DNA Aggregation Induced by Polyamines and Cobalthexamine

(Received for publication, September 26, 1995, and in revised form, December 20, 1995)

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We have studied the precipitation of short DNA molecules by the polycations spermidine, spermine, and cobalthexamine. The addition of these cations to a DNA solution leads first to the precipitation of the DNA; further addition resolubilizes the DNA pellet. The multivalent salt concentration required for resolubilization is essentially independent of the DNA concentration (between 1 µg/ml and 1 mg/ml) and of the monovalent cation concentration present in the DNA solution (up to 100 mM). The DNA aggregates are anisotropic; those observed in the presence of the polyamines spermidine and spermine generally contain a cholesteric liquid crystalline phase that flows spontaneously. In contrast, this phase is never seen in the presence of cobalthexamine. We propose that the ability of polyamines to condense DNA in fluid structures is an essential feature of their biological functions.

Multivalent cations with a charge of 3+ or greater induce the condensation of DNA in aqueous solution (reviewed in Ref. 1). In extremely dilute DNA solutions, one can observe the monomolecular collapse of long chains; with more concentrated DNA solutions (of short or long chains), aggregation sets in. Electrostatic forces appear to be predominant in DNA condensation. For highly charged polyelectrolytes there is a strong electrostatic repulsion between the chains. One expects the addition of multivalent cations to decrease this repulsion. DNA condensation by multivalent cations has been analyzed within the framework of the counterion condensation theory developed by Manning (2). This theory predicts the fraction of the DNA charges neutralized by a given cation: a saturating trivalent cation for instance should neutralize 92% of the DNA charges. The experimental data indicate that condensation is usually coupled with an isotropic to an anisotropic transition. In particular, high molecular weight DNA aggregates formed by spermidine, spermine, or cobalthexamine give a strong equatorial reflection when analyzed by x-ray diffraction (5, 6). Based on these data, several types of crystalline and liquid crystalline structures have been suggested for these DNA aggregates (5, 7). We have recently undertaken a study of these DNA aggregates using short (about 150 base pairs long) DNA molecules (8). In the case of the trivalent cation spermidine, we have found that the aggregate is generally biphasic and contains a liquid crystalline phase. In the course of these experiments, we have observed that the addition of excess spermidine leads to the resolubilization of the DNA aggregates. In this work, we describe this phenomenon, which is also observed with the cations spermine and cobalthexamine. We compare the precipitation by multivalent cations with the classical salting out obtained at high salt concentration (9) and with the formation of coacervates (10). We analyze the structures obtained with the three cations and discuss their biological significance.

MATERIALS AND METHODS

DNA Precipitation—Short DNA fragments were prepared from calf thymus as described in Ref. 8. The length of the fragments obtained ranged from 130 to 600 base pairs with 50% of 146 (± 7) base pairs. DNA precipitation was induced by the addition of the following polycations: spermidine (3HCl, Fluka) (initial concentration, 392 mM; prepared in a TE buffer: 10 mM Tris HCl, pH 7.6, 1 mM EDTA), cobalthexamine (3HCl, Fluka) (250 mM in TE buffer), and spermine (4HCl, Fluka) (400 mM in TE buffer). Two types of experiment were performed. First, the DNA concentration was kept constant at 1 mg/ml, and the salt concentration was varied. The NaCl concentration was varied from 4 mM to 1.2 M; the multivalent cation concentration was increased from 1 to 225 mM. In a second series of experiments the DNA concentration was varied from 1 µg/ml to 1 mg/ml. The NaCl concentration was kept constant at 25 mM, and the multivalent cation concentration was increased from 0 to 100 mM for spermidine and spermine. Following the addition of the salts to the solutions, the sample was vortexted, incubated at room temperature for 35 min, and centrifuged at 11,000 × g for 7 min. The amount of DNA remaining in the supernatant and in the pellet was determined by two methods: measurement of the absorbance at 260 nm or of the radioactivity of 32P-labeled DNA. For the UV absorbance, an aliquot of the supernatant was recovered and diluted in a TE buffer solution containing a NaCl concentration identical to that of the sample. For the second method, 1 µg of DNA was treated with alkaline phosphatase, and 5' end-labeled with T4 DNA kinase to a specific activity of about 5 × 107 cpm/µg. The labeled DNA was purified by gel filtration on a Sephadex NAP-5 column, and the eluted material (about 500 µl) was concentrated on a Centricon-10 microconcentrator (Amicon) to a final volume of about 50 µl. In a standard precipitation experiment, 10,000 cpm of this labeled DNA was added as a radioactive tracer. The radioactivity present in the pellet cannot reliably be determined by Cerenkov counting (in the 'H channel of the liquid scintillation counter). For this reason, the pellet was resuspended in 2 × NaCl, and the radioactivity present in the supernatant and in the pellet was measured in a toluene-based scintillation fluid. The concentration of multivalent cations required to either precipitate or resolubilize DNA was taken at the midpoint of the corresponding transitions (half-point concentration between zero precipitation and the maximal value obtained in precipitation).

* This work was supported by Grant 6473 from the Association pour la Recherche sur le Cancer and by Grants DSPT5 and ACC-SV5 from the Ministe`re de l'Enseignement Supe´rieur et de la Recherche. The authors are grateful to the Ministère de l'Enseignement Supérieur et de la Recherche sur le Cancer and by Grants DSPT5 and ACC-SV5 from the Ministe`re de l'Enseignement Supe´rieur et de la Recherche. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Polarizing Microscopy—DNA was precipitated from a 1 mg/ml solution using various monovalent and multivalent salt concentrations. An aliquot of the pellet was recovered and deposited between slide and coverslip. The sample was sealed with DPX resin (Fluka) in order to prevent evaporation. The preparations were observed between linear crossed polars in a Nikon Optiphot microscope.

RESULTS

DNA Precipitation at a Fixed DNA Concentration—DNA precipitation from a dilute solution (1 mg/ml) by spermidine, spermine, and cobalthexamine has been studied for multivalent cations concentrations ranging from 0 to 225 mM. The corresponding precipitation curves are shown on Fig. 1. At low NaCl concentration (25 mM), a common feature is observed for the three polycations: an increasing concentration leads first to an almost complete precipitation of the DNA, and further increase leads to the resolubilization of the pellet. This soluble state observed at high multivalent cation concentrations can be reached either directly without precipitation (in which case one may speak of a suppression of the precipitation) or by resolubilization of a pellet (the term that we use here). The phenomena of precipitation and resolubilization observed here are fully reversible, as noted in the case of precipitation in our previous work (8).

In the case of the trivalent cation cobalthexamine, the amount of DNA remaining in the supernatant could not be determined by the absorption at 260 nm, because of the high absorption of cobalthexamine at this wavelength. For this reason, the radioactivity method alone was used for this cation. In the case of spermidine and spermine, the two methods (spectroscopic and radioactivity) have been used and can be compared (Fig. 1, A and B). For spermidine, there is a clear quantitative difference in the amount of DNA remaining in solution at about 10 mM spermidine (maximum of precipitation) determined by UV absorption (less than 5%) and by radioactivity (about 40%). This seems to be due to fractionation: we have analyzed the length of the fragments in the pellet and in the supernatant by gel electrophoresis. The supernatant fragments are shorter than the pellet fragments, and the differences were quantified by densitometry of the gels on a PhosphorImager (data not shown). As a result, the mass amount of S' end-labeled DNA precipitated by spermidine is underestimated by the radioactive method. Qualitatively however, the shape of the two curves determined by UV absorption and radioactivity are similar. For spermine, the two curves obtained at 25 mM (Fig. 1B) are in much better agreement. We observe, however, some scattering of the experimental data in the resolubilization zone for the UV determination. This is apparently due to the precipitation of the aliquot of the supernatant recovered for the UV measurement, when it is diluted in the TE buffer. Because of the experimental difficulties encountered with cobalthexamine and spermine, we have chosen to use the radioactivity method for the three cations.

Several pieces of information can be extracted from a comparison of Fig. 1 (A, B, and C). 1) The amount of multivalent cations required to precipitate DNA varies: the tetravalent spermine is the most efficient. The trivalent cobalthexamine is about four times more efficient that the trivalent spermidine. Similar observations have been made previously in studies of the condensation of DNA by these cations (4, 11, 12). Spermine and cobalthexamine can lead to an almost complete precipitation. This implies that they are more efficient in the precipitation of small fragments as previously observed for spermine at low DNA concentrations (13). 2) The amount of multivalent cations required to resolubilize DNA varies but with a different order; the midpoint for this transition corresponds to 50 mM spermidine, 90 mM spermine and 220 mM cobalthexamine. 3) The presence of an increased NaCl concentration prevents the condensation of DNA by these cations (4, 11, 12). Spermine and cobalthexamine can lead to an almost complete precipitation. This implies that they are more efficient in the precipitation of small fragments as previously observed for spermine at low DNA concentrations (13).
The competing action of NaCl differs in the precipitation region and in the resolubilization region. In the precipitation region the presence of an increased amount of NaCl (100 mM instead of 25 mM) increases the concentration of spermine or cobalthexamine required for precipitation. This competition between mono- and multivalent cations has already been observed by several authors (3, 4, 15, 16). In contrast, the resolubilization by excess spermine or cobalthexamine is only slightly affected by the presence of 100 mM NaCl rather than 25 mM NaCl, with the midpoint remaining unchanged. In the case of spermidine, resolubilization is also not affected by a change from 4 to 55 mM NaCl (data not shown).

DNA Concentration Effects—DNA precipitation by polyamines has been studied for different DNA concentrations ranging from 1 \( \mu \)g/ml to 1 mg/ml (Fig. 2). In these experiments the NaCl concentration was kept constant at 25 mM. We observe again the behavior described above. The addition of polycations first leads to precipitation; further addition resolubilizes the DNA aggregate. The amount of multivalent cations required for DNA precipitation increases with DNA concentration at low concentrations (between 1 \( \mu \)g/ml and 50 \( \mu \)g/ml). Above 50 \( \mu \)g/ml, this concentration dependence is no longer observed for spermidine (Fig. 2A). The efficiency of the precipitation varies also with DNA concentration; it decreases from a maximal value of 60% (for 1 \( \mu \)g/ml) to 30% (for 50 \( \mu \)g/ml) and increases back to 60% (at 1 mg/ml). Using the data of Fig. 2A, one can show that between 1 and 50 \( \mu \)g/ml, the concentration of spermidine at the transition midpoint increases linearly with DNA concentration according to the equation:

\[ [\text{spermidine}]_{\text{agg}} = a[\text{DNA}] + \beta \]  

(Eq. 1)

where \( a \) and \( \beta \) are constant parameters.

This linear dependence has been previously observed for DNA condensation by polyamines (11) and cobalthexamine (4). In the case of spermidine, we obtain \( a \sim 36 \) and \( \beta \sim 0.55 \) (the DNA concentration being expressed in terms of monomer concentration; 1 mg/ml corresponds to 3 \( \mu \)ml). These values agree with those obtained previously (11). In contrast with the linear dependence observed for precipitation, the resolubilization by an excess polyamine is essentially independent of DNA concentration between 1 \( \mu \)g/ml and 1 mg/ml. This is easily seen for the resolubilization by spermine (Fig. 2B) and is illustrated in a better way for spermidine below (see Fig. 3A).

The data of Fig. 2 (A and B) have been used to draw a schematic phase diagram shown in Fig. 3 (A and B). This phase diagram is composed of three regions: two monophasic regions and one region where phase separation occurs. The boundaries of this phase separation region correspond to the concentration of polycations required to precipitate (lower bound) or resolubilize (upper bound) DNA. The choice for these boundaries (midpoint of the transition) is somewhat arbitrary; however,
The overall shape of the phase diagram is not modified by values of the polycation concentrations corresponding from 20 to 72% of the DNA remaining in solution (data not shown). The phase separation region is much wider for the tetravalent cation spermine (Fig. 3B) than for the trivalent spermidine (Fig. 3A). As observed above, the upper bound is essentially independent of the DNA concentration (Fig. 3A illustrates clearly this fact for spermidine). This contrasts with the lower boundary. Phase diagrams similar to those obtained here were described for another anionic polyelectrolyte (polystyrene sulfo-nate) in the presence of the trivalent cation LaCl3 and the tetravalent cation Th(NO3)4 (17). For this polymer also the presence of increasing amounts of NaCl shrinks the phase separation region. For DNA in the presence of spermidine, the lower boundary gives a funnel-shaped phase separation region. The change in slope of the lower boundary coincides with the minimum efficiency of the precipitation process. We do not understand this correlation.

Nature of the DNA Aggregates—DNA aggregates obtained in the presence of polyamines or cobalthexamine have been observed by polarizing microscopy. The nature of the aggregates obtained in the presence of polyamines depends on the NaCl and polycation concentrations, in contrast with the aggregates obtained in presence of cobalthexamine, where a unique phase is usually observed. In the presence of spermidine, there exist two types of condensed structures: a cholesteric liquid crystalline phase and a more concentrated phase (8). The relative amounts of the two phases are modulated by ionic conditions: one finds either a pure cholesteric phase (not illustrated), a biphasic state (Fig. 4A), or a pure concentrated phase (not illustrated). These structures have been analyzed in greater detail elsewhere.1 Pure concentrated phases are seen for spermidine concentrations just above the aggregation concentration, whereas pure cholesteric phases are found for NaCl or spermidine concentration close to the resolubilization concentration. We observe the same phenomenon for spermine; in Fig. 4B a pure concentrated phase is seen in the presence of 10 mM spermine and 25 mM NaCl. The structure is that of a dense cluster of germs that cannot always be seen individually. Between crossed polars, the intensity of the transmitted light is more intense than in the homologous phase with spermidine, which suggests a denser packing of the DNA helices. A pure cholesteric phase is observed in the presence of 90 mM spermine, 25 mM NaCl (Fig. 4C). In this last figure we note the presence of spherical germs with concentric layers, a texture characteristic of a cholesteric liquid crystal (18). A common feature of the cholesteric phases obtained in the presence of spermidine and spermine is their fluidity; they both flow spontaneously. For both phases the helical pitch is much larger than the cholesteric helical pitch obtained in the presence of NaCl alone (about 20 versus 2.5 Å (19)). DNA condensed in the presence of cobalthexamine yields a single phase (Fig. 4D), which is highly birefringent. This suggests a high packing density of the molecules, in agreement with interhelical spacing measured by x-ray diffraction: 27.5 Å (5) or 28.2 Å (6) for cobalthexamine compared with 29.2 Å for spermine (5) and 29.4 Å for spermidine (5, 6). This structure does not flow spontaneously. The fluidity of this phase is therefore highly restricted but can be established by squeezing the sample be-

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1. D. Durand, J. Doucet, and F. Livolant, submitted for publication.
between slide and coverslip. The structure is probably that of a columnar hexagonal liquid crystal. The time required for the appearance of the structure shown in Fig. 4D is much longer than the time required for the organization of the condensed states in the presence of polyamines. Following the addition of cobalthexamine to a DNA solution, the condensed phase first presents a structure identical to the structure obtained in the presence of spermine (Fig. 4B). This organization lasts a few days. The evolution to the texture seen in Fig. 4D requires about 1 week. In contrast this state is reached in a few hours with polyamines. The structure shown in Fig. 4D is the only one seen with cobalthexamine; cholesteric phases have never been seen, not even when an excess of cobalthexamine is present (corresponding to 55% DNA resolubilization).

**DISCUSSION**

Precipitation of Polyelectrolytes by Salts: Salting Out versus Complex Coacervation—The precipitation of polyelectrolytes by salts is often called “salting out.” It is useful to clearly distinguish between a classical salting out behavior and the phenomenon observed here. The classical salting out describes the precipitation observed in the presence of high concentrations (typically 1 M or greater) of salts (usually monovalent). The precipitation is thought to be due essentially to the reduction of the activity of water in the solution that results from the high concentration of the hydrated salt ions (9). The activity of the polyelectrolyte is therefore increased, and its solubility decreases. The classical salting out behavior can be observed for DNA in presence of LiCl; precipitation takes places for concentrations greater than 9 M (20, 21). In contrast with the high monovalent salt concentrations required in a classical salting out, the precipitation by multivalent cations occurs at low concentrations that are not expected to greatly modify the activity of water. We are not therefore dealing with a classical salting out here.

Bungenberg de Jong has described the precipitation of numerous polymers and colloids under various conditions (10). According to his nomenclature, the precipitate can be an ordered solid (a true crystal) or can be in an amorphous state, either solid (a flocculate) or liquid (a coacervate). He has specifically described the precipitation of numerous polyelectrolytes in presence of micro-ions or polyelectrolytes and introduced the term of complex coacervation to describe the phase separation occurring in such system. In our experiments, we observe such a phase separation, but the precipitate, instead of being amorphous, is a highly ordered fluid. Nevertheless we propose that it should be considered as a complex coacervate (as already done for polyelectrolyne-DNA complexes (22)). We note here that the term “complex coacervation” is often specifically used to describe a complex between oppositely charged polyelectrolytes (23). However the original definition of Bungenberg de Jong encompasses the condensation of DNA by $3^+$ and 4$^+$ cations (p. 336 in Ref. 10).

The difference between complex coacervation and a classical salting out can be illustrated in the following manner. Starting from DNA spermidine complex coacervate, the addition of increasing amounts of LiCl leads first to the resolubilization of the DNA aggregate (at about 0.2 M LiCl) and then to the reprocipitation corresponding to the classical salting out (which is observed at 12 M LiCl) (data not shown).

Precipitation/Resolubilization of Polyelectrolytes by Multivalent Salts—Bungenberg de Jong has shown that solutions of highly charged polyelectrolyte chains precipitate upon addition of multivalent salts and resolubilize with further addition of the salts; this behavior has been observed for several types of polyelectrolytes including nucleic acids (sodium nucleate) and for various multivalent cations, including cobalt hexamine and an hexavalent cation (hexol nitrate). Similar observations have been also reported for polystyrene sulfonate (17) and DNA (this work). How can we explain precipitation and resolubilization? Counterion condensation alone is not sufficient to explain the appearance of an attractive force between similarly charged polyelectrolytes in the presence of multivalent cations (1). Net electrostatic attractive forces are obtained when charges fluctuations are taken into account. Correlated counterion fluctuations can lead to an attractive interaction, as first proposed by Oosawa (24). The effect of charge fluctuations on the interaction of highly charged polyelectrolyte in the presence of multivalent cations has also been recently studied by Olvera de la Cruz et al. (25). The authors used a model where condensed ions are considered as a random charge along a flexible polymer. They showed that the precipitation by multivalent salts can be explained by a short-range electrostatic attraction and that the resolubilization at high salts concentration is due to the screening of the short range electrostatic attraction. Their model accounts almost quantitatively for the experimental data obtained for the precipitation of polystyrene sulfonate. Another mechanism that could also explain the resolubilization phenomenon is that of a charge reversal. There are several reasons why such a mechanism should be considered. First, it is well known experimentally that the resolubilization in presence of multivalent cations can correlate with a charge reversal; several examples (for instance sodium nucleate plus an hexavalent cation) are discussed by Bungenberg de Jong (10) who notes that transgression of solubility usually correlates with charge reversal. In the case of DNA, we know that already 90% of the charge is neutralized in the DNA aggregate (3, 4); because DNA resolubilization is produced by increasing the concentration of multivalent cation, resolubilization should be accompanied by an increased binding of the cation. The possibility of a charge reversal is therefore not unlikely. We note in this respect that the charge reversal of DNA in aqueous MgCl$_2$ solution has been observed for concentrations in excess of 1 M MgCl$_2$. We have tested this hypothesis with a DNA sample dissolved in a buffer containing 70 mM spermidine (in which 70% of the DNA is soluble). This sample was submitted to electrophoresis in an agarose gel equilibrated in the same buffer, which was circulated during electrophoresis. The DNA migrates toward the anode (data not shown). This indicates that the net charge of DNA remains negative and seems to rule out the charge reversal hypothesis.

To summarize, a purely electrostatic description of the behavior of polyelectrolytes in the presence of multivalent cations (25) accounts qualitatively for the phenomena of precipitation and resolubilization. It is clear however that this description is not sufficient to account for all the phenomena observed, in particular the existence of two distinct phases in equilibrium and their liquid crystalline nature in the case of polyanymes. In addition, we observe that cations carrying the same $3^+$ charge have different behaviors; both the nature of the aggregates and the concentrations required for precipitation and resolubilization differ. Thus there exist specific ion effects depending not only on their charge but also on their structure.

Several forces are supposed to be implicated in the stabilization of the DNA aggregates: van der Waals’ interactions (26), cross-links by condensing counterion (5), and hydration forces (6). We have argued previously that the existence of cross-links with spermidine is incompatible with the fluidity of the condensed phase.$^2$ We can extend this reasoning to the case of spermine. Our findings support the delocalized binding expected from the counterion condensation theory (2) that is

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$^2$ A. Papon and U. P. Strauss, unpublished results.
obscured for spermine in NMR (27) and photoaffinity cleavage experiments (28). We conclude from these observations that the existence of localized sites and salt bridges is unlikely here. This does not rule out their existence for specific DNA sequences or in nonaqueous solvents.

It is worth mentioning that the existence of the two types of aggregated phases reported here in the presence of spermidine and spermine has also been observed with short oligonucleotides (dodecamers) in the presence of spermidine, spermine, and cobalthexamine (29, 30). These experiments and ours cannot be directly compared, because the solvent conditions are different. The oligonucleotides are aggregated in the presence of methylpentane-diol or Polyethylene glycol 4000.

Biological Implications—The biological implications of our results can be discussed from three points of view: physiological, biochemical, and evolutionary.

From the physiological point of view, polyamines are ubiquitous compounds that are involved in numerous cellular processes (31, 32). Their binding to DNA and also to RNA suggests that these compounds play an important role in nucleic acid function. The concentration of polyamines increase markedly upon stimulation of RNA synthesis; they have also been implicated in protein synthesis. Here we have observed that spermidine and spermine are able to condense DNA into highly fluid anisotropic structures (cholesteric liquid crystal). In contrast, the inorganic cation cobalthexamine only yields an anisotropic structure that does not flow spontaneously. We propose that this ability to interact with nucleic acids in a dense phase while preserving some fluidity is an essential feature required for their biological functions. This proposal makes specific predictions that can be tested. 1) The ability to condense nucleic acids into highly fluid anisotropic structures should also be observed with other nucleic acid structures such as RNA or nucleosomal DNA. In the case of RNA, liquid crystalline structures have already been observed for transfer RNA in the absence of polyamines (33). We expect these nucleic acids to give rise to highly fluid anisotropic structure when condensed by polyamines. 2) There exist prokaryotic and eukaryotic mutants deficient in polyamine biosynthesis that are auxotrophic for polyamines (32). The addition of synthetic polynucleotide analogs can sometimes restore growth in these mutants. We expect the ability of these polyamines to restore growth to correlate with their ability to give rise to highly fluid anisotropic structures with nucleic acids in vitro. In particular, analogs that do not yield such structures should not be able to restore growth.

We would like to make it clear that we do not propose that DNA is condensed in cellular systems by polyamines alone (a point of view already expressed by Gosule and Schellman (34)). Clearly, several factors are involved in the stabilization of compact forms of DNA such as specific proteins (histones in eukaryotes or histone-like proteins in prokaryotes), macromolecular crowding (35), or DNA supercoiling. It appears that these different factors are able to cooperate in the stabilization of compact forms of DNA; macromolecular crowding for instance decreases the amount of cobalthexamine (6) or histone-like proteins (36) required for DNA condensation, and DNA supercoiling facilitates DNA condensation by divalent cations (37). Our proposal deals rather with the required fluidity of the condensed structure present in the cells; the factors that are physiologically involved in DNA compaction should preserve fluidity.

The biological significance of the resubilization observed at higher polyamine concentrations is at present unclear, because the concentrations required (typically about 50 mm) appear unphysiological (the polyamines intracellular concentrations are in the millimolar range (about 10 mm) (31, 32)). It is possible, however, that there exist locally high concentrations of polyamine that are involved in decondensing rather than condensing processes or that the concentration required for an in vivo effect is lower than in vitro experiments. In this respect, we note that the highest concentrations in polyamines are generally found at the G1 phase of the cell cycle (32). The suggestion has been made that these high concentrations are required in the cell’s preparation for DNA synthesis.

From the biochemical point of view, it is well known that polyamines are often used in vitro for the study of the functional properties of nucleic acids (see Ref. 8 and references therein). They can increase the efficiency of different enzymatic systems. It is also known that they can be present in sufficient concentrations to precipitate the nucleic acids (32). A correlation between the stimulatory effect and the aggregation of nucleic acids has been demonstrated in several cases leading to the suggestion that the aggregate should be fluid. For instance, the cationation of DNA by topoisomerases requires the aggregation of the DNA molecules by spermidine (16). The inorganic cation cobalthexamine is also able to stimulate these enzymatic reactions albeit generally in a less efficient manner. We propose that this lower efficiency of cobalthexamine results in part from the lack of fluidity of the condensed DNA phase. One way to investigate the role of the fluidity of the DNA phase in such experiments is to induce DNA condensation prior to the addition of the enzyme (in contrast with most experiments where DNA condensation and the addition of the enzyme are simultaneous). In an aggregate lacking fluidity the stimulatory effect on the action of the enzyme should be very low or even absent.

Finally, the experiments reported here can also be discussed from an evolutionary perspective. The ubiquity of polyamines among the cells is likely to reflect their antiquity. In addition to their compacting action and their role in the protection of DNA against shearing degradation and UV irradiation, polyamines can increase the biochemical activities of DNA as noted above. All these properties led Baeza et al. (38) to propose that “compact-forms of DNA induced by polyamines may represent a primordial DNA genome.” The observation that such aggregates are fluid strengthens this proposal.

We have seen above that such aggregates can be considered as coacervates. It is useful to recall here Oparin’s classic proposal on the role of coacervation in prebiotic chemistry (39): coacervation is considered as an essential concentrating process by which mixtures of randomly formed prebiotic polymers initially in dilute solutions are condensed into concentrated assemblies. The phase separation of the polymers into separate coacervate droplets is thought to provide the appropriate medium required for the evolution of these prebiotic systems. According to the current view, Oparin’s proposal suffers from the defect that it considered polyeptides rather than nucleic acids as a model for the primeval gene. The proposal of Baeza and co-workers as well as our present results should help reactivate Oparin’s coacervation model in the perspective of a DNA (or an RNA) world.

Acknowledgments—We thank Eric Gaillard for participation in experiments and Monica Olivera de la Cruz and Youri Timsit for useful discussions.

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