 min. Adsorption was determined by measuring the absorbance of the solution. As a control, chitosan (1.5 mg), prepared by precipitation from a 0.05N-HCl solution with added alkali, followed by washing with water and methanol, was used in this adsoption experiment. The experiments were performed in duplicate and the average values are presented in this paper: generally the duplicates showed good reproducibility.

Recovery of DNA from DNA–Chitosan DNA–chitosan (22.5 mg, containing 160 A_{260} units DNA) was treated with 0.1 N NaOH (5 ml) for 90 min at room temperature. The mixture was centrifuged, and the supernatant was taken and cooled in ice. Following addition of 100% trichloroacetic acid (0.65 ml), DNA was precipitated. After standing in ice for 0.5 h, the DNA was isolated by centrifugation, washed with ethanol and diethyl ether, and dried: 6.2 mg was obtained, which was found to contain 140 A_{260} units DNA (yield, 87%).

Results and Discussion

Preparation of Polynucleotide–Chitosan Complexes As Table 1 shows, the yields of chitosan complexes from DNA were almost quantitative and those from RNA and polyribonulceotides were 65—89%. D-Glucosamine did not give a precipitate with DNA.

The release of DNA from the DNA–chitosan complex into aqueous solution was measured at various pH values (1 mg DNA chitosan/1 ml buffer, 22 °C, 90 min with a vertical swirling at 30 rpm). The results were: pH/buffer/mol% released, pH 1/0.1 N HCl/2%, pH 9/0.1 M Tris–HCl/2.6%, pH 11/0.1 M glycine–NaOH/4.6%, pH 12/0.01 N NaOH/7.3%. At pH 2—7, no release was detected. Following treatment at 37 °C for 26 h in PBS, the release was less than 0.05%. Unlike the streptomycin–DNA complex, which dissociates in 1 M NaCl,[4] the release of DNA from the chitosan complex with 1 M NaCl in PBS was 0.4%. It was found that, at pH 13, the DNA was released from the complex: on treatment with 0.1 N NaOH at 80 °C for 1 h, about 90% of the DNA in the DNA–chitosan became soluble. When the residual solid was heated at 100 °C in 0.1 N HCl, the solid became soluble, and the absorbance due to DNA and its components amounted to about 10% of the total absorbance at 260 nm. The alkali-elutable DNA content in the complex was determined to be around 10 A_{260}/mg, which corresponded to about 50% of the weight of the precipitate (Table 1). The content can also be determined by treatment of the complex with nucleases; this indicates that the DNA in the complex is accessible to enzyme proteins.

Insoluble chitosan complexes were obtainable from RNA and other polynucleotides and the results in Table 1 show that all these complexes have similar high polynucleotide contents, about 50% in weight. On treatment with PBS for 90 min, less than 1% leakage of polynucleotide was noted from these complexes.

We explored the dependence of the precipitation on polynucleotide chain length, using oligo d(pT). It was observed that $d(pT)_{12-18}$ can be precipitated with an 84% efficiency from 1 A_{260} unit/0.1 ml PBS solution, but $d(pT)_4$ or $d(pT)_6$ did not precipitate.

An experiment showed that DNA was recoverable from

Table 1. Yields and Nucleotide Content of Polynucleotide–Chitosan Complexes

Polynucleotide	Precipitation efficiency[a] (%)	Weight of precipitate (mg from 10 mg polynucleotide)	Nucleotide content in precipitate[b] (A_{260}/mg)
DNA (calf thymus)	95	14	9.5
Denatured DNA (calf thymus)	97	14	8.5
DNA (salmon testes)	97	19	9.3, 9.6[c]
RNA (*E. coli*)	89	23	7.0, 8.5[c]
RNA (yeast)	79	18	10.5
Transfer RNA (yeast)	74	15	10.6
Poly I·Poly C	67	18	6.7
Poly A	72	13	16.5
Poly G	66	12	15.0
Poly U	65	13	9.4, 8.7[c]
Poly C	75	15	10.0

a) Determined spectroscopically from the absorbance of the supernatant of the reaction mixture and the washings of the precipitate. *b*) Determined, unless otherwise indicated, by treatment of duplicate samples with 0.1 N NaOH at 80 °C for 1 h. The A_{260} value is normalized to that of the parent polynucleotide at pH 7. The A_{260} values per mg of parent polynucleotides used were: native calf thymus DNA 13; denatured calf thymus DNA 17; native salmon testes DNA 18; RNA 19; transfer RNA 19; poly I·poly C 15; poly A 31; poly G 24; poly U 20; poly C 16. *c*) Determined by enzymatic digestion; for DNA with DNase I and snake venom phosphodiesterase, and for RNA and poly U with RNase A.

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DNA–chitosan by treatment with 0.1 N NaOH followed by acid precipitation, with a yield of 87%.

Chitosan samples with smaller viscosities (chitosan-500, -100, and -10 from Wako) precipitated calf thymus DNA quantitatively (98, 96, and 94%, respectively), and the products showed DNA contents and leakage properties similar to those of DNA–chitosan-1000. A chitosan from another source (Aldrich) showed similar reaction characteristics with DNA.

To explore whether the manner of precipitating the DNA–chitosan can affect the DNA content and precipitation efficiency, an experiment was performed in which the DNA had been diluted 3-fold with PBS before adding chitosan: thus, addition of 90 mg chitosan (in 30 ml) to 10 mg calf thymus DNA (in 30 ml PBS) resulted in 85% precipitation of DNA; the product weighed 16 mg with a DNA content of 7.8 A_{260}/mg. This experiment showed that the efficiency of complex formation and the quality of the precipitate were not greatly affected by this change in manipulation.

Since chitosan is soluble in acid, we treated DNA-chitosan in 0.05 N HCl for 90 min and attempted to quantify any chitosan released into the medium, using the fluorescamine assay[5]: only 0.6% of the chitosan in the DNA–chitosan was detected in acid solution.

Bisulfite Modification of DNA in the Chitosan Complex We investigated whether the DNA complexed with chitosan, thereby becoming insoluble, can be chemically modified by bisulfite, a single-strand specific, cytosine deaminating agent.[6] Table 2 and Fig. 1 show that deamination of the cytosine in the DNA of the complex took place almost as efficiently as in solution. The single-strand specific nature of this chemical reaction was retained for the DNA complexed with chitosan.

These results suggest that DNA bases in the complex can be reached by bisulfite and other ions (e.g., H^+ and

Table 2. Nucleoside Composition of Bisulfite-Treated DNA

Form of DNA	Bisulfite concentration[a]	Nucleoside (mol %)					Deamination % in DNA
		dA	dG	dT	dC	dU	(dU/[dC+dU])
Denatured	Exp.						
DNA–chitosan	1 M (i)	26.6	24.5	25.9	19.0	4.0	17.4
	(ii)	26.5	24.1	25.9	19.1	4.4	18.7
	2 M (i)	26.7	24.3	25.3	15.0	8.7	36.7
	(ii)	26.0	23.8	24.8	16.9	8.6	33.7
Denatured DNA							
(in solution)	1 M (i)	28.1	22.5	27.1	18.2	4.0	18.0
	(ii)	27.3	23.0	27.3	18.5	3.9	17.4
	2 M (i)	27.5	22.6	27.4	12.6	9.9	44.0
	(ii)	28.0	22.6	27.3	12.3	9.8	44.3
Native							
DNA–chitosan	1 M (i)	27.0	23.8	28.3	20.8	0	0
	(ii)	27.4	23.5	27.5	21.7	0	0
	2 M (i)	26.6	25.2	27.9	20.3	0	0
	(ii)	26.6	24.2	28.3	20.8	0	0
Native DNA							
(in solution)	1 M (i)	27.9	23.7	27.6	20.1	0	0
	(ii)	27.9	23.3	29.0	19.7	0	0
	2 M (i)	27.5	23.2	27.8	21.6	0	0
	(ii)	27.4	23.6	27.8	21.2	0	0

a) The reactions were carried out at 37 °C and pH 5 for 20 h. The nucleoside compositions were based on HPLC data (see Fig. 1 for an example).

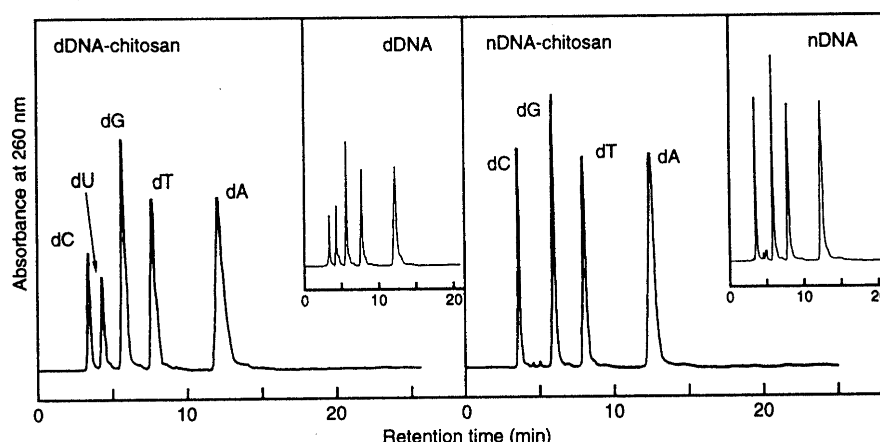

Fig. 1. HPLC Profiles of Nucleosides in the Digests of Bisulfite-Modified DNA–Chitosans

Denatured DNA (dDNA)- and native DNA (nDNA)-chitosans were treated with 2 M sodium bisulfite, and then enzymatically digested into nucleosides, and the mixture fractionated by HPLC. For comparison, DNA samples in solution were treated similarly: the insets show the results in these solution reactions.

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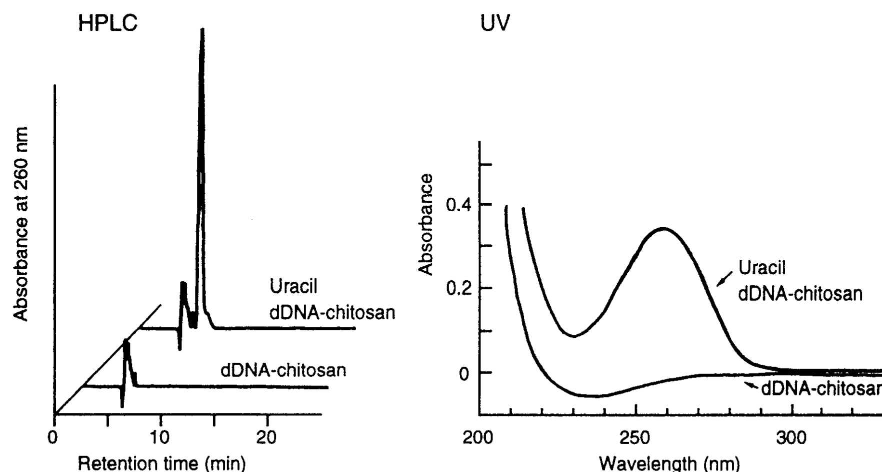

Fig. 2. Uracil Released from Uracil DNA–Chitosan by Uracil DNA Glycosylase
The peak at 5.6 min coincided with that of standard uracil. Results with DNA–chitosan treated with the enzyme are also shown for comparison.

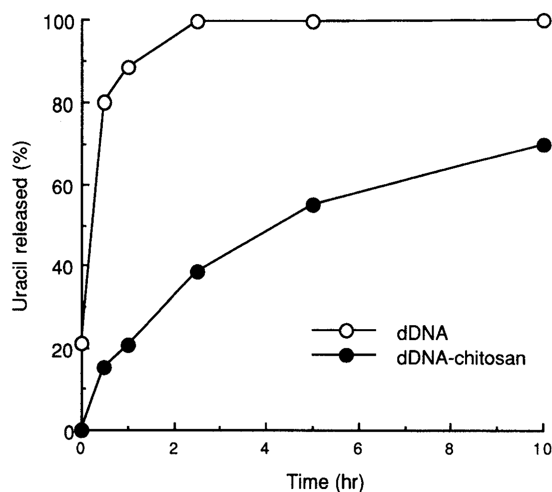

Fig. 3. Time-Course of Uracil Release from Uracil-Containing DNA–Chitosan and from Uracil-Containing DNA by Uracil DNA Glycosylase

●, uracil-containing denatured DNA—chitosan; ○, uracil-containing denatured DNA (average data from duplicate experiments using two pairs of samples from single lots).

OH^-) in the surrounding aqueous phase as easily as DNA bases in solution. Also, the results indicate that the double strandedness of DNA was retained in the complex. It should be noted that no release of DNA from the chitosan complexes was detected during the modification reactions.

Uracil DNA–Chitosan as a Substrate for Uracil DNA Glycosylase Uracil DNA–chitosan prepared as described above by treatment of denatured DNA–chitosan with bisulfite was subjected to uracil DNA glycosylase. Following a 5 h digestion under the conditions described in the Materials and Methods section, about 50% of the uracil in the substrate was released into solution: from DNA containing 140 nmol uracil, 70 nmol uracil (as quantified by HPLC), or 72 nmol uracil (as quantified by UV), was released (Fig. 2). No base release was detected from DNA–chitosan untreated with bisulfite. Furthermore, it was confirmed that the remaining DNA in the chitosan complex contained 62 nmol deoxyuridine as analyzed by the nuclease–HPLC procedure.

Figure 3 shows the time-course of uracil release from this DNA–chitosan substrate. This figure also shows the uracil release from a sample of bisulfite-treated denatured DNA in solution. The solution reaction reached completion at 2.5 h, whereas the reaction with the DNA–chitosan proceeded at a rate approximately 1/4 that of the solution reaction, and uracil-release continued even after 10 h of incubation. By analyzing samples of DNA–chitosan and DNA treated for 10 h following nuclease digestion and subsequent HPLC, it was confirmed that 30% of the uracil in the DNA–chitosan was still unreleased, whereas no uracil remained in the DNA sample.

These results suggest that the active site of uracil DNA glycosylase can have access to the majority, but not all, of the uracil nucleotides in the DNA–chitosan complex. The uracil cleaving reaction is considerably slower for the DNA–chitosan substrate than for DNA in solution. It was noted that the removal of uracil caused no release of DNA into the aqueous phase.

Table 3. Adsorption of Ethidium Bromide, Heterocyclic Amines, and Other Compounds to DNA– and RNA–Chitosans

Compound	μM	Adsorption[a] (%)		
		DNA–chitosan	RNA–chitosan	Chitosan
Ethidium bromide	20	92.9	94.3	0
Glob-P-1	50	25.7	31.5	16.7
Glob-P-2	50	20.5	20.9	5.2
Glu-P-1	20	12.5	15.4	3.7
Glu-P-2	20	15.7	17.5	4.4
IQ	20	15.8	23.7	0.8
MeIQ	20	27.1	41.6	2.3
MeIQx	20	11.2	25.8	0.6
PhIP	40	13.0	17.0	3.9
Trp-P-1	10	89.5	93.8	7.1
Trp-P-2	20	86.7	85.9	7.6
Norharman	20	30.8	23.0	0.6
8-Methoxypsoralen	50	29.6	11.8	1.6
4-Nitroquinoline 1-oxide	40	0	0.8	0.4

a) Native calf thymus DNA–chitosan containing 5 μmol nucleotide/3 mg, and yeast RNA–chitosan containing 4 μmol nucleotide/3 mg were used in 1 ml of reaction mixture in PBS containing individual compounds. For control, chitosan 1.5 mg was used.

Binding of Compounds to Polynucleotide–Chitosan These

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Table 4. Adsorption of 4 Heterocyclic Amines to Various Polynucleotide–Chitosans

Compound	μM	Adsorption[a] (%)							
		DNA–chitosan	Denatured DNA–chitosan	RNA–chitosan	Poly G–chitosan	Poly A–chitosan	Poly C–chitosan	Poly U–chitosan	Chitosan
IQ	20	15.8	34.3	23.7	52.5	27.5	1.2	0	0.8
MeIQx	20	11.2	53.9	25.8	57.8	25.0	2.6	1.2	0.6
PhIP	40	13.0	28.1	17.0	28.4	21.1	8.3	13.0	3.9
Trp-P-2	10	86.7	88.4	85.9	92.2	72.3	13.0	17.7	7.6

a) Nucleotide contents in 3 mg polynucleotide–chitosan complexes used in 1 ml adsorption experiments were: DNA, 5 μmol; denatured DNA, 3 μmol; RNA, 4 μmol; poly G, 5 μmol; poly A, 4 μmol; poly C, 4 μmol; and poly U, 4 μmol.

insoluble polynucleotide–chitosans are expected to be useful in directly measuring the affinity of compounds for polynucleotides. We examined ethidium bromide, heterocyclic amines, and several other compounds for their binding. Carcinogenic heterocyclic amines are known to be formed on cooking meat and fish.[7] More than 10 different compounds in this class have been identified and most of them have three fused aromatic rings. These compounds are strongly mutagenic to bacteria.[8] No extensive studies, however, have been performed regarding the non-covalent binding of these compounds to polynucleotides.[9,10]

The adsorption of ethidium in PBS to DNA–chitosan was very rapid, almost instantaneous. For Trp-P-2 and Glu-P-1, we examined the time required for equilibration and found that a plateau of adsorption was reached following a 90-min swirling period. With this technique, adsorption of compounds to large excesses of DNA– and RNA–chitosans were investigated and the results are summarized in Table 3. Ethidium was adsorbed to DNA– and RNA–chitosans almost quantitatively but not to chitosan itself. All of the heterocyclic amines tested showed adsorption to DNA and RNA. Although they were adsorbed to varying degrees, individual compounds had similar affinities for DNA and RNA. Norharman and 8-methoxypsoralen, both three-ring polycyclics, behaved in a manner similar to heterocyclic amines. 4-Nitroquinoline 1-oxide, a carcinogen, did not bind to either DNA or RNA.

More extensive studies were performed on 4 representative heterocyclic amines and the results are given in Table 4. These compounds were found to bind to denatured DNA more strongly than to native DNA. The results with homopolyribonucleotides suggest that binding occurs preferentially to purine nucleotides, particularly to guanine nucleotides. It is noteworthy that many heterocyclic amines including IQ, MeIQx, PhIP and Trp-P-2 are known to form adducts in DNA mainly with guanine.[9]

We determined the saturation levels for the binding of ethidium and Trp-P-2 to DNA. For example, a sample of DNA–chitosan (3.9 mg, containing 22.1 A_{260} units calf thymus DNA) was treated with 1 mM ethidium bromide in PBS (5 times with 1 ml aliquots of solution for 30 min), and the saturation level was found to be 0.54 mg ethidium bromide in total, which corresponded to 1 ethidium bromide molecule per 2.2 nucleotides, a value greater than the binding ratio (1/4) reported in the literature for DNA in solution.[11] Trp-P-2 was found to saturate DNA–chitosan at a reagent/nucleotide ratio of 1/8.

To establish that this adsorption is reversible, DNA–chitosan (3 mg) on which Trp-P-2 (17 μg) had been adsorbed was eluted with PBS (1 ml). The elution was done 9 times and each time the % elution was measured: $9.5 \pm 1\%$ elution was observed each time. Having shown that the adsorption was reversible, we evaluated the dissociation constant for Trp-P-2 to DNA-chitosan using 4 different Trp-P-2 concentrations, 5, 10, 50, and 100 μM, with DNA–chitosan at 2.3 mM (as nucleotide). The association constants were calculated on the basis that 8-nucleotide blocks are the binding targets. Thus, the K_a ([Trp-P-2·DNA–chitosan]/[Trp-P-2]·[DNA–chitosan]) was $1.95 \times 10^3\ M^{-1}$ at 5 μM, $1.92 \times 10^3\ M^{-1}$ at 10 μM, $1.85 \times 10^3\ M^{-1}$ at 50 μM, and $1.89 \times 10^3\ M^{-1}$ at 100 μM Trp-P-2 (mean ± S.D., 1.90 ± 0.04, $\times 10^3\ M^{-1}$).

It could be presumed from these observations that a) RNA, being more abundant in cells than DNA, may serve as a reservoir for carcinogenic heterocyclic amines, or conversely, may serve as a protection against the genotoxic attack of these agents on DNA and b) the attack of metabolically transformed, reactive heterocyclic amines on DNA may involve initial attachment to a single-stranded portion at a guanine and adenine moiety.

Conclusion

We have established a method for preparing polynucleotide–chitosan complexes. Oligo- and polynucleotides having chain lengths larger than 15—20 nucleotides can be generally precipitated by this procedure. Single-stranded or denatured polynucleotides may be recovered using this procedure. We have shown that the DNA in the chitosan complex is reactive towards enzymes and DNA-modifying and -binding agents.

In the application of the DNA–chitosan to treatment with reagents and enzymes, the most obvious advantage over solution reactions is the quickness and ease of manipulation. Agents can be renewed simply by centrifuging the mixtures and removing the supernatants. Another advantage is that the large DNA content of the chitosan complex allows small-scale manipulations. Hopefully, this new class of polynucleotide derivatives may prove useful in the chemistry and biochemistry of nucleic acids and in related enzyme studies.

References and Notes

1) Abbreviations used: Glob-P-1, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; Glob-P-2, 2-amino-9*H*-pyrido[2,3-*b*]indole; Glu-P-1,

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2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; Glu-P-2, 2-aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQ, 2-amino-3,4-dimethylimidazo-[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine; Trp-P-1, 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole.

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