Polyisobutylcyanoacrylate Nanocapsules Containing an Aqueous Core as a Novel Colloidal Carrier for the Delivery of Oligonucleotides

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Purpose. The goal of the present paper was to encapsulate oligonucleotides in a new particulate carrier in order to protect them from enzymatic degradation.

Methods. Nanocapsules with an aqueous core containing oligonucleotides were prepared by interfacial polymerization of isobutylcyanoacrylate in a W/O emulsion. Ultracentrifugation and re-suspension in water yielded a dispersion of these containing an aqueous core nanocapsules. Zeta potential measurements and quenching of fluorescence of fluorescein-bounded oligonucleotides were used to study the localization of the oligonucleotides. Oligonucleotide degradation studies were carried out in fetal calf serum.

Results. Polydisperse nanocapsules of size ranging from 20 to 400 nm were obtained. Oligonucleotide loading did not significantly influence the zeta potential, suggesting they were located within the core of the nanocapsules. Fluorescence quenching assays confirmed this localization. When encapsulated in the nanocapsules and incubated in the presence of serum, the oligonucleotides were efficiently protected from degradation by nucleases, whereas oligonucleotides adsorbed onto nanospheres were protected less efficiently.

Conclusions. This paper describes, for the first time, a nanotechnology able to encapsulate oligonucleotides rather than adsorbing them at the surface of a solid support. Such a formulation has great potential for oligonucleotide delivery.

KEY WORDS: nanoparticles; nanocapsules; nanospheres; polyisobutylcyanoacrylate; oligonucleotides; antisense.

INTRODUCTION

Antisense oligonucleotides (ODNs) are synthetic fragments of DNA with base sequences complementary to a mRNA. As a result of their ability to hybridize to mRNA targets, ODNs can selectively modulate the expression of individual genes (1). Thus, they have a great potential for the treatment of several diseases such as cancer (2,3) and viral infections (4). In addition, they may be useful tools in understanding the physiological role of a number of proteins involved in cellular message transduction (5). However, the intensive use of ODNs in therapeutics has been hampered by their poor stability in biological fluids and their low intracellular penetration (6). The use of chemically modified ODNs such as phosphorothioates or methylphosphonates (7) has been proposed as a solution to these problems.

These modified molecules, however, have been shown to display some aptameric effects and therefore, to be less specific than natural phosphodiester molecules (8,9). This explains why they generally exhibit more toxicity than phosphodiesters when administered *in vivo* (10,11). As an alternative strategy, colloidal carriers such as polymeric nanospheres have been proposed to improve the administration of phosphodiester ODNs (12,13). Due to their hydrophilic and polyanionic character, ODNs interact poorly with polymeric materials which are generally hydrophobic and slightly negatively charged, as is the case for polyalkylcyanoacrylate (PACA) and poly lactic acid (PLA). Therefore, the association of ODNs with nanoparticles is difficult. In most of cases, ODNs have been associated with the surface of nanospheres through electrostatic interactions; Chavany *et al.* (14) took advantage of the formation of ions pairs between the negatively charged phosphate groups of the nucleic acid chain and a positively charged compound such as cetyltrimethylammonium bromide (CTAB) which was preadsorbed onto polyalkylcyanoacrylate nanospheres. In general, this type of surface association was found to be rapidly reversible in the presence of protein-rich biological medium (15). Moreover, when associated through electrostatic forces, ODNs adopt conformation which make them unlikely to hybridize with their biological target (16). Thus, for optimal activity, the ideal carrier system would be ODNs entrapped within the internal core of polymeric nanocapsules in order to mask them and to protect them from interactions with proteins. To date, all the methodologies described for preparing nanocapsules involve the preparation of emulsions, either O/W emulsions which lead to nanocapsules with an oily core suspended in water (17–19) or W/O emulsions which lead to nanocapsules with an aqueous core suspended in oil (20). Oil-based nanocapsules are unable to encapsulate the water-soluble ODNs and aqueous nanocapsules in an oily phase are not suitable for i.v. administration. Therefore, in this study, we have developed a new process of preparation of nanocapsules with an aqueous core containing ODNs, which were successfully suspended in an aqueous medium. We have demonstrated that the ODNs are located in the core of these nanocapsules, which explains why this carrier protects ODNs more efficiently against enzymatic degradation than the nanospheres described previously.

MATERIALS AND METHODS

Materials

A 20-mer phosphodiester oligothymidylate, used as a model ODN was obtained from Eurogentec (Belgium). γ^{33}ATP (111 TBq/mmol) was from Dupont de Nemours (France) and T4 polynucleotide kinase from New England Biolabs (MA, USA). The scintillation liquid used was Ultima Gold™ (Packard Bioscience, Netherlands). Miglyol 812 (a medium chain triglyceride) was supplied by Lambert Riviere (France) and Montane 80 (sorbitan monooleate) by Seppic (France). Isobutylcyanoacrylate (IBCA) was from Loctite (Ireland), cetyltrimethylammonium (CTAB) and calcein from Sigma (France). Dextran 70 was obtained from Fluka (France).

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Oligonucleotide Radiolabelling

ODNs were labeled at their 5′ end using γ^{33}ATP and T4 polynucleotide kinase. Purified ODNs were recovered by gel filtration using a Bio-Spin 6 column (Biorad, Richmond, CA, USA) which was centrifuged at 900 g for 1 min. Purity was controlled by electrophoresis on a 20% polyacrylamide gel followed by analysis of radioactivity using an Automatic-TLC-Linear Analyzer® (EG&G Berthold, France) as described by Aynie *et al.* (21).

Nanocapsule Preparation and ODN Encapsulation

200 μl of ethanol were added to 800 μl of demineralized water containing the 20-mer oligothymidylate at various concentrations from 0 to 10 μM and adjusted to pH 7.4 by addition of 0.1 M NaOH. This solution (1 ml) was added to an organic phase containing 8 g of Miglyol 812 and 1.5 g of Montane 80 under vigorous stirring using an Ultraturrax (1 min, 24 000 rpm). 100 μg of the monomer, IBCA were then added slowly to the emulsion which remained under mechanical stirring at 500 rpm. After 4 h, polymerization was complete and the water droplets were surrounded by the polymer, thus leading to the formation of nanocapsules with an aqueous core containing the ODNs. Resuspension of nanocapsules in an aqueous medium was achieved by ultracentrifugation at 37 000 g for 30 min in a centrifuge tube which was filled with 700 μl of demineralized water and 700 μl of the suspension. After removal of the oily phase, the interface, and the water phase, the pellet was re-suspended in 1.5 ml of demineralized water under vortex agitation (1 min) followed by sonication (30 s). To remove residual oil, the suspension was re-centrifuged once more and the pellet was re-suspended again in 700 μl demineralized water as described above. This resulted in the preparation of nanocapsules dispersed in an aqueous medium with an aqueous core containing the ODNs (ODN*in*NC).

Preparation of Empty Nanocapsules

For fluorescence quenching assays, nanocapsules were also prepared without ODN in their core. In this case, ODNs were either simply added to the nanocapsule suspension (*NC + ODN*) or adsorbed to the surface of the nanocapsules (NC + CTAB + ODN) (14) by pre-adsorbing a hydrophobic cation, CTAB, onto the particle surface. This was followed by ion-pairing formation between the positive charges of the CTAB and the negative charges of the phosphate groups of the ODNs. In practice, 100 μl of a CTAB solution (1.5 mM) containing ODNs at 10 μM was added to 100 μl of the nanocapsule suspension and 800 μl Tris-HCI (10 mM, pH 7.4) under slow magnetic stirring to obtain a final volume of 1 ml. Stirring was then maintained for 2 h.

Nanosphere Preparation and ODN Adsorption

Nanospheres with ODN adsorbed onto their surface (NS + CTAB + ODN) were prepared to provide a control for ODN degradation studies, by an emulsion polymerization process as described previously (14,22). This control also allowed to compare the ODN-protection provided by the two systems in similar conditions.

Particle Size and Zeta Potential Measurements

Nanocapsule diameter was determined after dilution (1/10) in demineralized water using dynamic laser light scattering (Nanosizer ND4, Coultronics, FRANCE). The zeta potential of nanocapsules prepared with various concentrations of ODN was determined as follows: 200 μl of the samples were diluted in 2 ml of a 0.1 mM KCI solution adjusted to pH 7.4 and analyzed with a Zetasizer 4® (Malvern Instruments, UK).

Freeze Fracture Electron Microscopy (FFEM)

A small drop of the suspension containing 30% glycerol as cryoprotectant was deposited on a thin copper planchett and rapidly frozen in liquid propane. Fracturing and replication (using Pt carbon) were performed with a Balzers Baf 301 freeze-etch unit. The replicas were washed and examined in a Philips 410 electron microscope.

Freeze Drying

A small drop of the aqueous dispersion was spread on a carbon coated grid (which was previously submitted to a glow discharge) and rapidly frozen in liquid propane and transferred to the cold stage of the freeze etch unit. It was freeze dried at −80°C for an hour and shadowed with Pt-C.

Determination of ODN Encapsulation Yield

Radiolabeled ODNs were mixed with unlabeled ODN (1/10). This isotopic dilution was used at different total concentrations ranging from 0 to 10 μM to prepare the nanocapsules as described previously. After the first ultracentrifugation, radioactivity was counted in Ultima Gold™ with a liquid scintillation system LS6000TA (Beckman, CA) in supernatant and pellet. The final encapsulation yield was calculated using the following equation:

$$\%\ Encapsulation = \left[\frac{\text{Pellet Radioactivity}}{\text{Total Radioactivity (pellet + supernatant)}}\right] \times 100$$

Localization of ODNs in Nanocapsules Aqueous Core

In order to investigate the localization of the ODNs in the nanocapsules, collisional quenching of fluorescence studies were carried out using fluorescein-labeled ODN and potassium iodide (Kl) as an aqueous quencher. Potassium iodide at different concentrations (0 to 1 M) and 0.001 M sodium bisulfite were added to the samples. Sufficient KCl was added to keep the ionic strength constant and equivalent to a 1 M Kl solution. Quenching of the fluorescent ODN in the nanocapsule suspension (ODN*in*NC) was compared to different controls: free fluorescein-labeled ODN in solution (FREE ODN), fluorescein-labeled ODN adsorbed onto the surface of the nanocapsules using CTAB (NC + CTAB + ODN), fluorescein-labeled ODN mixed with a 150 μM solution of CTAB (CTAB + ODN), and labeled ODN mixed with empty nanocapsules prepared without ODNs (NC + ODN). The fluorescence intensity of the fluorescein-labeled ODN was measured at 520 nm using excitation at 494 nm using a Perkin-Elmer spectrofluorimeter (LS 50 B). The excitation and emission slits were 5 mm. Data were analyzed

according to the Stern-Volmer equation for collisional quenching:

$$\frac{F_0}{F} - 1 = K_{sv}.Q,$$

where F_0 and F are the fluorescence intensities in the absence and in the presence of quencher, respectively; Q, the concentration of the quencher and K_{sv}, the Stern-Volmer quenching constant. K_{sv} is a reliable indication of the bimolecular rate constant for collisional quenching of fluorescein through the aqueous phase (23,24). In another experiment, K_{sv} was determined after degradation of the polymer using a 1M NaOH solution (incubation times ranging from 0 to 24 h). The pH of each preparation was adjusted to pH7 before fluorescence measurement.

Calcein Release from Nanocapsules

Nanocapsules were prepared as described by using an aqueous phase containing 2 mg/ml calcein. Release experiments were performed in water at pH 7.4 with nanocapsules as prepared and after dilution 100 times. Fluorescence was measured using a Perkin-Elmer spectrofluorimeter (LS 50 B). Excitation and emission wavelengths were 489 and 519 nm, respectively.

Stability of ODNs in the Presence of Serum

Nanocapsules were prepared as described using a 10 μM solution of 20 mer ^{33}P radiolabeled oligothymidylate (ODN*in*NC). Free ODNs (FREE ODN) and ODNs associated with nanospheres by ion-pair formation with CTAB (NS + CTAB + ODN) were used as controls. 150 μl of the different preparations: "ODN*in*NC", "FREE ODN" or "NS + CTAB + ODN" were mixed with 350 μl of fetal calf serum at 37°C for various time intervals ranging from 0 to 60 min. After incubation, the mixture was immediately placed at 70°C for 15 min to inactivate serum enzymes. ODN release from nanocapsules was obtained by incubation with NaOH (4M) for 12 h at 37°C.

ODN integrity was assayed for each preparation at different times interval by electrophoresis on a 20% polyacrylamide-7M urea sequencing gel (PAGE) followed by analysis using a multichannel radioactivity counter (Automatic TLC-Linear Analyzer®, Berthold, Germany)(21). The free 20 mer oligothymidylate migrated 3.7 cm in our experimental conditions and γ^{33}ATP approximately 12.5 cm (data not shown). The amount of intact ODN for each sample was evaluated by integration of the region corresponding to the first half of the 20-mer oligothymidylate peak (0 to 3.7 cm) to avoid interference from peaks corresponding to partially degraded ODNs.

RESULTS

Particle Size and Morphology

The mean diameter of the particles, measured by laser light scattering, was 350 nm with a standard deviation of 100 nm. FFEM confirmed this large size polydispersity. Most of the observed particles revealed a fracture plane which propagated at their periphery, revealing concave and convex fractures. In rare cases, it was possible to observe an internal peripheral matrix (Fig. 1a). In addition, freeze drying evidenced the presence of some collapsed particles (Fig. 1b).

Encapsulation Yield and Zeta Potential

For a 1 μM aqueous solution of ODN, 70% of the ODN was associated with the pellet. Figure 2a shows the yield of the ODN encapsulation depended on the initial concentration of ODN in the aqueous phase. The encapsulation rate (%) decreased gradually with increasing ODN concentration, whereas the total amount of encapsulated ODN increased (Fig. 2b) up to a concentration of 5 μM at which a plateau was reached corresponding to a value of 2.5 nmol ODN per mg of polymer.

Nanocapsules without ODN had a zeta potential of -38 ± 5.7 mV. When ODNs were encapsulated, all values were around -40 mV (data not shown).

Localization of the ODN in Nanocapsules by Quenching of Fluorescence

The Stern-Volmer plots are shown in Fig. 3. The Stern-Volmer constant was low when the ODNs were inside the

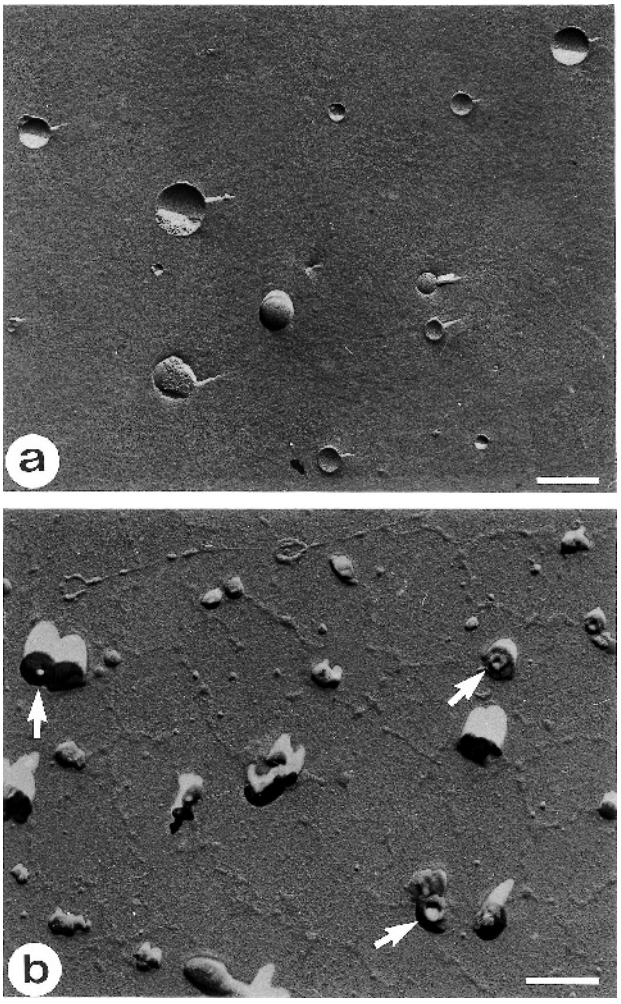

Fig. 1. Electron microscopy. (a) Freeze fracture; (b) Freeze drying. Bar = 300 nm.

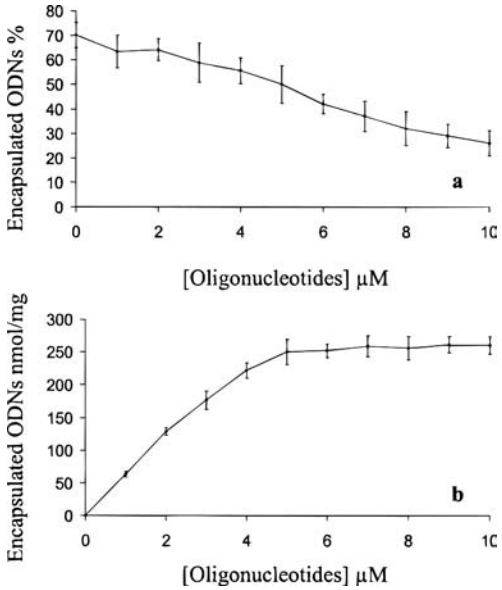

Fig. 2. Encapsulation yield (a) and amount per mg of polymer (b) of the oligonucleotides in the PIBCA nanocapsules as a function of the oligonucleotide concentration in the aqueous phase used for the preparation (n = 4). Encapsulation yield is expressed as a range of the total amount of oligonucleotide introduced.

nanocapsules "ODN*in*NC" (K_{SV} = 0.64 M^{-1}), whereas the value of K_{SV} was 4.03 M^{-1} for "FREE ODN". These data show the fluorescein-labeled ODNs were almost inaccessible to the quencher when they were located in the aqueous core of nanocapsules whereas they were highly accessible to the quencher when free in solution. Intermediate values of K_{SV} were obtained with the following controls: "CTAB + ODN" (K_{SV} = 3.56 M^{-1}), "NC + CTAB + ODN" (K_{SV} = 3.48 M^{-1}) and "NC + ODN" (K_{SV} = 2.52 M^{-1}). Even after 10-times dilution of the samples, no change in KSV was observed indicating the polymer concentration had no influence on the Stern-Volmer constant (data not shown). When nanocapsules were degraded by incubation with a 1 M NaOH solution, K_{SV} increased dramatically from 0.64 M^{-1} to 3.73 M^{-1} after 24 h (Fig. 4). This increase of K_{SV} represents a transition from a state where fluorescein bound to the ODN was not accessible to the quencher to a state in which it became accessible to Kl after the hydrolysis of the polymer by NaOH had occurred. Thus, after 24 h, the major part of the fluorescein-labeled ODNs was released from the inner aqueous core of the nanocapsules.

Calcein Release Experiments

Incubation of calcein loaded nanocapsules in water (pH 7.4) did not lead to any increase in fluorescence values over 24 h. When diluted 100 times, the sample displayed a proportional decrease of fluorescence values which did not vary during the 24 h of the experimental period (data not shown).

Stability of the ODN Nanocapsules in the Serum

Free oligothymidylate was rapidly degraded in serum (Fig. 5a). In this case, the initial 20-mer peak at 3.7 cm, corresponding to intact ODN decreased after only 30 s in serum and moved rapidly to lower molecular weights, indicating a significant part of ODN was completely degraded to ^{33}P monothymidilate (at 12.5 cm) and lower molecular weight compound ($\gamma^{33}P$) which ran out of the gel (>14 cm). Integration between 0 and 3.7 cm indicated that 50% of the oligothymidylate were degraded at 30 s and less than 5% remained intact after 60 min incubation. The oligothymidylate within nanocapsules showed a radically different degradation profile (Fig. 5b). The main peak obtained for each experimental time point remained at a distance of 3.7 cm which means that intact ODN was still present during the course of the incubation period. However,

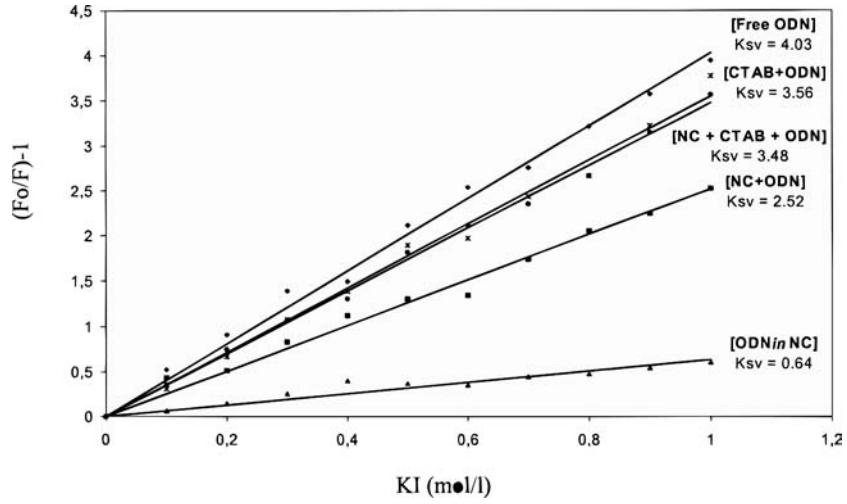

Fig. 3. Potassium iodide Stern-Volmer quenching of fluorescein labelled oligonucleotides in the different formulations (from the higher K_{sv} to the lower): Free oligonucleotides [Free ODN], oligonucleotides complexed to CTAB [CTAB + ODN], oligonucleotides adsorbed onto the nanocapsules via CTAB [NC + CTAB + ODN], free oligonucleotides mixed with empty nanocapsules [NC + ODN] and encapsulated oligonucleotides in the nanocapsules [ODN*in*NC].

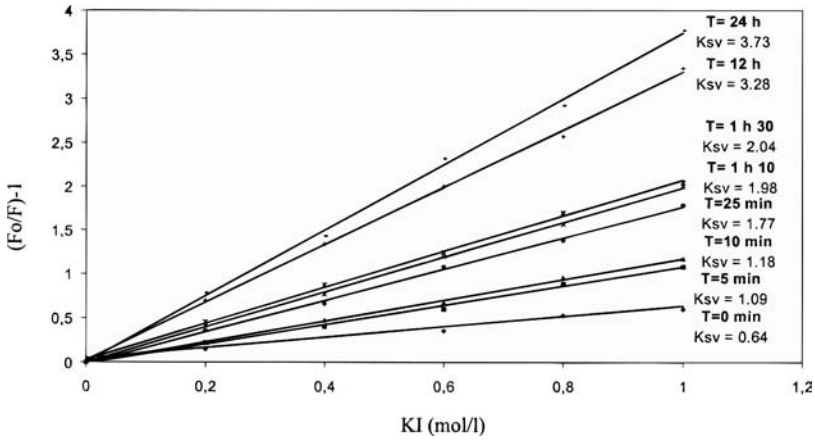

Fig. 4. Potassium iodide Stern-Volmer quenching of fluorescein labelled oligonucleotides encapsulated in nanocapsules progressively degraded in a 1 M NaOH solution.

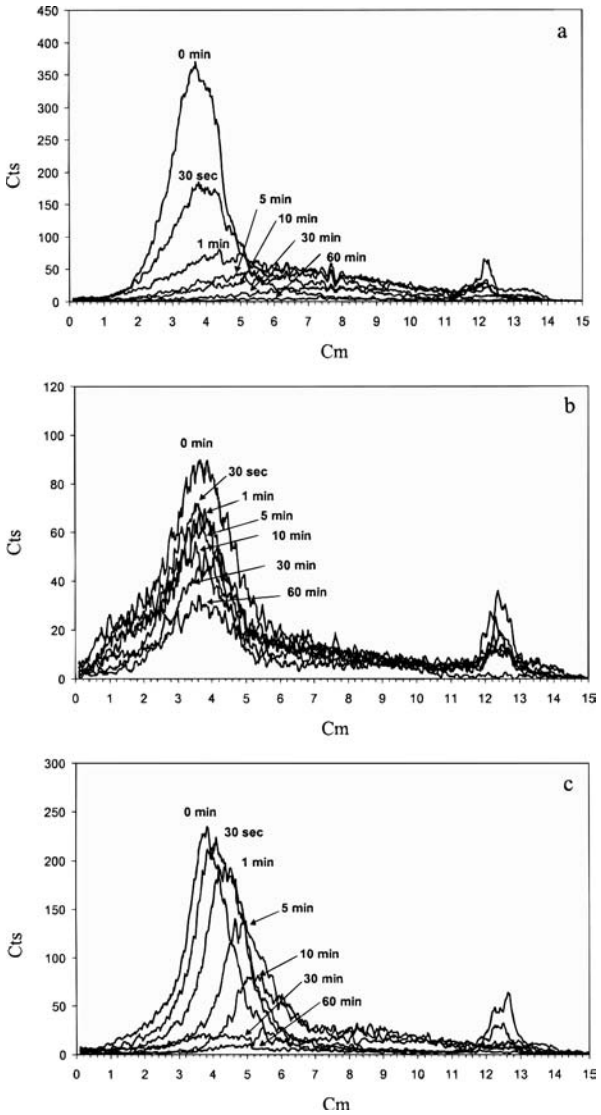

Fig. 5. Electrophoresis chromatograms of free oligothymidylate (a), oligothymidylate encapsulated in nanocapsules (b) and oligothymidylate adsorbed on the surface of nanospheres (c) after incubation in serum.

the intensity of the peak decreased, showing a part of the ODN was degraded. The fate of the encapsulated ODNs seemed to be all or nothing: fully protected or fully degraded, corresponding respectively to encapsulated ODN and to ODN released after the polymer forming the wall of the nanocapsules had been degraded. Indeed, the ODN that leaked out followed the same kinetic of degradation as the free ODN. For this nanocapsule formulation, integration of the 0-3.7 cm region indicates that 35% of the ODNs remained intact after 60 min of contact with serum. When oligothymidylate was adsorbed onto the surface of nanospheres (NS + CTAB + ODN), the degradation profile was completely different (Fig. 5c): even after 30 s, the main peak moved progressively to lower molecular weights while these peaks also decreased gradually in intensity. In this case, the complete pool of initial ODN was progressively degraded. This type of protection was given by the adsorption of ODN to the surface of the particles which could not prevent the initiation of degradation at extremity of the ODN.

DISCUSSION

Zeta potential, fluorescent-ODN quenching, electron microscopy, and degradation studies were able to demonstrate the ODNs were located in the interior of the prepared particles and that they had a nanocapsule structure.

As shown by Fig. 2, the ODN could be encapsulated efficiently. Increasing the concentration of ODN in the aqueous phase used for the preparation led, however, to a decrease in the yield of encapsulation, but the total amount of encapsulated ODN still increased until it reached a plateau at 5 μM. This profile may be explained by the fact that, at high concentration, a part of the ODN was located at the interface, which could hamper the polymerization of the monomer.

In a previous study, we showed the adsorption of ODN onto the surface of nanospheres had a dramatic effect on their zeta potential due to the negative charges provided by the phosphate groups of the ODNs (22). In contrast, in the present case, increasing the amount of ODN in the formulation had no effect on the zeta potential, suggesting the main part of the ODNs is located inside the nanocapsules rather than at their surface.

For a wide proportion of the particles observed after freeze

fracture, the sections occurred around the surface of the particles. This type of fracture may be explained by the presence of a fracturable membrane which is due to the presence of span at the interface. The freeze dried sample micrographs displayed particles which were partly collapsed due to the existence of a large internal aqueous cavity.

Quenching of fluorescence studies also suggested that fluorescent ODNs were located in the aqueous core of the nanocapsules, surrounded by a polymeric wall, thus inaccessible to the quencher. On the contrary, when fluorescent-ODNs were free in solution, fluorophores were highly accessible and important quenching occurred. Similar quenching could be obtained with nano-encapsulated ODNs only after performing the hydrolysis of the nanocapsule polymer wall, thus releasing the ODNs. Completely shielding the ODN by the nanocapsule would have resulted in a Ksv value of zero. However, the obtained value of Ksv with ODN nanocapsules was still very low (0.64 M^{-}) and this increment could be explained by the presence of a small portion of non-encapsulated ODNs in the mixture. Self-quenching of the fluorophores was shown not to occur in a wide range of concentration (5–100 μM) (data not shown). It was noteworthy that free ODNs mixed with empty nanocapsules presented a Stern-Volmer constant intermediate between those obtained with nano-encapsulated and free ODNs. Indeed, it was found that when mixed with empty PIBCA nanocapsules, fluorescein-labeled ODNs were partially adsorbed onto their surface due to hydrophobic cohesion forces involving the heterocycles belonging to the fluorescein group (data not shown). This adsorption may partially decrease the accessibility of the quencher and therefore, reduce the Stern-Volmer constant. When ODNs were mixed together with CTAB, the ionic interaction between CTAB and phosphates rendered the fluorescein fully accessible to the quencher. When CTAB was mixed with ODNs and empty nanocapsules, the ODNs were found adsorbed onto the surface of the capsules (data not shown) by electrostatic interactions with CTAB. In this case, the fluorescein marker which was primarily not involved in binding, may have remained accessible to the quencher. Figure 6 depicts a model for fluorescent ODNs localization and quenching in the different preparations. When the nanocapsules were degraded by hydrolysis after addition of NaOH (25,26), electrophoresis showed that released ODNs migrated to 3.7 cm (Fig. 5b at t = 0 min), corresponding to the 20-mer oligothymidylate. This result indicates intact ODNs can be released from degraded nanoparticles, thus conserving their affinity for the targeted sequence.

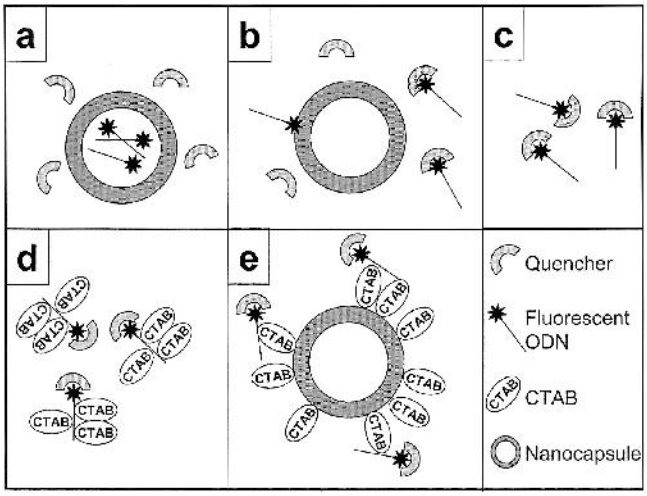

Fig. 6. Model for fluorescent oligonucleotides localization and quenching for (a) [ODNinNC], (b) [NC + ODN], (c) [FREE ODN], (d) [CTAB + ODN] and (e) [NC + CTAB + ODN].

The possibility that porous nanospheres with ODNs located in their core had been prepared instead of nanocapsules can be excluded because it has been clearly shown that due to their negative charges and to the absence of hydrophobic interactions, ODNs do not interact spontaneously with the PACA polymer (14). For this reason, ODN loading onto nanospheres requires a hydrophobic cation such as CTAB. Thus, if ODNs are not able to adsorb to the surface of PACA in the absence of CTAB, they would not be expected to associate with porous nanospheres; on the contrary, high loading efficiency was observed here. Moreover, in the procedure used for the preparation of the nanocapsules, although washing was performed with water, no significant ODN release or desorption was observed. Similarly, after dilution of the fluorescent-ODN-nanocapsules, the Ksv value remained unchanged (data not shown), confirming no release occurred. Again, in the case of a porous nanosphere structure, we would predict a strong desorption of the ODN during washing or dilution. Finally, when calcein was encapsulated, no increase of fluorescence with time was observed over 24 h and when the sample was diluted, no dequenching occurred. This again argues in favor of a core shell nanocapsule structure rather than a porous nanosphere system which would have released calcein after dilution.

Our studies also demonstrated that nano-encapsulation was able to protect ODNs against degradation by serum nucleases and that this protection was much more efficient than that obtained with CTAB-coated nanospheres (NS + CTAB + ODN), which allow only simple adsorption of ODNs onto the surface (14) rather than encapsulation. Moreover, the pattern of degradation of the nano-encapsulated ODNs was totally different from that of adsorbed ODNs. Indeed, when ODNs were adsorbed onto the surface of the nanospheres, they were degraded progressively by exonucleases, as was also observed by Chavany on PAGE gel electrophoresis (27). In contrast, when the ODNs were encapsulated, a large proportion of the ODNs remained intact while a part was degraded, the latter corresponding to the ODN fraction progressively released as the polymeric envelope was hydrolyzed under the action of serum esterases (25,26). This pattern of degradation also confirmed the nanocapsule structure of the particles since if the ODNs had been simply adsorbed to the nanocapsules surface, a similar degradation pattern to that of adsorbed ODNs would have been observed.

Intracellular uptake and release of ODN into the cell cytoplasm is an important challenge. As far as PACA polymers are concerned, it has been previously observed that the uptake of ODN by U937 cells was dramatically increased when adsorbed onto nanospheres and that cell internalization occurred through an endocytotic/phagocytotic pathway (27). Furthermore, additional experiments have shown that when associated with PACA nanospheres, intact ODN were detected in both the nuclear and the extra nuclear fractions (27), and were able to provoke a specific inhibition of mutated Ha-ras-mediated cell proliferation (28). These observations demonstrate ODN delivered by

nanospheres escaped from the lysosomes. How they do so remains unclear but this may be attributed to PACA polymer and/or its degradation products. Thus, it is to be expected that the nanocapsules described here would behave similarly, allowing cytoplasmic and nuclear delivery of ODN.

The advantage of nanocapsules compared to other nanoparticulate strategies is that, as shown in the paper, the ODN are encapsulated and not simply adsorbed at the surface of the particles. As a consequence, a better protection from degradation by nucleases is expected as well as a reduced burst release after administration in vivo. Since anti-ras ODN-coated nanospheres have been shown to be efficient against tumor growth after intratumoral administration (28), we could expect to obtain still greater efficiency with ODN encapsulated within these newly designed nanocapsules. Additionally, since after intravenous administration, ODN-coated nanospheres were not effective, probably because of rapid desorption/degradation in the blood stream (15), encapsulation technology would appear very interesting. In comparison with liposomes, nanocapsules should be more stable in biological media, especially in blood; this type of polymeric system is less susceptible to destabilization by protein interactions.

When administered by the I.V. route, the nanocapsules would certainly be taken up by the macrophages of the reticuloendothelial system, as was observed with PACA nanoparticles (15). Targeting the macrophages may, however, be useful therapeutically, for example, as demonstrated by Chakraborty *et al.* who have shown that low concentrations of anti-Leshmania ODNs could specifically reduce the amastigote burden in cultured macrophages (29). Moreover, modulation of the biodegradability of polyalkylcyanoacrylates is possible (30) and, therefore, the release of the ODNs may be controlled by changing the length of the alkyl chain.

CONCLUSIONS

This study describes, for the first time, a successful methodology for ODN encapsulation. This new carrier has shown its ability to protect ODNs to a greater extend than nanospheres on which ODNs were simply adsorbed by ionic interactions. In addition, these nanocapsules containing an aqueous core do not necessitate the use of positively charged compounds to ensure ODN association, thus avoiding the protein interaction and toxicity associated with this type of molecule. This technology may also offer interesting perspectives for DNA and peptide transport and delivery.

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