# Torus-shaped particles formed due to intermolecular condensation of circular DNA upon interaction with synthetic tripeptide

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The morphology of complexes between relaxed circular plasmid pBR322 DNA and tripeptide L-Val-L-Val-L-Val-NH-NH-Dns (TVP) at different peptide/DNA ratios was studied by electron microscopy The results show that interaction of TVP with circular DNA leads to the formation of perfect torus-shaped particles The torus parameter measurements offer the possibility to conclude that DNA condensation observed is of intermolecular nature On the basis of the analysis of the structures corresponding to the early stages of DNA compaction the model for intermolecular condensation of circular DNA into torus-shaped particles is proposed

*Circular DNA condensation* *Synthetic tripeptide* *Electron microscopy* *Torus-shaped particle*

## 1 INTRODUCTION

DNA condensation in vitro with the formation of torus-shaped particles was studied by many authors as a valuable model for DNA packing in bacteriophages and viruses. The formation of toruses was visualised by electron microscopy upon interaction of DNA with histone H1 [1,2], polylysine [3], polyamines [4,5], polyethyleneglycol [6] and some other agents. The corresponding molecular mechanisms were also considered theoretically [7,8]. However, neither the details of interactions involved nor the organization of compact particles is completely clear at present.

We studied DNA condensation upon interaction with synthetic tripeptide L-Val-L-Val-L-Val-NH-NH-Dns (TVP). Physicochemical data show that TVP is capable of $\beta$-structure formation in aqueous solution [9]. The investigations of DNA complexes with TVP are of particular interest since these peptides can interact specifically with certain nucleotide sequences on DNA [10]. It was shown that oligopeptides of this type can condense DNA upon binding to it. Interaction of TVP with calf thymus DNA leads to the formation of unusual compact rod-like structures in which DNA filaments are associated in a side-by-side way [11].

Here, we present data about intermolecular condensation of relaxed circular pBR322 DNA upon interaction with TVP leading to the formation of perfect torus-shaped particles.

*Abbreviation.* Dns, 5-dimethylaminonaphthalene-1-sulfonic group

## 2 MATERIALS AND METHODS

TVP was synthesized as described earlier [9]. The DNA–TVP complexes were prepared by direct mixing of DNA and peptide in the presence of 25% methanol and 1 mM Na-cacodylate buffer (pH 7.0). The DNA concentration in all experiments was $2 \times 10^{-5}$ M (base pairs) and that of TVP varied from $5 \times 10^{-6}$ M to $10^{-4}$ M. The droplets of DNA-peptide mixtures were applied directly on electron microscopic grids covered by collodion films. The excess of liquid was removed

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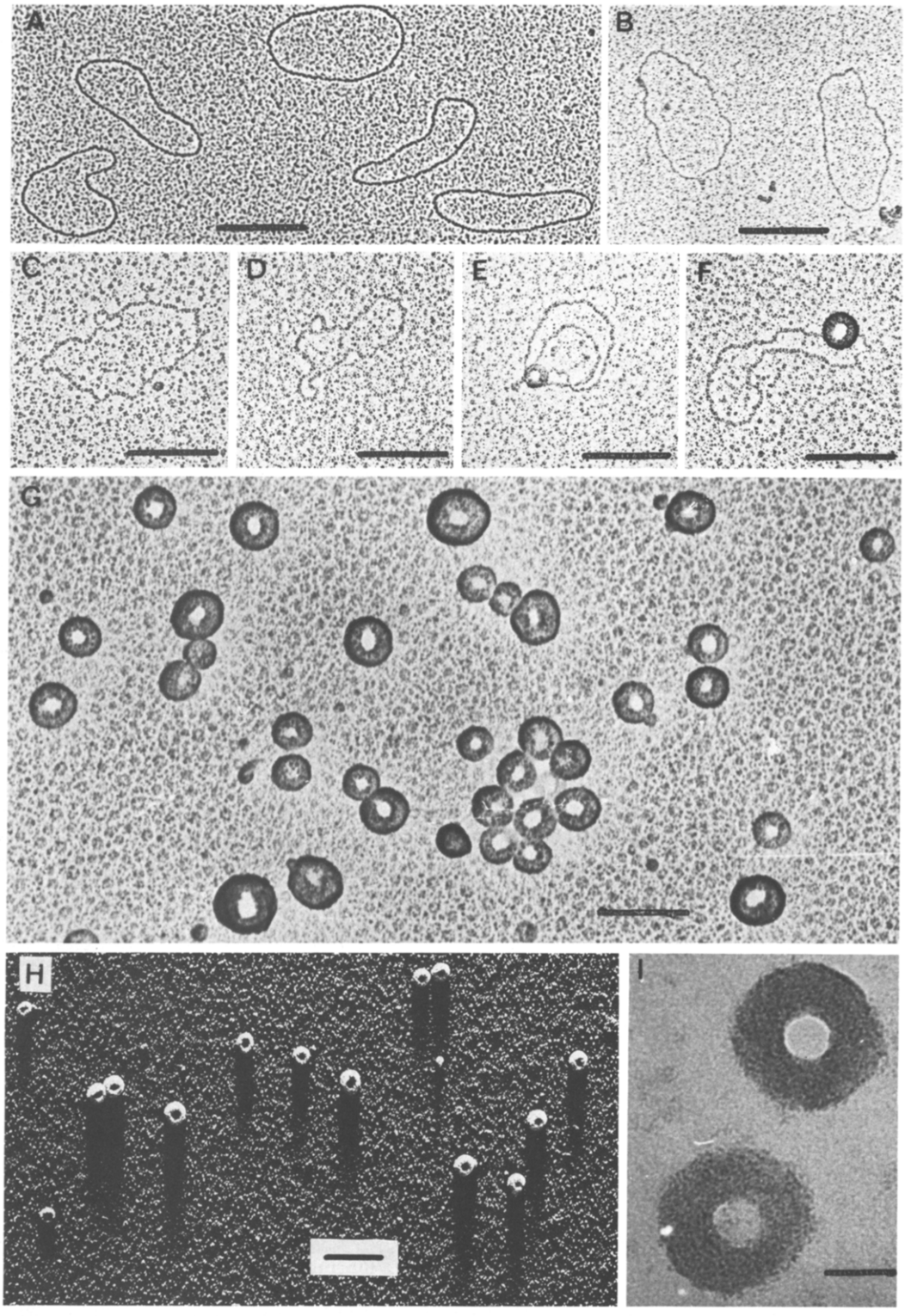
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Fig 1 Electron micrographs of pBR322 DNA and DNA–TVP complexes (A) DNA in the absence of TVP. Spreading with cytochrome *c* Bar, 0 5 $\mu$m (B) DNA in the presence of $1 \times 10^{-5}$ M TVP Bar, 0 5 $\mu$m (C–F) Selected intermediate structures at TVP concentrations of C,D, $3 \times 10^{-5}$ M, E,F, $7 \times 10^{-5}$ M. Bars, 0.25 $\mu$m (G–I) Torus-shaped particles of condensed DNA at TVP concentration $1 \times 10^{-4}$ M G,H: bars, 0 25 $\mu$m I bar, 400 Å Contrasting: A–G, rotary shadowing; H, line shadowing, I, uranyl acetate staining

with blotting paper and the grids were air dried. The samples were contrasted by rotary shadowing or uranyl acetate staining and analysed in a JEM 100CX ('JEOL', Japan) electron microscope.

## 3. RESULTS AND DISCUSSION

Fig.1A shows the micrograph of a relaxed plasmid pBR322 DNA preparation, spread with cytochrome *c*, in the absence of TVP. Most of the molecules had the appearance of open circles. The preparation was practically free of linear DNA molecules.

In fig.1B–I the micrographs of pBR322 DNA are shown at various TVP/DNA ratios. At peptide concentrations lower than $2 \times 10^{-5}$ M the major part of the material in the preparations had the appearance of open circular DNA molecules with a thickness of 40–60 Å after contrasting by rotary shadowing (fig 1B). When TVP concentration was raised over $3 \times 10^{-5}$ M the DNA in the preparations was present mainly in a condensed form At peptide concentrations $\sim 10^{-4}$ M practically all of the material on the preparations had the morphology of dense torus-shaped particles of various sizes (fig.1G; for convenience we call them toroids). Along with the toroids sometimes on certain fields structures were observed corresponding to the description of the 'spheroids' in [4]. The toroids had the same structure with clearly observed holes both on shadowed (fig.1G,H) and uranyl acetate-stained preparations (fig.1I).

The height of the toroids was measured from the length of the shadows on the preparations after shadowing at known angle from one direction (fig.1H). For more than 50 toroids the particle height was found to be coincident with the width of the toroid, measured on the same micrograph. These results show that the observed particles are not 'flat' but have a shape close to a perfect torus.

In order to evaluate the number of DNA molecules contained in a toroid one should estimate the apparent volume of the particle. To accomplish this the relevant dimensions of the toroids were measured on the stained preparations where the structures are not covered with a shadowed metal layer. The results of the measurements are given in fig.2. If the particles possess perfect torus-like shape their volume can be easily calculated according to the equation:

$$v = \frac{1}{32\pi}(C_o + C_i)(C_o - C_i)^2,$$

where $C_o$ and $C_i$ designate the length of outer and inner circumference of the torus, respectively. The apparent volumes of toroids estimated in the above-described way varied from 3 to $20 \times 10^8$ Å$^3$. Using estimates for DNA packing in torus-shaped structures [12] one can approximately calculate the volume occupied by one pBR322 DNA molecule in a toroid, which is equal to $\sim 10^7$ Å$^3$. So a conclusion can be drawn that most of the toroids contain 50–150 DNA molecules. This means that the compaction observed is of intermolecular nature.

The analysis of the structures corresponding to early stages of DNA condensation can provide important information about organization of DNA in toroids. However such 'intermediate' structures were observed rarely at TVP/DNA ratios sufficient for DNA condensation. Some of these struc-

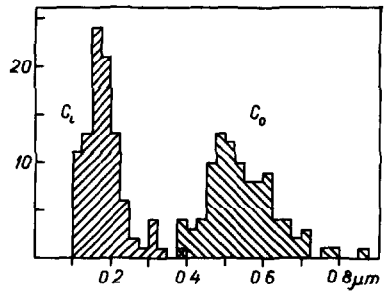


Fig 2 Results of the measurements of the length of outer ($C_o$) and inner ($C_i$) circumferences of the toroids $C_o$ = 5440 ± 920 Å; $C_i$ = 1840 ± 520 Å $n$ = 97

tures are shown in fig.1C–F. The morphology analysis of 'intermediate' structures led us to a model for intermolecular compaction of circular DNA molecules with the formation of toroids upon interaction with TVP. This model is presented schematically in fig.3.

The proposed scheme of compaction is based on the following experimental results and arguments. (i) The binding of TVP to DNA is known to be a highly cooperative process. (ii) The peptide binds to DNA in a self-associated form containing elements of antiparallel $\beta$-structure. (iii) The $\beta$-associated oligopeptides bound to DNA segments of the same or different DNA molecules can aggregate forming $\beta$-sandwiches stabilized by hydrophobic interactions [9–11,13].

In fig.3 the peptide bound to DNA is designated by dashes. Double dashes symbolize the hydrophobic contacts between peptide molecules bound to neighbouring DNA segments. The first step of compaction is probably an interaction between peptide molecules bound to different segments of the same DNA molecule, arranging these segments in a side-by-side way. Then the region of the DNA segments' contact propagates and a small toroid is formed which serves as a kind of a bobbin for the remaining part of the same molecule or the other molecules (fig.3).

Peptide–peptide interactions are not only responsible for initiation of the compaction process but also stabilize the resulting compact structures. This means that once formed a toroid is stable due to $\beta$-sandwich formation between peptide molecules situated on DNA filaments lying side by side. This suggestion can explain the fact that toroids behave like very stable particles in the course of electron microscopic sample preparation.

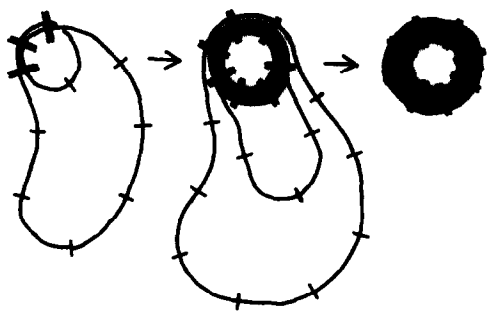

Fig.3. Schematical presentation of succession of compaction events leading to the intermolecular condensation of circular DNA with the formation of toroids.

In general, the data presented show that interaction between TVP and DNA can condense circular relaxed DNA and generate compact toroids, resembling 'doughnuts' or 'toruses', observed under the action of polyamines, histone H1 and other agents [1–6]. DNA in the described compact particles is arranged circumferentially as was proposed in [5] and [14] on the basis of biochemical and electron microscopic data.

The mechanisms involved in the formation and stabilization of compact torus-shaped particles upon peptide–DNA interactions may have important bearing for DNA compaction in vivo.

## REFERENCES


[1] Olins, D E. and Olins, A L. (1971) J Mol. Biol. 57, 437–455.

[2] Hsiang, M.W and Cole, R.D. (1977) Proc. Natl. Acad. Sci USA 74, 4852–4856.

[3] Haynes, M., Garret, R.A. and Gratzer, W.B. (1970) Biochemistry 9, 4410–4416

[4] Gosule, L.C., Chattoraj, D K. and Schellman, J A. (1978) in: Advances in Polyamine Research (Campbell, R A et al eds) vol.1, pp.201–215, Raven, New York.

[5] Marx, K A. and Ruben, G.C (1983) Nucleic Acids Res. 11, 1839–1854.

[6] Evdokimov, Yu.M., Platonov, A.L., Tikhonenko, A.S and Varshavsky, Ya M (1972) FEBS Lett 23, 180–184.

[7] Manning, G.S. (1980) Biopolymers 19, 37–59.

[8] Grosberg, A.Yu , Erukhimovitch, I Ya. and Shakhnovitch, E.I (1981) Biophizika 25, 929–941.

[9] Streltsov, S.A., Khorlin, A.A., Surovaya, A.N., Gurskey, G V., Zasedatelev, A.S., Zhuze, A.L and Gottikh, B.P. (1980) Biophizika 25, 929–941.

[10] Gursky, G.V., Zasedatelev, A S , Zhuze, A L , Khorlin, A.A., Grokhovsky, S.L., Streltsov, S.A , Surovaya, A.N , Nikitin, S.M., Krylov, A.S., Rethinsky, V.O., Mikhailov, M V , Beabealashvili, R S. and Gottikh, B P (1983) Cold Spring Harbor Symp. Quant. Biol 47, 367–378

[11] Makarov, V L., Streltsov, S.A , Vengerov, Y Y , Khorlin, A A. and Gursky, G V. (1983) Mol Biol (Moscow) 17, 1089–1101

[12] Earnshaw, W C. and Casjens, S.R. (1980) Cell 21, 319–331.

[13] Gursky, G.V , Tumanyan, V G., Zasedatelev, A.S., Zhuze, A L., Grokhovsky, S.L. and Gottikh, B.P (1975) Mol Biol (Moscow) 9, 635–651

[14] Marx, K.A. and Reynolds, T.C. (1982) Proc. Natl Acad Sci. USA 79, 6484–6488