Vol. 107, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 30, 1982 Pages 538-545

SOLUBILIZATION AND CONDENSED PACKAGING OF NUCLEIC ACIDS IN REVERSED MICELLES

V.E. Imre and P.L. Luisi (≠)

Technisch Chemisches Labor der ETH, Zürich, Switzerland

Received May 28, 1982

Summary: RNA and DNA can be solubilized without denaturation in isooctane solutions with the help of reverse micelles formed by di(2-ethyl-hexyl) sodium sulfosuccinate and small amounts of water (down to 0.5%,v:v). With respect to aqueous solutions, RNA (mol. weight 20,000-30,000) in the hydrocarbon micellar solutions shows a decreased absorbance in the 260 nm region, accompanied by an increase of ellipticity. This is attributed to a higher conformational rigidity of the guest biopolymer, and most probably to an increase of base pair stacking. While spectra of low molecular weight samples of DNA (ca. 5000 Daltons) show practically no difference with respect to water, the CD spectrum of the 250,000 Daltons sample is dramatically changed and becomes reminiscent of that of the condensed ψ form. The above spectroscopic effects can be continuously modulated by changing the water content of the micellar system. This offers the possibility of using DNA-containing reverse micelles as models for condensed packaging of DNA in vivo (as in certain phage heads or chromatin).

Proteins can be solubilized in hydrocarbon solvents via aqueous reverse micelles (1-4). Most probably, the proteins are embedded in the aqueous inner core (the so-called "water pool") of the reverse micelle and are protected from the apolar environment by a layer of water and the surfactant shell. With di(2-ethyl-hexyl) sodium sulfosuccinate (from now on AOT), enzymes retain their activity (2-4) and a series of questions concerning the structure and stability of these micellar aggregates, their potential for technological applications, the peculiarity of the enzyme's environment and the enzyme mechanism have been discussed (5-8) and recently summarized in a review (9).

In one of our previous papers (3) we reported on the activity of ribonuclease in the reverse micellar system n-octane/AOT/water with the cyclic substrate citidine-2':3'-phosphate. In this connection, the question arose as to whether and to what extent the polymeric substrate RNA and other polynucleotides might be solubilized in the same micellar system.

(≠) To whom correspondence should be addressed.

0006-291X/82/140538-08$01.00/0

At first glance this proposal may appear surprising. In fact, AOT is an anionic surfactant and since RNA (and DNA) is a negatively charged polyelectrolyte at pHs above neutrality, strong repulsive interactions and a correspondingly high instability of the system should be expected. However, the solubilization of RNA as well as of DNA in micellar hydrocarbon solution takes place, and in this communication we describe some basic features of these novel macromolecular aggregates. Some preliminary experimental observations have been mentioned earlier (10,11).

EXPERIMENTAL

Materials: Di(2-ethyl-hexyl) sodium sulfosuccinate (AOT) was obtained from Serva and purified as suggested in literature (12). Isooctane was puriss. from Fluka. RNA (ex torula), nominal molecular weight 20,000-30,000, was obtained from Calbiochem. Low molecular weight DNA and DNA, with a nominal molecular weight of 1.2×10^6 were obtained from Fluka and Sigma. Isooctane solutions were 50mM in AOT. For chromatography of DNA, Ultrogel A6 was obtained from LKB.

Methods: Nucleic acids were solubilized in the isooctane micellar solutions by two ways, either with the direct injection techniques (i.e. adding a few microliters of concentrated -between 2 and 5 mg/ml- RNA/DNA buffered stock solution), or in the case of RNA,by extraction from solid state (i.e. mildly shaking the biopolymer powder with the AOT hydrocarbon solution containing the desired amount of water). The extraction proceeded very slowly under our conditions, and actually after 4 days of RNA extraction, the optical density of the hydrocarbon solution (1.4 at 260 nm in 1 cm cells) continued to increase. The solubilization with the injection procedure was performed by adding 10-50 µl of stock solution of nucleic acid (usually in borate buffer pH= 9.0) to the dry AOT isooctane solution. The volume of the added buffer solution corresponds to the final water content in the micellar system.

The precision of the injection procedure was checked by plotting the optical densities of the so prepared RNA solutions versus the number of microliters of the stock solution added to the hydrocarbon micellar solution. A very satisfactory linearity was obtained. Small and slow fluctuations in the optical density of the micellar solutions were observed over long time intervals. More particularly, there was a decrease of ca. 3.2% in the first 4 hours after solubilization, the decrease of the optical density reached a maximum of ca 12% after 20 hours, and afterwards it increased again to reach the initial reading after ca. 4 days. These equilibirium fluctuations of very high molecular weight micellar aggregates are not too surprising in view of the analogous effects found in liposomes and vesicles (25). These slow changes are with all likelihood connected with structural transformations of the micelles and/or of the guest biopolymer and will not be discussed in detail in this report. The slow decrease of absorbance with time has practically no influence on the results presented in this paper, since the spectra were collected rapidly (within 30 min. at the most for CD runs) and with micellar solutions not older than 2 hours (the ΔOD changes due to solution aging are then one order of magnitude smaller than those reported in Fig 2 and 3 between water and hydrocarbon solution). A low molecular weight DNA sample from Fluka was purified with a Ultrogel A6 (13) column, and the fraction with $K_{av} = 0.67$ was used for our studies. Ultracentrifugation runs yielded a sedimentation coefficient of 1.38 s and a diffusion coefficient of 1.36×10^{-6} cm^2/sec., which on the basis of the

Svedberg equation (and using a partial specific volume of 0.5) gives a molecular weight of 5000 Daltons. The experimental uncertainty (difficult to determine precisely in this case) should be within 10%.
A high molecular weight sample (nominal molecular weight 1.2 x 10^6) was obtained from Fluka and used for qualitative runs without further purification. Clear micellar solutions could also be obtained with this sample; however the 259 nm optical density rapidly decreased. It could be shown that the material was absorbed on the glass walls: When the micellar solution was pipetted out of the glass cell, and this was washed with pure isooctane and dried, and water was finally added to this glass cell, the optical density of the water solution corresponded quantitatively to that for the DNA which was "lost" in the initial micellar solution. The large optical density changes observed in our first communication (10) are partly due to such loss of material. This process can be slowed down by using silanized cells.
High molecular weight calf thymus DNA purchased from Sigma was subjected to sonication (6 minutes total time at a power of ca. 125 watts, with intermittent cooling), 1:1 extraction with phenol (first neutralized and then water-saturated) and ethanol precipitation by adding 3 volumes of ethanol after treatment with diethylether to remove phenol. The precipitated DNA was washed with pure ethanol, and dried under water vacuum. The DNA fraction was characterized with an analytical ultracentrifuge (Beckmann Model E) and a sedimentation coefficient of 6.5 s was obtained, which gave a molecular weight of ca. 250,000 Daltons according to the literature standardization (14).
The final water content of the hydrocarbon phase was 0.5 - 2.3% (v:v), or, expressed in terms of the parameter w_0 (the molar ratio $[H_2O]/[AOT]$), between $w_0 \approx 5$ and 26. All studies were carried out at room temperature. Detailed information about pH, w_0, concentration, etc. of typical experiments reported here are given in the figure legends.
Spectroscopic studies were carried out with the instrumentation and the procedures described earlier for the case of proteins in micellar solution (5-6).

RESULTS AND DISCUSSION

Clear micellar solutions of DNA and RNA in the AOT/isooctane/water system could be obtained only in a restricted range of pH, concentration, and w_0 values. Fig. 1 shows the results of the extraction of RNA from solid state using our standard conditions. Notice that the percentage of solubilized biopolymer does not increase monotonically when the water content of the micellar system is increased, but it has a maximum at w_0 18 (ca. 1.6% water v:v). This is in agreement with the results obtained in the case of proteins, for example lysozyme (5) and ribonuclease (3). Also the enzymatic activity is at its maximum at w_0 values well below the maximum percentage of water (5,6). It is difficult at the moment to give a satisfactory explanation of this phenomenon. However, it is well established that in the case of "unfilled" micelles (no guest molecules other than water) there are critical values of w_0 around which some physical properties of the reverse micelles change rather drastically (15, 16). It is now of interest to compare the spectroscopic

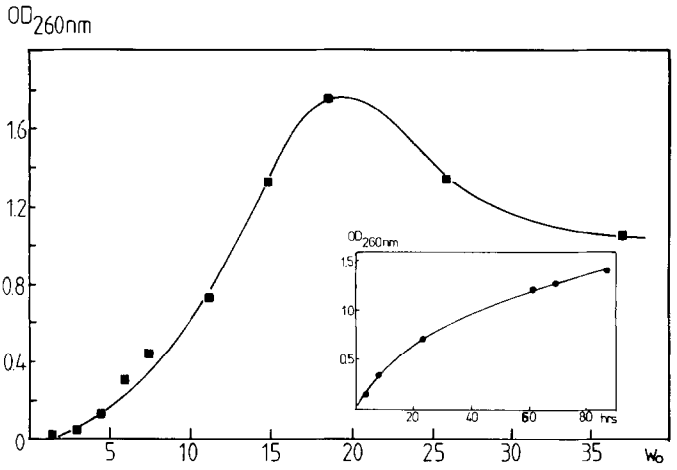

Fig. 1: Extraction of RNA from the solid state to the supernatant isooctane/AOT solution, as a function of w_o (w_o = $[H_2O]/[AOT]$). The optical densities (1 cm light path) were measured after 72 hrs. of mildly shaking. The water concentration (w_o) was set by solubilizing borate buffer pH=9.0 in the isooctane/AOT solution before the extraction experiments. Under our conditions ([AOT]=50 mM) w_o-values of 10 and 30 correspond to water contents (v:v) of 0.9 and 2.6% respectively. The insert shows the time progress of the extraction (at w_o=14.8).

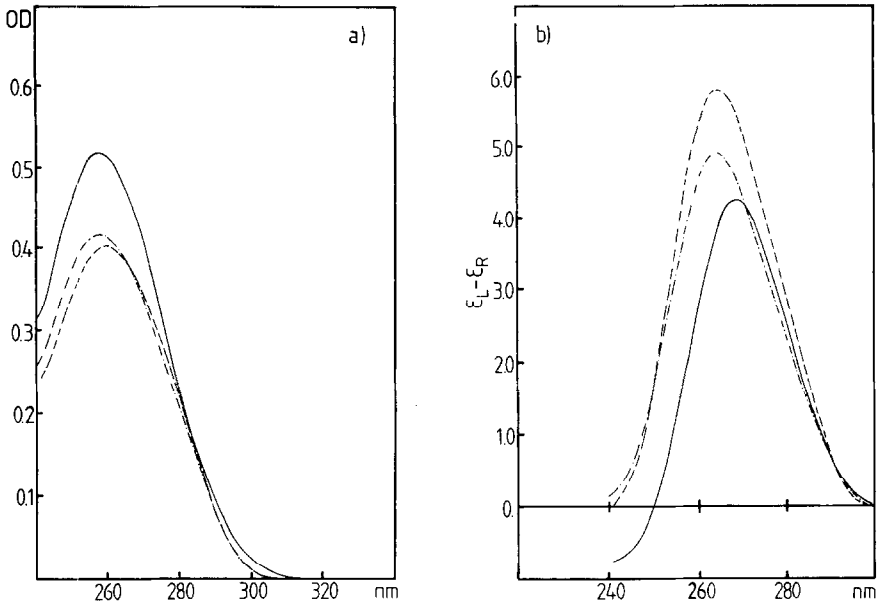

Fig. 2: Absorbtion (a) and ellipticity (b) properties (1 cm light path) of RNA (mol. weight 20,000-30,000 Daltons) in water and in hydrocarbon micellar solutions. (——) RNA in aqueous solution borate buffer pH=9.0; (---) in isooctane/AOT solution at w_o=14.8 (prepared from an aqueous stock solution borate buffer pH=9.0); (—·—) same as (---) but w_o=22.2 . These solutions have been prepared by the injection procedure, namely adding the same number of microliters of an aqueous stock solution to 3 ml of water or of hydrocarbon micellar solution. [AOT]=50 mM. The ellipticities in this and in next figure are given in (degrees x l./nucleotide-mol x cm) under the assumption that the molar concentration in the micellar solutions and in water are the same. The extinction coefficient in water for RNA was taken as 7000 (26) per nucleotide.

properties of polynucleotides in the micellar solution and in water. For the low molecular weight DNA (≈5000), negligible differences between the micellar hydrocarbon and the water solutions were observed (ΔOD_{260} of the order of 1.5% at w_0 = 18.5). This shows that the micellar environment per sê has no, or very modest, effects upon the chromophore system of the nucleotides. More interesting are the results in the case of RNA with molecular weight 20'000-30'000, as shown in Fig. 2. Note that, with respect to an aqueous solution, the RNA micellar solution has a lower absorbance and a higher ellipticity. This effect is larger, the smaller w_0. These spectroscopic changes are most likely due to conformational changes of the guest RNA molecules. In this regard one should recall that DNA and RNA solutions are often characterized by hypochromic effects, which are usually attributed to base stacking. Accordingly, the decrease of absorbance of RNA in the micellar solutions can be ascribed to an increase of base stacking with respect to water solutions, and the consequent increase of "conformational rigidity" could then be the primary cause of the increased ellipticity. This interpretation is in keeping with the data of Brahms, Michelson and Van Holde (21). They investigated the CD properties of poly(adenosine) at different temperatures (from -2 to +70°C). They found that the elipticity increased by decreasing the temperature, which was then interpreted to reflect a decreased flexibility of the polynucleotide.

This interpretation is in agreement with the results and interpretations which we found in our studies of proteins. For lysozyme (5) and α-chymotrypsin (6), for example, the marked increase of ellipticity in the 190-230 nm region was ascribed to an increase of conformational rigidity of the polypeptide chain. The rigidity was attributed to increased H-bonding, due perhaps to a lower dielectric constant and, more generally, to the peculiar structure of water in the micellar inner core. With enzymes, this effect increased when the size of the biopolymer-containing micelles was decreased (5,6). This is analogous to the results reported in Fig. 2b. When a DNA sample with molecular weight 250,000 was solubilized in the micellar hydrocarbon solutions, surprising results were obtained, as shown in Fig. 3. The first observation is the striking increase of ellipticity with respect to normal water solution values. Equally striking are the shifts in position of λ_{max} of the CD maxima and the position of the cross-over point. Also notice that there is a sizeable contribution also at very high wavelengths (till and over 500 nm). Parallel to these large changes in CD values, the absorption properties of the micellar solutions (Fig. 3a) show hypochromic effects and a slight blue shift with respect to water solution. Although these

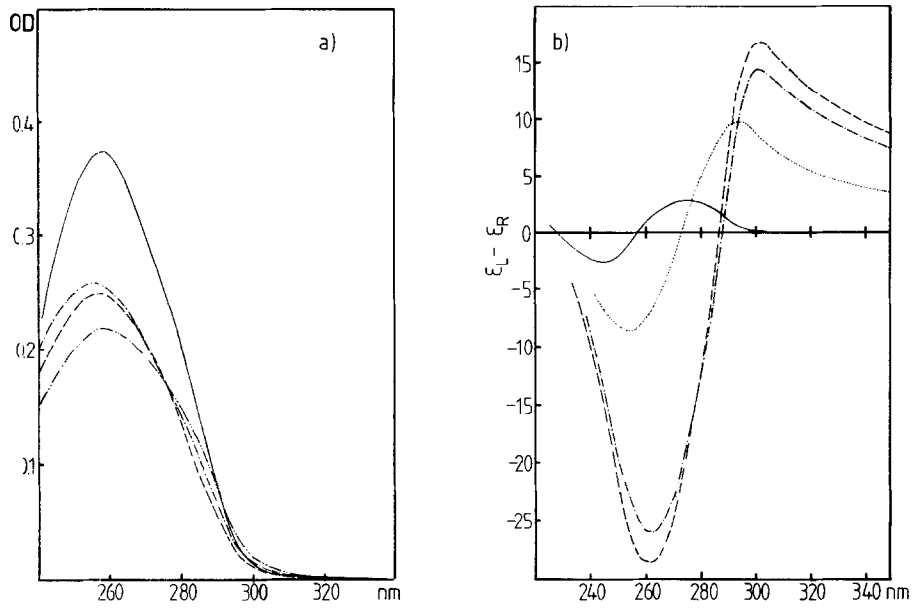

Fig. 3: Absorbtion (a) and ellipticity (b) properties (1 cm light path) of DNA (mol. weight ca. 250,000 Daltons) in water (——) and in isooctane/AOT (50 mM)/water micellar solutions: (----) w_o=18.5 (prepared from an aqueous stock solution borate buffer pH=9.0); (—·—·) w_o=18.5 (prepared from an aqueous stock solution tris buffer pH=7.5); (·····) same as (----) but w_o=25.9; (—···—) same as (----) but w_o=14.8; (—·—·—,in a)) same as (----) but w_o=22.2 . The extinction coefficient of DNA in water was taken as 6450 (27) per nucleotide.

absorption perturbations are significant and in line with those obtained for RNA, most likely the changes observed in the CD spectra are not caused by the perturbations of the chromophores per sè. It is also important to recall that low molecular weight DNA samples (5000 Dalton, see experimental) do not show changes either in absorption or in ellipticity, with respect to water solution (data not shown). It appears therefore that the striking CD properties of the high molecular weight DNA in the micelles arise from a changed tertiary structure of the guest biopolymer.

An important observation from Fig. 3 is that these changes are directly influenced by w_0 (as already reported, w_0 directly influences the size and the physical properties of the water pools of reverse micelles (12,16)). Also notice from Fig. 3 that when the pH of the stock solution is varied from 9 to 7.5, at constant w_0, there are no significant changes in the form or in the intensity of the spectrum. This observation shows that the "pH-jump" on going from the stock water solution to the water pool (17-20) is not responsible for the CD changes.

It is difficult to give a clear cut interpretation of the high ellipticity elicited by the micellar environment for DNA. However, it is important to remark that similar features have been reported in literature and are generally ascribed to tertiary structure effects (23-25). In particular a spectrum with intense positive and negative CD bands, quite similar to the spectra of the micellar AOT solutions shown in our Fig. 3, was obtained by Jordan and coworkers studying water solutions of DNA in the presence of salt and ionized polyacrylate. (23). Such spectra are broadly defined as ψ-spectra (23, 24, 25) and are attributed to a condensed form of DNA, i.e. to a tightly packed structure. The interest in this observation is that a compacted DNA form also exists in vivo, for example in condensed chromatin and in certain phage heads.

Summarizing briefly, at this preliminary stage the following potentials of nucleic acid-containing reverse micelles are apparent: i) The possibility of studying the conformation of nucleic acids as a function of an environment, where the physical properties can be continuously and simply modulated by changing the water content. This includes studies on ψ-DNA and/or supercoiling. ii) The possibility for new binding and assemblage studies. This would be based on the consideration that any sort of polar macromolecules inserted in the hydrocarbon micellar solution would tend to concentrate into the small water pool cavities. iii) Finally, recognizing the dynamic properties of reverse micelles (22), one may propose these systems as useful models for material exchange across interfaces in in-vivo systems.

ACKNOWLEDGEMENTS: Initial experiments on DNA and RNA containing micelles are presented in the Ph.D. Thesis of Dr. R. Wolf (11). We are grateful to Barbara Straub for carrying out CD-measurements and for the critical reading of the manuscript as well as to Dr.Hans Jäckle for the ultracentrifuge experiments.

REFERENCES:

1. Luisi P.L., Henninger F., Joppich M., Dossena A. and Casnati G. (1977) Biochem. Biophys. Res. Commun. 74, 1384-1389
2. Martinek K., Levashov A.V., Klyachko N.L. and Berezin I.V. (1978) Dokl. Akad. Nauk. SSSR (engl. edition) 236, 951-953
3. Wolf R., Luisi P.L. (1979) Biochem. Biophys. Res. Commun. 89, 209-217
4. Menger F.M. and Yamada K. (1979) J.Am. Chem. Soc. 97, 909-911
5. Grandi C., Smith R.E. and Luisi P.L. (1981) J.Biol.Chem. 256, 837-843
6. Barbaric S. and Luisi P.L. (1981) J.Am.Chem.Soc. 103, 4239-4244
7. Douzou P., Keh E. and Balny C. (1979) Proc.Natl.Acad.Sci.USA 76, 681-684
8. Martinek K., Levashov A.V., Klyachko N.L., Pantin V.I., Berezin I.V. (1981) Biochem.Biophys.Acta 657, 277-294
9. Luisi P.L. and Wolf R., in Solution Behaviour of Surfactants (Fendler E. and Mittal K. eds.) in press
10. Luisi P.L., Grandi C. and Wolf R. (1980) Proceedings of IUPAC Symposium on Macromolecules, Preprints Vol. 2, 531-534

11. Wolf R. (1982) ETH-Dissertation Nr:7027
12. Wong M., Thomas J.K. and Graetzel M. (1976) J.Am.Chem.Soc 98 2391-2397
13. Petrovic S.L., Petrovic J.S. and Markovic R.A. (1974) Prep.Biochem. 4, 509-522
14. Eigner J., Doty P. (1965) J.Mol.Biol. 12, 549-580
15. Wong M., Thomas J.K. and Nowak T. (1977) J.Am.Chem.Soc. 99, 4730-4735
16. Zulauf M. and Eicke H.F. (1979) J.Phys.Chem. 83, 480-486
17. Smith R.E. and Luisi P.L. (1980) Helv.Chim.Acta 63, 2302-2311
18. Fujii I., Kawai Y. and Nishikawa H. (1979) Bull.Chem.Soc.Japan 52, 2051-2055
19. Menger F.M. and Saito G. (1978) J.Am.Chem.Soc. 100, 4376-4379
20. Kitahara A. (1980) Adv. Colloid.Interface Sci. 12, 109-140
21. Brahms J., Michelson A.M., and Van Holde K.E. (1966) J.Mol.Biol. 15, 467-488
22. Eicke H.F., Shepherd J.C. and Steinemann A. (1976) J.Colloid Interface Sci. 56, 168-176
23. Jordan C.F., Lerman L.S. and Venable jun. J.H. (1972) Nature New Biol. 236, 67-70
24. Lerman L.S. (1973) in Physico Chemical Properties of Nucleic Acids Vol. 3, 59-76
25. Gray D.M. Edmondson S.P., Lang D. and Vaughan M. (1979) Nucleic Acids Res. 6, 2089-2107
26. Miura K.I., Kimura I., Suzuki N. (1966) Virology 28, 571-579
27. Hirschman S.Z. and Felsenfeld G. (1966) J. Mol. Biol. 16, 347-358