# The Dissolution of Polynucleotides in Non-aqueous Solvents using Macrocyclic Polyethers

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Crown ethers and cryptands aid the selective dissolution of certain polynucleotides, including double-helical and single-stranded forms of deoxyribonucleic acid in alcoholic and mixed alcoholic–non-aqueous solvent systems; deoxyribonucleic acids appear to be more easily solubilised than ribonucleic acids.

The association between polycrown ethers (*e.g.* polyvinylbenzo-18-crown-6) and polynucleotides (*e.g.* polyadenylate) in the presence and absence of cations has been reported[1,2] in aqueous solution. A consequence of this interaction was to cause the inhibition of RNA-dependent DNA polymerase enzyme activity. Monomeric crown ethers did not show similar associative properties with polynucleotides in water nor inhibition of this enzyme activity. We report here our recent discovery that some polynucleotides can be made soluble in non-aqueous solvents, in both the presence and absence of cations, by association with monomeric crown ethers and cryptands. Various other phase-transfer reagents (*e.g.* tetra-alkylammonium salts, polyethylene glycols, *etc.*) do not have the same solubilising power as crown ethers and cryptands.

The solubilities of a range of polynucleotides in methanol were examined in the presence of 18-crown-6 and cryptand [2.2.2]. Table 1 lists approximate weight ratios of polynucleotide : crown ether (cryptand) required to dissolve *ca.* 0.5—2 mg/ml of polynucleotide in methanol at 25 °C. As shown, most forms of DNA (salt or free-acid, double-helical, or single-stranded) are soluble in concentrations of up to 2 mg/ml. Samples of RNA, however, appear to be less soluble under similar conditions, with the exception of the diethylaminoethanol salt from Torula yeast. Simple nucleotides such as guanosine 5′-monophosphate and thymidine 5′-monophosphate are solubilised in methanol in the presence of 18-crown-6; most other nucleotides proved either insoluble (*e.g.* cytidine 5′-monophosphate) or soluble (*e.g.* uridine 5′-monophosphate) irrespective of crown ether (cryptand).

Surprisingly, crown ether and cryptand alcoholic solutions of DNA remain homogeneous on addition of non-polar solvents (*e.g.* $CH_2Cl_2$, $CHCl_3$, EtOAc, PhMe, $Et_2O$, *etc.*) in concentrations of at least 95% non-polar solvent. These non-polar solvents will not dissolve DNA directly without its initial dissolution in methanol. On dissolution of double-

**Table 1.** The dissolution of polynucleotides in methanol–18-crown-6 and cryptand [2.2.2] (C.E.) mixtures at 25 °C.

| Polynucleotide[a] | Suspension of polynucleotide (mg/ml)[b] | Weight ratio of polynucleotide: C.E. to effect solubilisation | |
|---|---|---|---|
| | | 18-Crown-6 | Cryptand [2.2.2] |
| Type of DNA: | | | |
| *Micrococcus lysodeikticus* (Type XI) | 0.8 | 1:55 | 1:36 |
| | 0.5 | | |
| Calf thymus (single-stranded) | 1.9 | 1:55 | 1:40 |
| | 0.80 | | |
| Calf thymus (double-helical Na salt) (Type I) | 1.7 | 1:28 | 1:20 |
| | 1.5 | | |
| Calf thymus (Type XV) | 2.1 | 1:19 | 1:20 |
| | 2.0 | | |
| Salmon testes (Type III Na Salt) | 1.4 | 1:100 | 1:44 |
| | 0.9 | | |
| *Escherichia coli* (Strain B) (Type VIII) | 1.1 | 1:24 | |
| Type of RNA: | | | |
| Torula yeast (diethylaminoethanol salt) (Type IX | 0.7 | 1:30 | |
| Torula yeast (Type VI) | 0.85 | in[c] (1:>200) | |
| Calf liver (Type IV) | 1.5 | in[c] (1:>200) | |
| Bakers yeast (Type III) | 1.75 | in[c] (1:300) | |
| (Type XI) | 1.7 | in[c] (1:250) | |

[a] Commercial grades of polynucleotides were purchased from Sigma Chemical Company. [b] Small portions of C.E. (*ca.* 2.0 mg) were added to a suspension of the polynucleotide in methanol at 25 °C with stirring, until the suspension dissolved. U.v. spectra of DNA solutions showed maxima at $\lambda_{max.}$ 208 and 260 nm repectively. [c] in = largely insoluble suspension.

helical DNA in methanol with 18-crown-6 a substantial increase in absorbance at 260 nm (*ca.* 30—40%) compared with its spectrum in water was observed† (Figure 1). Single-stranded forms of DNA in methanol–crown ether did not exhibit this hyperchromic effect. The increase in absorbance when double-helical forms of DNA dissolve in organic solvent–crown mixtures is consistent with the conversion of double-helical DNA into single-stranded DNA.[3] When samples of DNA in methanol–crown ether were dialysed against water the absorbance at 260 nm reduced to that of the native DNA, indicating complete reassociation to the double-helical form.

The ability to dissolve polynucleotide in non-aqueous solvents using crown ethers and cryptands offers a range of possible applications,[4] for *e.g.* the following. (i) These selective complexants may be used for the purification and separation of polynucleotides where the less soluble components (*e.g.* RNA, protein,[5] *etc.*) could be filtered off; (ii) immobilised crown ethers and cryptands may be used as chromatographic materials for polynucleotide separations; examples where polymeric crown ether materials are already used in chromatographic separations of cations, anions, and organic compounds (*e.g.* optically active compounds) are well known;[6] (iii) crown ethers may effect the specificity patterns of restriction endonucleases; (iv) the solubilisation of polynucleotides in organic solvents is likely to be very useful in chemical synthesis and modification of polynucleotides, especially with regard to the use of moisture-sensitive and non-polar reagents.

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† An additional shoulder at 270 nm, which is absent in aqueous solution, was also noted.

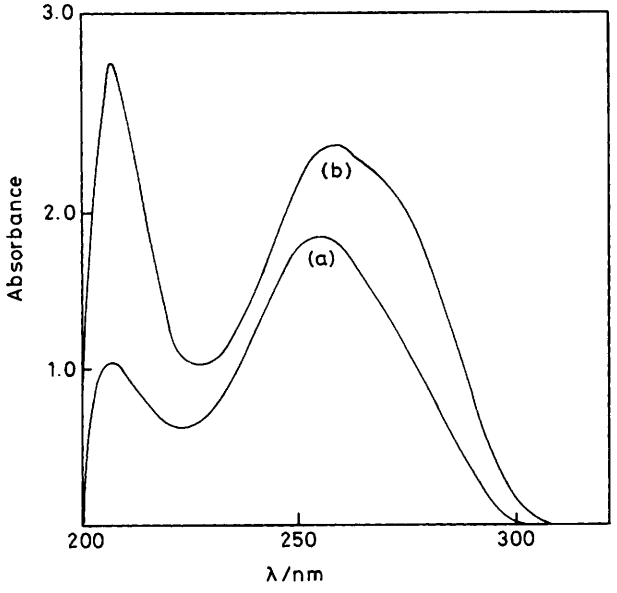


**Figure 1.** The u.v.-visible spectra of double-helical DNA [*Micrococcus lysodeikticus* (Type XI)] (a) in water (b) in methanol-18-crown-6 solution (concentration of DNA, 1 mg/ml).

## References

1 J. Pitha and J. Smid, *Biochem. Biophys. Acta*, 1976, **425**, 287.
2 J. Smid, *Pure Appl. Chem.*, 1982, **54**, 2129.
3 J. N. Davidson and W. E. Cohn, *Progr. Nucleic Acid. Res.*, 1963, **1**, 260, 273.
4 U.K. Pat. Appl. 8 405 763, filed on 6th March, 1984.
5 B. Odell and G. Earlam, *J. Chem. Soc., Chem. Commun.*, 1985, 359.
6 F. Vögtle, H. Sieger, and W. M. Müller, *Top. Curr. Chem.*, 1981, **98**, 156; J. Smid, *Ind. Eng. Chem. Prod. Res. Dev.*, 1980, **19**, 364; L. J. Mathias and K. B. Al-Jumah, *Polym. News.*, 1979, **6**, 9.