Behavioural Differences Between Native DNA and Denatured DNA

Native (N-)DNA derived from different sources interacts with a variety of cationic compounds to form a clot, fibrous or of other formations. Polyanions such as dextran sulphates, polyphosphates, polyethylene sulphonates, RNA, double stranded RNA, single stranded DNA, heat denatured (D-)DNA etc., however, interact differently by forming a simple precipitate (ppt) or granular ppt or exhibiting turbidity (td). Such a difference between N-DNA and other anionic polyelectrolytes has not been reported in the literature[1-3]. It was of interest, therefore, to make a study of the complexes of DNA with basic compounds. DNA was used in a concentration of 0.2 mg/ml in standard citrate saline. The concentration of aqueous solutions of the various polyanions (Na salts) as also of many of the basic compounds (as their salts) was 1 mg/ml. The weakly interacting cationic compounds were, however, employed in higher concentrations of 10 mg/ml or more. The test was performed by adding an equal volume or more of a basic compound to a convenient volume of N-DNA or any other polyanion. A gentle shaking evinced a clot with the former, but td/ppt with the latter. The sensitivity of detection for DNA of 5 μg was comparable with the methods already known excepting the oscillopolarographic technique[4].

The clot or ppt formed in a system was subjected as such to the dissolving action of KCl[5] (0.5–3.0 M) to find out the titre or binding strength of the interactants. For dissolving KCl-insoluble complexes of DNA with acriflavine (Acr), toluidine blue (TB), methylene blue (MB), cinchonidine, decamethylene diisothiouronium (DDIT) etc., the centrifuged clot or ppt was treated with the decomplexer, 10 M urea or 5–10% deoxycholate. A complete dissolution was effected by gradual addition and occasional scratching with a fine spatula for 24 h or more in the cold. The affinity of these compounds for DNA may be assumed to be much higher than that of the former group. This may be due to the operation of forces in addition to the primary electrostatic interactions in their formation. From a dissolved complex involving a dye, the latter was quantitatively extracted with *n*-butanol in order to determine the amount from its standard curve. Since the amount of a dye bound by N- or D-DNA was found to be practically the same, it appears that the number of binding sites for the dye on the DNA molecule does not undergo perceptible alteration on heat denaturation.

The order of relative binding strength of some basic compounds forming KCl soluble complexes with DNA has been found to be as follows: poly-L-lysine > protamine > histones > < dimethyl cetyl sulphonium > cetyl pyridinium (CPB) > viomycin > neomycin > polymyxin > lysozyme > kanamycin ~ spermine > dihydrostreptomycin ~ $[Co(NH_3)_6]^{+3}$. Of the 2 forms of the compound[6], $[Co(en)_2Cl_2]^{+1}$ (en = ethylenediamine), only the cis form produced a clot with N-DNA. Among the dimethyl alkyl sulphoniums and the polyamines, the affinity for DNA increased progressively from the lower to the higher homologues. This property is analogous to the protection afforded to DNA by polyamines against heat denaturation and breakage by hydrodynamic shear[7].

Thus, if acridines, histones and polyamines are endowed with the property of protecting[7-9] DNA and also of clot formation, one may infer that some of the clot-forming compounds such as basic antibiotics, basic dyes, alkaloids, diamidines, metal complexes[6], DDIT, lysozyme, cytochrome c, polybasic amino acids etc. may exhibit a protective effect towards DNA.

The binding strength (affinity) of the 2 forms of DNA for a representative group of basic compounds shows 3 patterns: (a) N-DNA > D-DNA (onium salts of nitrogen

[1] L. S. LERMAN, J. molec. Biol. *3*, 18 (1961).
[2] M. MOSKOWITZ, Nature *200*, 335 (1963).
[3] E. W. JOHNS and J. A. V. BUTLER, Nature *204*, 853 (1964).
[4] E. PALEČEK, Biochim. biophys. Acta *94*, 293 (1965).
[5] C. SINGH, Ind. J. Chem. *2*, 67 (1964).
[6] C. SINGH, J. Chromat. *18*, 194 (1965).
[7] H. TABOR and C. W. TABOR, Pharmac. Rev. *16*, 245 (1964).
[8] H. TABOR, Biochemistry *1*, 496 (1962). – H. P. VON HAHN, Experientia *21*, 90 (1965). – R. C. C. HUANG and J. BONNER, Proc. natn. Acad. Sci., USA *48*, 1216 (1962), and J. molec. Biol. *8*, 54 (1964).
[9] B. D. MEHROTRA, Ph.D. thesis (Indiana University, USA, 1963).

Recovery of N-DNA from its mixtures with D-DNA

Basic compound	Amounts of N- and D-DNA in a system in μg		
	System I	System II	System III
	$N_{50} + D_{100}$	$N_{50} + D_{50}$	$N_{100} + D_{50}$
	DNA in the clot[a], μg	DNA in the clot, μg	DNA in the clot, μg
1. CPB, 1 mg/ml	N-DNA lost in the ppt or as non-workable small bits or thready mass	50	128 (+ 28)
2. Protamin, 1 mg/ml	58 (+ 16)[b]	77 (+ 54)	140 (+ 40)
3. Acriflavine, 1 mg/ml	135 (+ 170)	95 (+ 90)	141 (+ 41)
4. Cobalt complex, 5 mg/ml $[Co(NH_3)_6]Cl_3$	As in CPB. The loss was, however, greater and recovery impossible	12 (– 76)	60 (– 40)

[a] The clot after its separation from the system was dissolved in a drop or two of 0.1 N NaOH and warmed after adding M KCl solution. The volume was made up with the latter and the amount of DNA determined colorimetrically by BURTON's method[13]. [b] For compounds 1–3, the values in parenthesis indicate the amount of D-DNA which is co-precipitated and it is expressed as % of the N-DNA initially present in the system. For compound 4, however, the values in parenthesis represent the loss of the N-DNA expressed as % of the N-DNA initially present in the system.

and sulphur), (b) N-DNA ~ D-DNA (Acr and hemoglobin, pH 3.5) and (c) N-DNA < D-DNA (MB, TB). Different modes of binding of N- and D-DNA with the various cationic compounds can be inferred from this. Different binding patterns of DNA have also been reported by other investigators[10,11] using techniques quite distinct from the present one. As evident from the Table, the influence of D-DNA on the clot formation by N-DNA is conditioned both by the nature of the cationic compound and the proportion of the two forms of DNA in the system.

The ultrasonicated DNA (N_2 atmosphere, 12 Kcs, 2.5 A, 60 min)loses its clot-forming ability to different extents as tested with different basic compounds. The change is, however, drastic and fast in an atmosphere of air.

The unique behaviour of clot formation by N-DNA may be due to its high viscosity as well as large extension in space of its molecules as compared to other polymers[12]. Preliminary experiments with some of the systems indicate that DNA is recoverable from the clots. LERMAN[1] has reported the recovery of N-DNA from its complexes with acridines[14].

Zusammenfassung. Es wird ein Test angewendet, der native und denaturierte DNS unterscheiden lässt. Er besteht auf einer Verklumpung nativer DNS bei Zugabe von kationisierten Stoffen, während denaturierte DNS nur eine Trübung, respektive einen Niederschlag gibt.

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Central Drug Research Institute, Lucknow (India), November 30, 1965.

[10] C. HARTMANN and M. LIERSCH, Angew. Chem. Internat. Ed. *3*, 648 (1964).

[11] E. O. AKINRIMISI, J. BONNER, and P. O. P. TS'O, J. molec. Biol. *11*, 128 (1965).

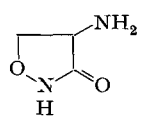
[12] C. TANFORD, *Physical Chemistry of Macromolecules* (John Wiley & Sons Inc., New York 1961), p. 406; P. DOTY, J. cell. comp. Physiol. *49*, 27 (1957).

[13] K. BURTON, Biochem. J. *62*, 315 (1956).

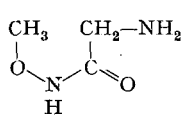
[14] The authors wish to thank Dr. M. L. DHAR, Director, Central Drug Research Institute, Lucknow, for his interest in the work and Dr. C. R. KRISHNA MURTI for valuable suggestions and Prof. Q. VAN WINKLE (Ohio, USA) for useful criticisms. Thanks are also due to several companies and scientists for generous gifts of biochemicals.

Glycine Methoxyamide, Acyclic Analog of Cycloserine

There has been considerable speculation on the molecular mechanism by which cycloserine (1) inhibits certain enzymes in microbiological systems[1]. The formation of a Schiff base with pyridoxal followed by opening of the isoxazolidone ring are the principal steps in the proposed mechanism. It seemed, therefore, of interest to synthesize the acyclic analog of cycloserine, glycine methoxyamide (GMA) (2), to determine whether the isoxazolidone ring was necessary to antibiotic activity. GMA does not appear in the chemical literature, but was readily synthesized by the procedure of KNOBLER[2] et al., using glycine N-carbonic anhydride and methoxyamine. The purified product of this reaction showed no activity against 29 representative bacteria, fungi and yeasts, demonstrating clearly that the ring is necessary for biological activity.



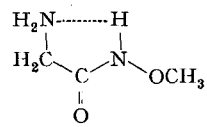
(1)



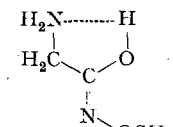
(2)

The structure of GMA was established by elemental analysis, IR-, UV- and NMR-spectroscopy. The solid-state IR-spectrum of GMA showed major bands at 6.1 (C=O), 6.22 (N–H), 6.52 (amide II) and 9.44 μ (C–O). Much hydrogen bonding of the α-amino group was indicated by broad absorption in the 3.0–4.0 μ region. These results are consistent with those of EXNER[3], but, in our opinion, do not support either the amide (3) or imide (4) structure.

UV data, λ^{MeOH} = 214 (log ε, 3.07), 222 nm (log ε, 3.38), was similar to that for cycloserine and NMR peaks in NaOD/D_2O appeared at δ 3.72 (–O–CH_3) and 3.35 (–CH_2–) which are consistent with the structure. The expected paramagnetic shifts to δ 3.78 and 3.55 occurred when the solution was acidified with trifluoroacetic acid.



(3)



(4)

It seemed pertinent to compare the ionization constants and metal binding affinities of GMA with those of cycloserine since large differences in these properties might account for the difference in biological activity between these two compounds. In our hands, cycloserine showed pKa values of 4.53 (–CONHO–) and 7.31 (–NH_2) in agreement with those reported by NEILANDS[4]. GMA, however, was much less acidic than cycloserine, having 7.20 (–CONHO–) and 9.51 (–NH_2) pKa values. This large difference of 1.8 pK units in the acidity of the cyclic and acyclic alkoxyamides is quite surprising. The flat cycloserine ring[5] undoubtedly stabilized the anion by facilitating overlap of the nitrogen *p*-electrons with the carbonyl group, thus increasing the acidity almost one

[1] (a) C. H. STAMMER and J. D. MCKINNEY, J. Org. Chem. *30*, 3436 (1965); (b) M. YA. KARPEISKY, R. M. KHOMUTOV, E. S. SEVERIN, and YU. N. BREUSOV, in *Chemical and Biological Aspects of Pyridoxal Catalysis* (Ed., E. E. SNELL, P. M. FASELLA, A. BRAUNSTEIN, and A. R. FANELLI; Macmillan, New York 1963), p. 323.

[2] Y. KNOBLER, S. BITTNER, and M. FRANKEL, J. chem. Soc. *1964* 3941.

[3] O. EXNER and B. KAKAC, Coll. Czech. Chem. Comm. *28*, 1656 (1963), favor the amide form for hydroxamic acids.

[4] J. B. NEILANDS, Arch. Biochem. Biophys. *62*, 151 (1956).

[5] R. PEPINSKY, Rec. Chem. Prog. *17*, 145 (1956).