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Circular dichroic properties and average dimensions of DNA-containing reverse micellar aggregates

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Abstract

With the aim of investigating the compartmentation of nucleic acids and surfactant aggregates, we have studied the circular dichroic properties of DNA solubilized in reverse micelles. DNA incorporated in AOT/isooctane reverse micelles (AOT = bis-2-ethyl-hexyl sodium sulfosuccinate) assumes an anomalous circular dichroism (CD) spectrum with the characteristic features of a psi spectrum. Older literature observations could therefore be confirmed that attribute these spectral changes to the fact that the reverse micelles induce the formation of a condensed form of DNA. A dynamic light scattering (DLS) characterization of the DNA-containing micellar solutions was carried out, and three populations of aggregates in a polar solvent are observed, with an average radius centered at 5, 100 and 1000 nm, respectively, all three containing DNA. Several forms of DNA, including a plasmid, have been investigated. The formation of 1-μm-large aggregates depends on the DNA concentration and such aggregates disappear in the course of a few hours. Conversely, the 100 nm aggregates are stable for at least 1 day and contain DNA in a normal spectral state at low concentration and in a condensed form—it is the characteristic psi spectrum—in a higher concentration range. The solubilization of DNA in reverse micelles brings about unexpected larger structures in hydrocarbon solution, and whereas the very large component can be with all likelihood be attributed to clusters of smaller reverse micelles, the components at 100 nm radius appear to be a quite stable and characteristic feature of DNA-containing reverse micelles.

Keywords: Reverse micelle; Psi spectrum; Condensed form of DNA; Circular dichroism; Light scattering

1. Introduction

This paper deals with the interactions between DNA and reverse micelles with the aim to determine, mostly on the basis of circular dichroism (CD) studies, whether and to what extent specific nucleic acid structures result from such interactions. Although reverse micelles, formed in organic detergent solutions, are less interesting from a biological point of view than micelles or liposomes that form in water, they offer the advantage of a compulsory compartmentation. In fact, all water-soluble components added to the hydrocarbon detergent system must go into the water pool of the reverse micelles—and in this sense reverse micelles provide one with a well-defined microreactor. Particular emphasis is given to the DNA packaged in reverse micelles as a model for the condensed form of DNA. This form of DNA occurs upon chromosome formation during metaphase and is thought to play a role in the regulation of gene expression. It is now accepted that this particular DNA structure acquires very peculiar and characteristic CD features, the so-called psi spectrum. This DNA form has been simulated in vitro with the use of certain polymers [1–3].

The question of the dimension of reverse micelles containing DNA has been investigated with few detergents systems. Triton B reverse micelles (100 mM, $w_0 = 6$) with lambda DNA have an average radius of 10–15 nm [4] and Span 80/Tween 80 11:2 reverse micelles (88 mM, $w_0 = 38$) with plasmid DNA have a mean particle radius of ca. 32 nm [5]. Hanley et al. [6] hinted that the average radius of AOT (bis-2-ethyl-hexyl sodium sulfosuccinate) reverse micelles (50–100 mM) with DNA (0.1 mg/ml buffer) in hexane is 100–150 nm whereas the mean radius of AOT reverse micelles without DNA is about 10 nm (w_0 used was 6 for 100 mM AOT reverse micelles but the water content of the reverse micelles at lower AOT concentrations remains unknown).

The question, whether the psi form of DNA could also be obtained in the compartment of reverse micelles, has been addressed by an early preliminary communication from our group [7]. In the present paper, we reconsider this work by

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using the classic AOT reverse micellar system but extending the investigations to different DNA types and lengths, and varying the concentration as well. Circular dichroic studies are supplemented by dynamic light scattering measurements in order to determine the dimensions of the aggregates and their stability over time.

2. Materials and methods

2.1. Materials

DNA from herring testes was purchased from Fluka (Buchs, Switzerland). Lambda DNA and pBR322 DNA, a plasmid-cloning vector, was obtained from New England BioLabs (Beverly, MA, USA). AOT was purchased from Serva or Sigma (Buchs, Switzerland).

2.2. Methods

AOT/isooctane solution was filtered with Millex®-GV 0.22 μm filters (Millipore, Bedford, US) to remove dust. DNA was solubilized in the isooctane micellar solutions by direct injection techniques (i.e. adding a few microliters of DNA stock solution (4 or 0.5 mg/ml). To extract the DNA from reverse micelles, the solution was simply transferred in a glass vial with a large surface. After 12 h, a precipitation could be obtained. The supernatant was removed and the resulting DNA dried with nitrogen and resuspended in water. For reference experiments, the same number of microliters of water or buffer was added to AOT/isooctane or DNA solution was added to water or buffer. The length of herring DNA was determined by gel electrophoresis according to Sambrook et al. [8]. It was 500–1000 bp unless otherwise stated. Lambda DNA was 48502 bp long [9] and the length of pBR322 DNA was 4361 bp [10].

UV/VIS absorption spectra were recorded at 25° on a Cary 1E spectrophotometer from Varian using quartz cells of 1 cm length unless otherwise stated. CD spectra were measured on a JASCO J-600 spectropolarimeter equipped with a thermostated cell holder. Quartz cells of 0.5 cm path length were used. Samples were scanned nine times at a rate of 50 nm/min. Molar ellipticities were normalized per base pair. The concentration of DNA was determined with UV spectroscopy. Dynamic light scattering (DLS) measurements were performed on an apparatus consisting of an Innova 70 coherent argon ion laser ($\lambda = 488$ nm), a digital autocorrelator ALV 5000 and an ALV laser goniometer model ALV/SP-125 S/N 49. Samples were measured 10 times at 60°. DLS spectra were normalized to the integral of the peak around 5 nm.

3. Results and discussion

As already mentioned, preliminary observations have indicated that reverse micelles can induce the condensed form of DNA. This results in an anomalous spectrum, usually called the psi-form[1], and one distinguishes between +psi and −psi spectrum depending on whether the long wavelength band has a positive or negative signal. Early work by Jordan et al. [11] also obtained CD spectra with intense positive and negative bands studying aqueous solutions of DNA in the presence of salt and ionized polyacrylate.

It is assumed that in vivo DNA condensation is brought about by basic proteins: in fact, DNA in the nucleus of a higher eukaryote is intimately associated with basic nuclear protein rich in lysine or arginine. It is not so easy to reproduce in the laboratory this protein-induced condensation process, although interesting experiments have been performed with poly(arginine) and other polymers [2,3,12]. Apparently, due to the high negative charge of the DNA phosphate backbone, an increase in the degree of charge neutralization of the DNA should result in an extensive condensation and in the separation of DNA in the form of insoluble compact structures. It is commonly accepted that a high degree of intermolecular interaction among many chains is the basis of this highly condensed form, with the consequent formation of large wreathed coils.

This consideration about the dimensions of the DNA aggregate is important. It has been in fact reported that the CD spectrum of an optically active molecule may change drastically when it becomes part of a larger aggregate [13]. In particular, if this aggregate has dimensions similar to the wavelength used in the CD experiment, two kinds of changes or anomalies may occur [2]. Firstly, long tails may appear in the CD at wavelengths outside the absorption bands of the constituent molecules [14]. Secondly, as the size of the aggregate grows, the magnitude and the form of the CD bands changes [15]. These perturbations within the absorption bands give rise to the already mentioned psi-type anomalies.

On the basis of the above considerations, one may argue that the formation of the psi form of DNA may be facilitated by a forced compartmentation in a very small volume and by the presence of high charge density. If this is so, then the use of reverse micelles appears a suitable choice. In reverse micelles, the dimension of the water pool is dictated by the molar ratio of water to surfactant, w_o. For a typical w_o value of 18 in the isooctane/AOT/water reverse micelle system, the water pool radius is around 5 nm and the water pool is extremely rich in negative charges of the AOT polar head groups [16]. When the water-soluble DNA is added to the reverse micellar system, it can only go in the water pool, and given the consequent high local concentration, it is conceivable that DNA may assume a condensed form.

Let us consider now some experiments. When a DNA sample with a length of 300 bp was solubilized in the

[1] This nomenclature originated from the fact that the phenomena have been most commonly observed in solutions of condensed DNA particles, where the agent used to condense the DNA was a polymer at relatively high salt concentration (hence "psi" = polymer and salt-induced CD spectra).

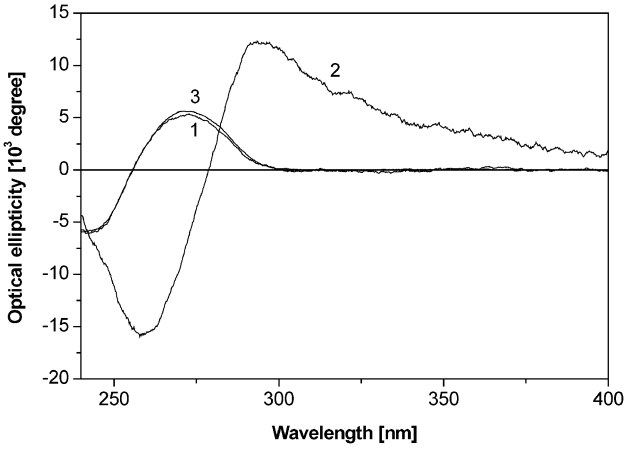

Fig. 1. CD spectrum of herring DNA in water, 66 μg/ml (1), of 4 mg herring DNA/ml water in reverse micelles consisting of AOT (50 mM)/isooctane/water (w_0 = 18.5) (2) and herring DNA in water after extraction from the reverse micelle isooctane solution (3). T = 25 °C.

micellar hydrocarbon solution, results as shown in Fig. 1 were obtained. The first observation is the striking increase of optical ellipticity with respect to normal water solution values. Equally striking are the shifts in position of λ_{max} of the CD maxima and position of the crossover point. Also notice the sizable contribution at very high wavelength, where normally DNA does not have a UV absorption contribution. Most of these observations are in keeping with our preliminary study [7].

These are several questions concerning the micelle DNA complex that we have not addressed in the preliminary paper. One is whether the formation of the micellar condensed DNA form is reversible or not. We have now tackled this question and we can conclude that this type of DNA condensation is reversible. In fact, after extraction from the reverse micelles and resolubilization in water, the normal DNA aqueous spectrum was recovered (Fig. 1). This suggests that only secondary interactions are involved in the formation of the condensed form, and also suggests that the functionality of DNA is not lost when it is entrapped in reverse micelles. By inference, one may suggest that the functionality of DNA in vivo may not be lost when DNA is temporarily engaged in a condensed psi form.

One other important question is relative to the dimensions of the DNA-containing reverse micelles. Two different limit cases might occur: either the reverse micelles maintain their original small size, forcing the DNA macromolecules

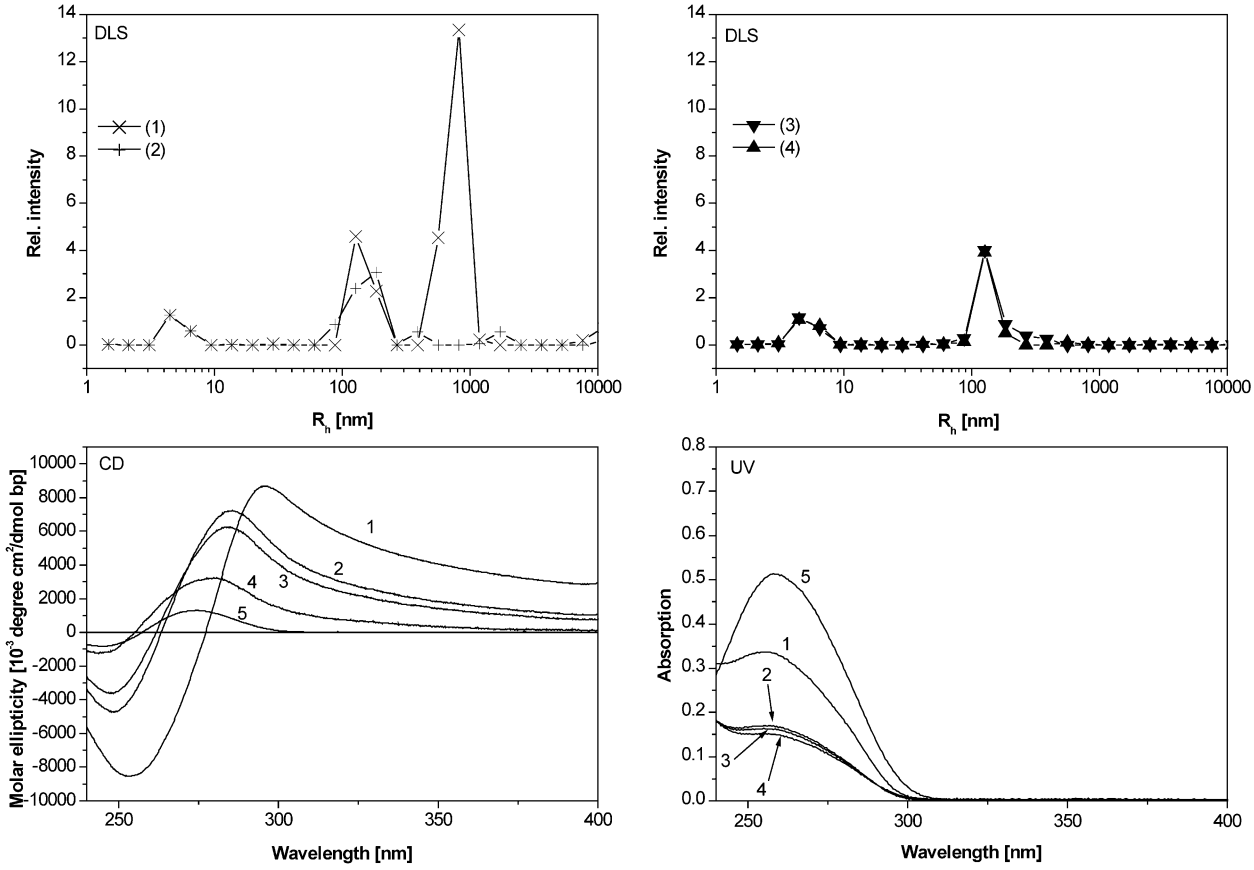

Fig. 2. DLS, UV and CD measurements on AOT reverse micelles (97 mM) in isooctane, w_0 = 18.5, containing aqueous herring DNA solution (4 mg/ml water) in comparison to the same amount of added DNA in water. AOT micelles after 10 min incubation, 84 μg DNA/ml solution (1), after 5 h incubation, 42 μg DNA/ml solution (2), after 1 day incubation, 40 μg DNA/ml solution (3), after 8 days incubation, 38 μg DNA/ml solution (4), 127 μg DNA/ml solution in water (5). T = 25 °C, UV cells with 0.2 cm path length were used.

inside their 5 nm radius aqueous compartment; or larger aggregates are induced by the presence of the large DNA guest molecules, obtained by the fusion and re-organization of several initial reverse micelles.

A highly sensitive DLS apparatus was used in order to characterize the size of the reverse micellar aggregates. The system studied was the classic AOT/isooctane reverse micellar system with a w_o of 18.5. CD and UV spectra were collected in parallel to DLS data.

Some typical results are collected in Fig. 2. Light scattering data suggest for the "empty" micelles (i.e. micelles containing water but without DNA) an average dimension centered around a 5 nm radius. After addition of DNA, two additional populations are observed, which are remarkably shifted towards larger sizes, with water pool radius of 100 and 1000 nm, respectively. This indicates a remarkable increase of the average micellar size after incorporation of DNA. It should be noted that all these solutions are transparent and without noticeable light scattering, as shown by the UV spectra of Fig. 2. This lack of significant scattering also suggests that the percentage of these large structures in solution is negligible. Fresh AOT/isooctane/DNA reverse micelles containing the same added amount of DNA as DNA in water showed a significantly smaller UV absorption at 260 nm than the latter. This hypochromic effect is attributed to a changed tertiary structure of the DNA [7]. From 5 h on up to 8 days, the DNA concentration of AOT/isooctane/DNA reverse micelles (according to UV) changed little even though the molar ellipticity changed significantly within this time. While all these spectra show the characteristic shape of DNA absorption, the corresponding CD spectra show the characteristic psi form.

We studied the time stability of these three forms, by repeated DLS studies for up to 1 week. The larger aggregates were not stable with time. In fact, as shown in Fig. 2, this population was already substantially smaller after 5 h, and vanished completely within 1 day. The population around 100 nm instead appeared to be stable for at least 8 days, as judged by the DLS spectra. These 100 nm species showed even after 8 days a psi-type CD spectrum.

Since all these observations had been made with herring DNA, it appeared important to check whether they were also present in other DNA types with different lengths and forms.

Fig. 3 reports the case of a 500–1000 bp herring DNA sample together with the cases of lambda DNA (48502 bp) and pBR322 plasmid (4361 bp). In all these cases, a relatively small concentration of 0.5 mg/ml DNA was used, which is ca. one order of magnitude lower than the investigated case of herring DNA shown in Fig. 2 (4 mg/ml). In particular, Fig. 3A shows the UV absorption spectra in buffer and in reverse micelles. A significant hypochromic effect is again apparent by comparing the spectra of the lambda DNA in buffer and in reverse micelles. As far as the CD spectra are concerned (Fig. 3B), normal CD spectra as in aqueous solution are present indicating a B helix. When one compares

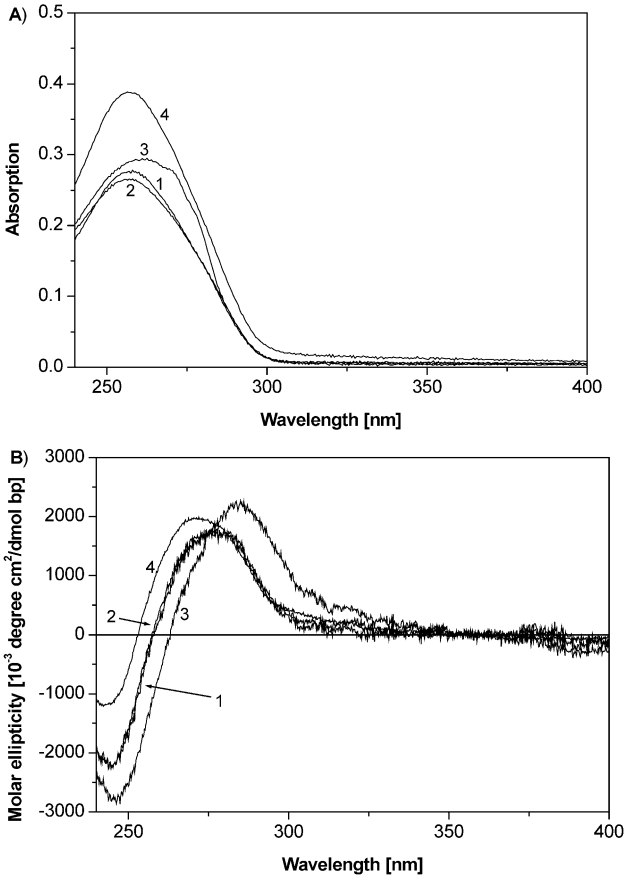

Fig. 3. (A) UV and (B) CD spectra of different kinds of DNA in AOT reverse micelles (99 mM) in isooctane, $w_0 = 18.5$, containing the same amount of added aqueous DNA (0.5 mg/ml buffer): herring DNA in 10 mM Tris, pH 8 (1), lambda DNA in 10 mM Tris, pH 8, 1 mM EDTA (2), pBR322 plasmid in 10 mM Tris, pH 8, 0.5 mM EDTA (3) and lambda DNA in 10 mM Tris, pH 8 (4). $T = 25$ °C.

these data with the data of Fig. 2 for herring DNA obtained at a DNA concentration of 4 mg/ml and with the data collected several years ago with the plasmid pBR322 at a DNA concentration of 2–8 mg/ml [17], both of them showing the typical psi form, one reaches the conclusion that the condensed form of DNA disappears at lower concentration. This confirms the notion that a higher concentration of DNA results in intermolecular interactions of an amorphous, interpenetrating coil of DNA molecules. This seems to be the structural prerequisite for the condensed form of DNA.

The DLS data for these three DNA types including the time dependence is presented in Fig. 4. Notice again that in the case of the plasmid, there are three well distinct species centered again at 5, ca. 100, ca. 700 nm, and that the highest component disappears after 1 week, whereas the component at 100 nm does not. In the case of the lambda DNA, only two peaks appear that are still present after 1 week. All this has been carried out at the same DNA concentration of 0.5 mg/ml, which shows that the 100 nm form is present also when there is no condensed DNA form in the CD spectra (see Fig. 3).

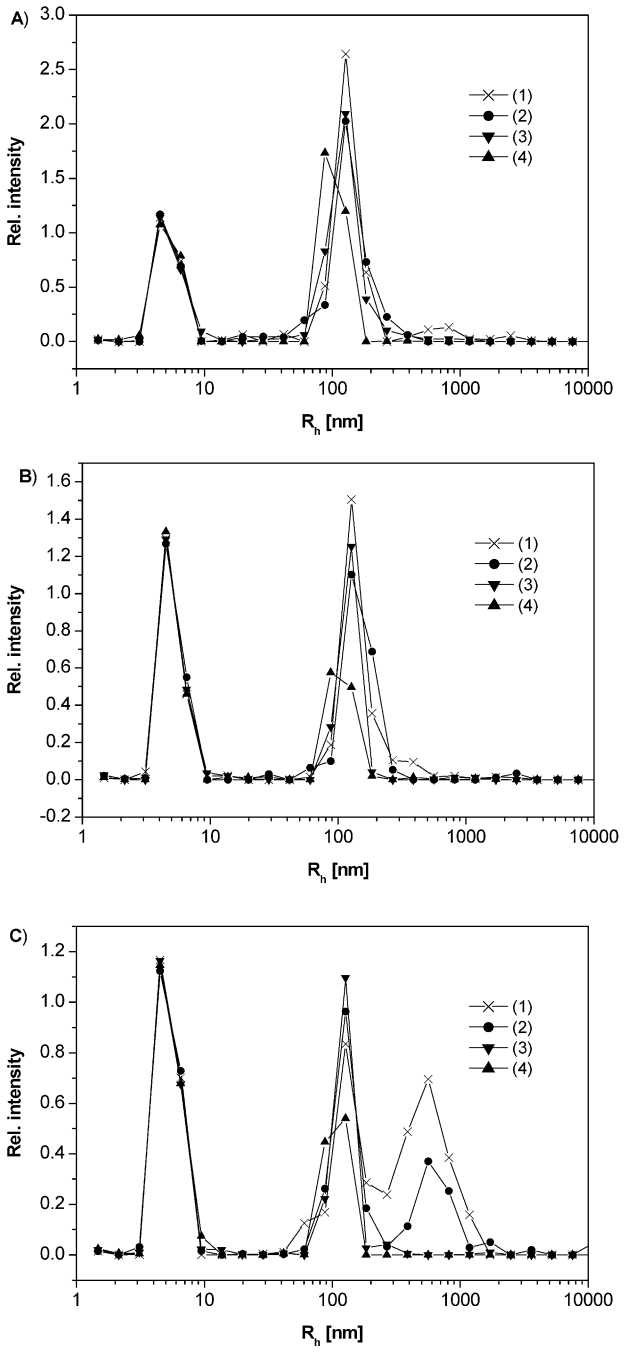

Fig. 4. DLS of several types of DNA in AOT (99 mM)/isooctane reverse micelles, $w_0 = 18.5$, containing the same amount of added aqueous DNA solution (0.5 mg/ml buffer), (A) herring DNA in 10 mM Tris, pH 8, (B) lambda DNA in 10 mM Tris, pH 8, 1 mM EDTA, (C) pBR322 plasmid in 10 mM Tris, pH 8, 0.5 mM EDTA; after 10 min incubation (1), after 5 h incubation (2), after 1 day incubation (3), after 8 days incubation (4). $T = 25$ °C.

A few observations can be made on the basis of the data of Figs. 2–4. Firstly, the existence of reverse aggregates with radius around 100 nm or larger in a clear and transparent solution is per se quite surprising. It is usually assumed that reverse micelles are small. Consider in this regard that the 500–1000 bp herring DNA used in these experiments, once assumed in the linear rigid form, should correspond to ca. 50–100 helical turns, i.e. it should have a length of ca. 170–340 nm. This would perhaps account for the aggregates having a radius of 100 nm; however, the same argument does not apply to the case of lambda DNA with 48502-bp length. It is indeed unlikely that DNA in solution maintains a rigid rod structure, and most likely it assumes a bent or a coiled structure that would reduce its linear dimensions so as to fit into reverse structures having a radius of 100 nm.

It should be clear, however, that we have not yet demonstrated that the 100 nm aggregates are regular reverse micelles — studies to clarify this point are in progress in our group. Also, the interpretation of the 1 μm aggregates remains problematic. Our data suggest that these are aggregates that disappear with time and do not occur at lower concentrations. Most probably, then, we are dealing with clusters of small 5 or 100 nm micelles, which disaggregate with time. However, one should also recall that literature shows that entire microorganisms can be solubilized in hydrocarbon reverse micellar solutions [18,19], where they are stable for several days. The dimensions of these microorganism-containing reverse micelles have not been studied to date, but presumably these are at least as large as the guest microorganisms, i.e. of the order of microns. In other words, it cannot be excluded that part or all of these very large aggregates are indeed single reverse micelles that owning to their extreme size are less stable and precipitate with time. Studies in our group are in progress to clarify this point, which is not easy as electron microscopy cannot be easily applied to reverse micellar systems and the physical separations of the three species by chromatography also does not appear readily feasible.

4. Concluding remarks

This work confirms that the simple solubilization of DNA into reverse micelles induces under various sets of conditions the formation of the supercondensed form of DNA, which results in a psi spectrum. This is most likely an aggregate of DNA molecules, a particular coiled quaternary structure whose formation is reversible. The fact that the very simple compartment of reverse micelles induces reproducibly and reversibly this form may turn out to be useful to study the details of this interesting and biologically important DNA form. To be noted is the fact that one obtains transparent solutions, which are amenable to NMR studies. Also, this feature is not confined to a particular type or length of DNA; it appears, on the contrary, to be a general phenomenon.

Another interesting aspect of this work is about the nature of the larger structures observed in the reverse micellar solutions. We have suggested here that the very large component—that is not so stable over time—can be ascribed to a cluster of smaller micelles. This hypothesis requires further study. However, the really challenging

question is about the nature of the ca. 100 nm structure, which is quite time-stable and which forms with all types of DNA tested in this study. It might be that this component is indeed a novel form of reverse structure, which can be micellar or not; or some form of very stable aggregate or cluster of the small 5 nm reverse micelles. As already mentioned, the elucidation of this structure is experimentally not easy. In fact, electron microscopy cannot be easily applied to hydrocarbon solutions, and also chromatographic separation techniques are not so established in the field of reverse micelles. This technique needs furthermore the assumption that such micellar aggregates can be separated from each other, which implies that they are not in chemical equilibrium with each other, but kinetically trapped forms [20]. At any rate, this study about the structure of these novel forms is now in progress in our group.

In closing, we would also like to mention what to us appears as a particularly challenging field of study emerging from this work. This concerns the possibility that different populations of reverse micelles can fuse and react with each other. It is known that reverse micelles are dynamic systems, characterized by a high frequency of collisions and exchange of the water pools with each other [21,22]. If this were so also for biopolymer-containing reverse micelles, the way would be opened to a very efficient modeling of reaction between biological compartments. This is presently also under study.

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