Electrophoretic mobility of DNA knots

SIR — Elsewhere in this issue[1], we report a study using Metropolis Monte Carlo simulations to show that populations of thermally distorted knotted polymeric chains maintain certain geometrical properties of the ideal representations of the respective knots. Here we show that, in real DNA knots undergoing gel electrophoresis, there is a linear relationship between speeds of migration of different types of DNA knots and the average crossing numbers of their ideal geometrical representations (the concept of average crossing number is explained in ref. 1).

Several classes of enzymes acting on DNA produce DNA knots, the most well-known being topoisomerases and enzymes participating in site-specific recombination[2–6]. By finding out the types of DNA knots formed, it should be possible to determine the mechanisms by which these enzymes are involved in the proper functioning of chromosomes. But it is not straightforward to determine which types of DNA knots are formed by different enzymes. It requires a laborious electron-microscopy technique, where knotted DNA molecules are covered with RecA or UvsX protein to distinguish between underlying and overlying segments of knotted molecules[7,8].

Studies combining gel separation of different knot types with electron-microscope observations have shown that the speed of migration of DNA knots increases with their increasing complexity. To a first approximation, knots with the same minimal crossing number co-migrate on gels[9]. Higher-resolution gels can be used to separate torus- and twist-type knots having the same minimal numbers of crossings[10], opening up the possibility of identifying a given type of knot simply by its position on the gel, without the need for the laborious processes mentioned above[7,11].

We therefore decided to analyse the high-resolution gels presented in ref. 10 to compare the migration distances of the knots. When we plotted these distances against the average crossing numbers of ideal representations of the corresponding types of knot, we obtained perfect linear correlation (Fig. 1*a*). To cross-check if knots other than twist and torus type also migrate proportionally to the average crossing number of their ideal geometric representations, we analysed gels where composite knots ('granny' knots) were run together with different prime knots of the same size[12]. Figure 1*b* shows that granny knots also follow the same rule. This linear relation between the speed of gel migration of the knots and their average crossing number has not previously been noticed, although it has been reported that knots with 'higher' energies migrate quicker than knots with 'smaller' energies[13].

Obviously, DNA knots cannot maintain their ideal geometrical forms in solution and especially during gel separation. But we have demonstrated that the mean values of the average crossing number calculated for a Boltzmann ensemble of thermally agitated molecules are linearly related to the mean crossing numbers of the ideal geometrical forms of these knots[1]. Thus the linear relations in Fig. 1 would be maintained if we used the mean crossing number calculated for the population of thermally agitated configurations of the analysed knotted molecules instead of the average crossing number of ideal forms.

The linear relation between average crossing numbers of knots and their speed of migration can be explained by the fact that the average crossing number is directly proportional to the compactness of knots as expressed by the mean of inverse distances within the analysed trajectory (Fig. 2). The mean of inverse distances in a molecule is an accepted measure of molecular compactness and is simply related to the sedimentation constant[14]. Although electrophoretic migration in gels is a more complex process than sedimentation, compact molecules migrate quicker than less compact ones with the same charge. Thus it may be not so surprising that different knotted molecules migrate on gels with a speed proportional to their average crossing number.

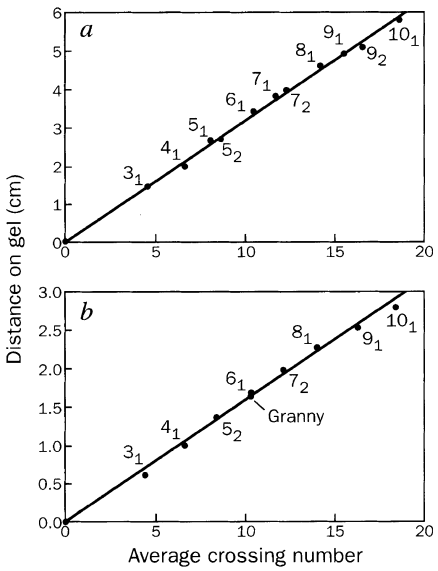

FIG. 1 Linear relation between electrophoretic migration of real DNA knots and the average crossing number of the ideal forms of these knots. *a*, Distances of different knots from the position of circular DNA measured from the image of a gel shown in Fig. 7 of ref. 10. Note that the knots analysed in ref. 10 were of twist and torus type only. *b*, Distance of migration of different knots measured on a gel (Fig. 4 of ref. 12). Note that the gel systems used in refs 10 and 12 are very similar.

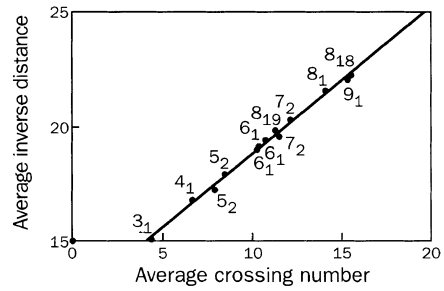

FIG. 2 Compactness of knots as a function of their average crossing number. Mean of inverse distances calculated for several ideal representations of knots is plotted against the average crossing number of these representations of knots. The values of the mean of inverse distances are normalized to the total axial length of a given knot.

Because there is no consensus about theoretical models that would allow gel migration of a given type of DNA knot to be predicted, our observation should be helpful to the study of DNA topology. Nevertheless, other gel systems may not preserve the perfect linear relation between migration speed and crossing number of different knots of the same size.

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1. Katritch, V. *et al. Nature* **384,** 142–145 (1996).
2. Wang, J. C. & Liu, L. F. in *DNA Topology and its Biological Effects* (eds Cozzarelli, N. R. & Wang, J. C.) 321–340 (Cold Spring Harbor Laboratory Press, New York, 1990).
3. Sumners, D. W. *Math. Intell.* **12**, 71–80 (1990).
4. Cozzarelli, N. R., Krasnow, M. A., Gerrard, S. P. & White, J. H. *Cold Spring Harb. Symp. Quant. Biol.* **49,** 383–400 (1984).
5. Stark, W. M., Boocock, M. R. & Sherrat, D. J. *Trends Genet.* **8**, 432–439 (1992).
6. Spengler, S. J., Stasiak, A. & Cozzarelli, N. R. *Cell* **42**, 325–334 (1985).
7. Krasnow, M. A. *et al. Nature* **304**, 559–560 (1983).
8. Griffith, J. D. & Nash, H. A. *Proc. Natl Acad. Sci. USA* **82**, 3124–3128 (1985).
9. Dean, F. B., Stasiak, A., Koller, T. & Cozzarelli, N. R. *J. Biol. Chem.* **260**, 4975–4983 (1985).
10. Crisona, N. J. *et al. J. Mol. Biol.* **243**, 437–457 (1994).
11. Wasserman, S. A., Dungan, J. M. & Cozzarelli, N. R. *Science* **229**, 171–174 (1985).
12. Kanaar, R. *et al. Cell* **62**, 353–366 (1990).
13. Simon, J. in *Mathematical Approaches to Biomolecular Structure and Dynamics* (eds Mesirov, J. P., Schulten, K. & Sumners, D. W.) 39–58 (Springer, New York, 1996).
14. Le Bret, M. *Biopolymers* **19**, 619–637 (1980).

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