when administered from day 1 to day 5. Further there was a significant reduction in the number of litters delivered on day 23 ($P < 0.01$) compared to the litters of the control group. This activity seems to be due to vicolide D because vicolide D alone had shown 71.4% antifertility activity at 50 mg per kg body weight[3]. The antifertility activity of vicolide B was 87.5% at this dose level[2].

The animals aborted on administration of vicolide B alone from day 8 to day 14 was 100%[1] but the mixture had shown 50% activity. The animals of this group showed significant reduction in the number of litters delivered on day 23 ($P < 0.02$) compared to the control group. The per cent animals aborted by the mixture on administration from day 14 to day 21 was in agreement with that of vicolide D at 100 mg per kg body weight[3].

This study suggests that the action of vicolide B is not fully exhibited when combined with vicolide D. This may be due to competition of vicolide D with vicolide B in the physiological system. Both vicolides may be substrates for the same enzyme because both were anioestrogenic in nature[2,3]. Chemically vicolides B and D are germacranolides. Vicolide D differs from vicolide B in having an epoxy group in the side chain. The antiprogestational activity exhibited by vicolide D is probably due to this group in the molecule. The inhibition of activity of vicolide B may also be due to this epoxy group in vicolide D. The diminished activity exhibited by vicolide B from day 8 to day 14 may be due to its inhibition by vicolide D. Vicolide D alone had shown negligible activity from day 8 to day 14 in rats at 100 mg per kg body weight[3].

We thus conclude that in a 1:1 vicolide B and D mixture the net antifertility activity will be due to vicolide D only.

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Silanization of DNA bound baked glass permits enhanced polymerization by DNA polymerase

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DNA can bind to pure silicon dioxide through the formation of hydrogen bonds. This bonding occurs when the hydration shell of DNA is disrupted by chaotropic agents and such binding can be easily reversed with water or buffers of low ionic strength. Single-stranded, supercoiled and denatured DNA when bound to flint glass and baked at 60°C for 2 h permit synthesis reaction using Klenow. The reaction was enhanced when the glass was silanized prior to synthesis reaction.

DOUBLE-STRANDED helical structure of DNA has been analysed for its periodicity by binding it to calcium phosphate and then subjecting it to digestion by DNase I (ref. 1). This was the first report which showed that such solid-state-bound DNA maintains a periodicity which is greater than the one postulated for the B-DNA structure, viz. ten base pairs per turn of helix. The actual value turned out to be 10.4 and this was totally corroborated by the solution structure analysis of DNA. Hence mere binding of DNA to a solid state matrix is known not to distort the structure strongly. Also such structures were found to be amenable to nuclease digestion. Recently it has been shown that binding of photolabile 5′-nitroveratryl thymidine onto an aminated glass surface allowed solid state chemical synthesis of dinucleotides when treated with phospharamidite-activated 2′-deoxycytidine (ref. 2). We describe here activity of DNA polymerase (Klenow fragment) on glass-bound DNA.

The substrate chosen for analysis was single-stranded closed-circular DNA, double-stranded closed-circular duplex DNA (form I) and denatured closed-circular duplex DNA (form I_d). The objective being to investigate how far does binding to a two-dimensional surface influence the tertiary structure of the DNA to

affect its substrate specificity to known enzymatic reaction.

DNA has a natural affinity to hydrogen bond with glass, when the hydration shell of DNA is disrupted with chaotropic agents like sodium thiocyanate, sodium iodide, etc. Hydrogen bond formation between glass and DNA is favoured at ice temperature and as the hydrogen bonding efficiency of DNA with glass is low, compared to water, the hydration shell of DNA is restored in the presence of water or buffers of low ionic strength[3]. The elution of DNA bound to flint glass is relatively low and, once baked even at 60°C, is not eluted, whereas DNA bound to pure silicon dioxide is eluted even after it is baked. Hence pure silicon dioxide is used in the presently available commercial protocol for purification of DNA.

Double-stranded covalently closed-circular pUC18 DNA (form I) and single-stranded circular M13 DNA (s. s. circular) were obtained by usual protocol[4]. Denatured covalently closed-circular duplex DNA (form I_d) was obtained by treating form I DNA with equal volume of 1 N NaOH at room temperature for 15 min followed by neutralization with 2× volume of 1 M Tris-HCl, pH 8.0 (ref. 5).

For Klenow-mediated synthesis reaction different forms of DNA were first treated with 2.5 volumes of 6 M sodium thiocyanate to disrupt the hydration shell of DNA followed by the addition of 20 μl of flint glass (20% powdered flint glass solution in 10 mM Tris-HCl, pH 8). The mixture was incubated in ice for 15 min and centrifuged at 4°C for 10 min. The supernatant was discarded and the glass pellet was washed twice with 100 μl of 50% isopropanol solution to remove any RNA if present as well as to ensure complete removal of sodium thiocyanate. The glass-bound DNA was then baked at 80°C for 2 h which would render the binding of DNA to glass irreversible. To this glass-bound DNA pellet 1 μl of Klenow polymerase enzyme, 1 μl of reagent mix (containing dTTP, dATP, dGTP, reaction buffer and primer), 1 μl of BSA from the Pharmacia random-primed oligo labelled reagent kit along with 10 μCi of α^{32}P dCTP in 1 μl were added. The synthesis reaction was allowed to continue for 2 h at 37°C and terminated by the addition of 0.2 M EDTA. The sample was then denatured at 95°C for 5 min, chilled, centrifuged at 4°C for 5 mins finally eluted with water —the newly synthesized strands go to the aqueous phase. The supernatant containing newly synthesized single-stranded DNA was subjected to 5% trichloroacetic acid precipitation for 30 min at 4°C. The mixture was recentrifuged and, finally, counts per minutes were recorded in Beckmann liquid scintillation counter LS 6000SE. The glass precipitate after first round of elution was subjected to two subsequent cycles of synthesis reaction and counts per minutes left in glass, TCA precipitable count, and stray count were recorded after each cycle of synthesis reaction (Table 1).

Results indicate that although the per cent of incorporation of radiolabelled dCTP during the first cycle of synthesis was relatively low (5%) there was increase in the amount of incorporation α-^{32}P labelled dCTP in the subsequent cycles of synthesis reaction. There was substantially greater amount of incorporation of radiolabelled dCTP in the newly synthesized DNA strand when the substrate was double-stranded circular DNA compared to single-stranded circular DNA. The reason being perhaps the greater accessibility of hexameric primer to anneal to double-stranded structure whereas single-stranded DNA template offered no such advantage. Lack of complementary strand may in fact result in inaccessible regions—regions which are in physical contact with glass.

Table 2 gives results of experiments carried out by binding denatured linearized double-stranded DNA to glass and then subjecting it to baking at 80°C for 2 h. This glass-bound baked DNA was then treated with

Table 1. Measured radioactive counts of different fractions of glass bound DNA undergoing Klenow synthesis.

Count	Sample	Set 1 (cpm)	Set 2 (cpm)	Set 3 (cpm)
Total input count	Form I	3700000	1003881.9	1745926.5
	Form I_d	2722969	1111181.9	1656890
	Single stranded circular DNA	2281174.8	919669.8	1925421.1
Residual Count on glass	Form I	55118.8	21213.3	47684.4
	Form I_d	54365.9	47522.9	21456.2
	Single-stranded circular DNA	25140.8	23076.3	29480.3
TCA ppt. count	Form I	85798.9	114773.1	497065.7
	Form I_d	178866.2	110588.5	387074.5
	Single-stranded circular DNA	116944.5	127949.6	308926.2
Stray count	Form I	2118772.1	778928.7	1201176.4
	Form I_d	2489736.9	935614.2	1248359.2
	Single-stranded circular DNA	2139089.4	834369.4	1587014.6
Incorporation (%)	Form I	4%	13%	28.5%
	Form I	7%	10.1%	23.4%
	Single-stranded circular DNA	5.2%	13%	16%

Table 2. Radioactive counts of different fraction of silanized glass bound DNA undergoing Klenow synthesis

Sample	Count	Set 1 (cpm)	Set 2 (cpm)
Linear DNA	Total count	3096771.7	2511984
	Count left on glass	348369.25	370550.59
	TCA ppt.	88736 53	934446.8
	Stray count	18610458	1518335
	Incorporation (%)	28.7%	37.2%

Figure 1. A photographic print of the autoradiogram of a strip of nitrocellulose to which pUC18 DNA was Southern blotted. The nitrocellulose filter was hybridized with Klenow synthesized pUC18 DNA using flint glass-bound baked pUC18 DNA as template.

50 μl bind silane (marketed by LKB). The treatment was carried out at 80°C for 20 min in the same eppendorf tube. Bind silane was removed as the supernatant after centrifugation and finally the silanized glass-bound baked DNA was used as substrate for generation of probes.

Glass-bound baked linearized pUC18 DNA was used as a template for synthesis reaction in a similar fashion as described above and the newly synthesized DNA was used as a probe to hybridized Southern blotted pUC18 DNA. Autoradiogram (Figure 2) of the strip of nitrocellulose filter showed the DNA synthesized is authentic pUC18 DNA.

We can conclude that DNA bound to flint glass is a good substrate for DNA polymerase. The advantage of using glass-baked DNA is that even sparingly available DNA samples once bound can be used repeatedly for the generation of probe DNA.

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Triplex formation between d-CGCTCT and the self-complementary oligonucleotide d-CAATCTCGCGA-GATTG-spectroscopic investigations

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We report here studies on triple helix formation by mixed purine–pyrimidine strands with a view to explore the general potential of oligomeric triplex formation as a recognition phenomenon of DNA sequences. It is observed that the hexamer d-CGCTCT-3′ with a purine at second position forms a stable triplex with a self-complementary DNA duplex d-CAATCTCGCGA-GATTG-3′ in the pH range 5–7, in the presence of 2M NaCl. ^{1}H NMR spectra in H_2O solution under these conditions show the imino resonances belonging to the Hoogsteen base pairs in the triplex. Thermodynamic parameters for the triplex formation have been determined by UV spectroscopy.

The existence of triplexes in DNA and RNA has been known for over three decades[1]. In the ensuing years a large number of investigators have used pyrimidine oligodeoxynucleotide triple helix formation to recognize extended purine sequences in double helical DNA[2–12]. From X-ray-fibre diffraction studies it has been observed that the third strand in the triplex sits in the major groove of the duplex, formed by the other two strands, is held to it by Hoogsteen base pairs[13–15]. The base triplets T.(AT) and C^+.(GC) thus formed are shown in Figure 1a. It may be noted that the latter requires the protonation of the cytosine residue and hence its formation is pH-dependent[3,9,17]. Formation of the base triplets such as G.(GC) and A.(AT) have also been recently reported[16]. Homopyrimidine oligodeoxynucleotides attached to DNA-cleaving agents have been used to cut duplex DNA[3,5]. Strobel *et al.*[6] established the usefulness of such sequences as probes for chromosome mapping and as antisense DNA for