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Influence of Silicic Acid on *In Vitro* Depurination of DNA

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Epidemiological and experimental studies have indicated an intimate association between asbestos exposure and cancer of pleural mesothelium (Wagner, 1974; Mossman et al., 1990). The molecular mechanism of asbestos mediated carcinogenesis however, remains unknown. Silicic acid dissolved from asbestos is considered to be responsible for some of the pathological alterations associated with asbestos toxicity (Rahman et al., 1973, 1974; Singh and Rahman, 1987). A possibility of exchange between silicic acid and phosphoric acid in DNA and RNA has been suggested (Iler, 1979). Various authors have reported an increased rate of sister-chromatid exchanges and chromosomal aberrations, increased turn-over of DNA synthesis and strand breakage in animals exposed to asbestos (Amachar et al., 1979; Price-Jones et al., 1980). Lindahl and coworkers (1977) have proposed that a significant number of purines may be spontaneously lost from mammalian cells under physiological conditions. Depurinated DNA is also implicated as an intermediate in excision repair of chemically induced damage in DNA (Karran et al., 1977; Weiss and Grossman, 1987). Earlier studies from this laboratory demonstrated the degradation of DNA on treatment with silicic acid (Rahman et al., 1984). In view of the involvement of the depurination process in various cellular mechanisms of DNA repair we have studied the influence of silicic acid on in vitro depurination of DNA.

MATERIALS AND METHODS

Calf thymus deoxyribonucleic acid (sodium salt average mol. wt. one million) was obtained from Sigma Chemical Company and was used without further purification. S1

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nuclease was purchased from Boehringer and sodium silcofluoride was a product of BDH, U.K.

Silicic acid solution was prepared by dissolving 2 gm sodium silicofluoride in 500 ml double glass distilled water containing 5 ml 10 N H_2SO_4. The required amount of solid $CaCO_3$ was added to remove fluoride and the liquid filtered. The pH was adjusted to 7.0 with 0.1 N NaOH and the solution passed through a 2x50 cm column of chelex resin (flow rate 10 ml/hour). The Si content of the silicic acid solution was estimated by the method of King et al. (1955). The solution was also analysed for calcium by the EDTA titration procedure using the dye, erichrome black T (Reilley et al., 1959) and by atomic absorption (Perkin Elmer 5000, Atomic Absorption Spectrophotometer). No calcium could be detected by the titration procedure and atomic absorption gave a value of less than 0.1 ppm. Before use, the solution was kept in boiling water for 10 minutes to depolymerize any polysilicic acid and then cooled and diluted to the desired concentration. DNA was depurinated by heating at pH 3.5 (Hadi and Goldthwait, 1971). For the experiment shown in Figure 1, 0.25 ml of a 2 mg/ml solution of DNA in TNE_4 (0.01 M Tris-HCl buffer pH 7.4, 0.01 M NaCl, 2 x 10^{-4} M EDTA) was added to 0.5 ml of 0.2 M citrate buffer pH 3.5. Silicic acid was also added from the stock solution in increasing concentration to obtain DNA nucleotide/ silicic acid molar-ratios of 1:0.25, 1:0.5, 1:0.75 and 1:1. The reaction mixture (1 ml) was incubated at 70° C for 60 minutes. The degree of depurination was estimated by alkaline hydrolysis of depurinated DNA (Tamm et al., 1953) and was carried out by adding NaOH to a final concentration of 0.1 M and leaving the solution at room temperature for 15 minutes. 0.2 ml of 10 mg/ml bovine serum albumin and 1 ml of cold 14% perchloric acid was then added to precipitate unhydrolyzed DNA. Acid soluble nucleotides were measured by the diphenylamine procedure (Schneider, 1957). In the case of experiment recorded in Figure 2, depurination of DNA in the presence of silicic acid at DNA nucleotide/silicic acid molar ratio of 1:1 was carried out as described above. Parallel control, which did not contain silicic acid was also incubated. 1 ml aliquots containing 500 µg DNA were removed at various time intervals and subjected to alkaline hydrolysis and estimation of acid soluble materials as already described. For experiment shown in Figure 3, depurination of DNA was done in the absence of silicic acid by the procedure as given above. After depurination, DNA solution was quickly quenched in ice, neutralized with NaOH and divided into two aliquots. To one of the aliquots silicic

acid was added in a DNA nucleotide/silicic acid molar ratio of 1:1. The other aliquot served as a control. Both the samples, were incubated at 70° C and different aliquots (1 ml) containing 500 µg DNA were removed at various time intervals and subjected to alkaline hydrolysis.

RESULTS AND DISCUSSION

Figure 1 shows the alkaline hydrolysis of DNA depurinated in the presence of increasing silicic acid concentration expressed as DNA nucleotide/silicic acid molar ratio. It is seen that the production of acid soluble material decreased with increasing silicic acid concentration. That this effect is not due to the increased ionic strength of the depurination solution, due to the presence of silicic acid, was demonstrated by another experiment where in place of silicic acid, NaCl was added in similar molar ratios (results not shown). Under these conditions no decrease in the extent of alkaline hydrolysis of DNA was seen, demonstrating a protective effect of silicic acid on the depurination process. Figure 2 gives the effect of time of depurination at 70° C at pH 3.5 in the presence of silicic acid. In agreement with the results of Figure 1 it is seen that the rate of depurination in the presence of silicic acid is almost half as much as in its absence. In order to confirm that the effect of silicic acid seen in the above experiments was on the depurination process itself and not on alkaline hydrolysis of depurinated DNA, the experiment shown in Figure 3 was carried out. DNA was depurinated in the absence of silicic acid, neutralized to pH 7.0 and subsequently treated with silicic acid at 70° C for various time periods before alkaline hydrolysis. Under conditions, as shown in the figure, silicic acid did not have any effect on the extent of alkaline hydrolysis. These results demontrate that silicic acid inhibits the depurination reaction but does not affect the alkaline hydrolysis of depurinated DNA.

S1 nuclease is a nucleolytic enzyme that is specific for single stranded nucleic acids but can also hydrolyze partially denatured regions or less stable secondary structures in double stranded DNA (Shishido and Ando, 1982). The extended period of heat depurination (60 minutes) at pH 3.5 employed in the previous experiments results in denaturation of duplex DNA due to extensive depurination (Hadi and Goldthwait, 1971). In order to preserve the double strandedness of DNA during depurination, the experiment shown in Table 1 was carried out. DNA was

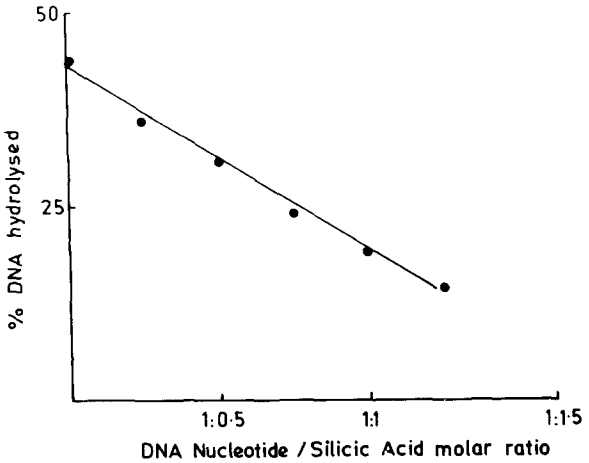

Figure 1 · Alkaline hydrolysis of DNA depurinated in the presence of increasing silicic acid concentration. The details of the experimental procedure is described in the text.

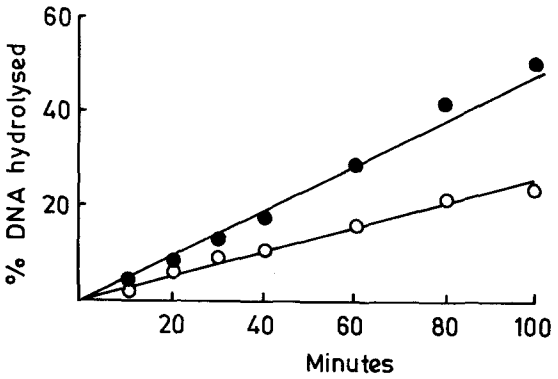

Figure 2 · Alkaline hydrolysis of DNA depurinated with silicic acid for various times. The details of the experimental procedure is given in the text. (●——●) without silicic acid, (O——O) with silicic acid.

depurinated for a shorter period of 5 minutes in the presence of silicic acid and subjected to S1 nuclease and alkaline hydrolysis. As shown in the table, under these conditions, no alkaline hydrolysis of depurinated DNA is observed indicating significnatly

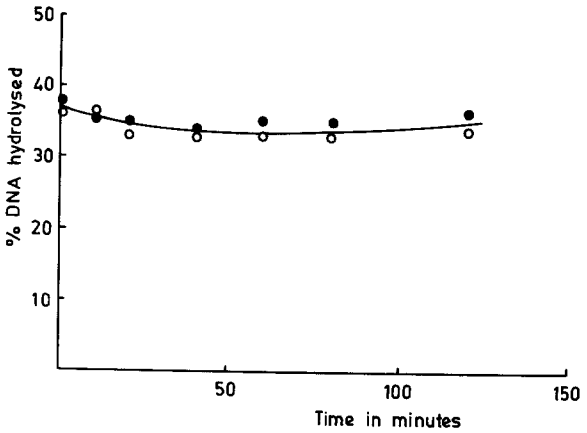

Figure 3 · Alkaline hydrolysis of depurinated DNA after treatment with silicic acid. The details of the experimental procedure is described in the text.(●—●) without silicic acid, (O—O) with silicic acid.

less depurination. Also, the DNA possibly remains at least partially double stranded as suggested by its incomplete digestion by S1 nuclease in relation to heat denatured DNA. In agreement with previous results, depurination with increasing molar ratios of silicic acid results in a progressively decreasing degree of S1 nuclease hydrolysis. Using denatured DNA it was earlier determined that S1 nuclease is not inhibited at the concentrations of silicic acid tested (results not shown). The results further suggest that depurination of DNA in the presence of silicic acid does not result in any gross distortion of the polynucleotide as the S1 nuclease is still also to recognize its substrate.

Our results show that silicic acid decreases the rate of heat depurination of DNA in vitro and suggests the possibility of its reaction with polynucleotides. The nature of the reaction and its exact toxicological significance however, remains unknown, and further **in vivo** studies are needed in this direction. Asbestos fibers have been considered to be able to enter various cell types as well as the nucleus (Haugen et al., 1982; Johnson and Davies, 1983), and thereby may increase the local concentration of silicic acid inside the cells or nucleus. It is tempting to

Table 1. S1 nuclease and alkaline hydrolysis of DNA depurinated inthe presence of silicic acid

DNA nucleotide/ silicic acid molar ratio	S1 Nuclease hydrolysis		Alkaline hydrolysis	
	µmole acid soluble DNA nucleotide	per cent DNA hydrolysed	µmole acid soluble DNA nucleotide	per cent DNA hydrolysed
Native DNA	0.06	7.0	0.0	0.0
Heat denatured DNA	1.18	98.0	0.0	0.0
Depurinated DNA	0.70	58.0	0.0	0.0
1 : 0.25	0.60	48.0	0.0	0.0
1 : 0.50	0.43	33.0	0.0	0.0
1 : 0.75	0.30	22.0	0.0	0.0
1 : 1.00	0.26	19.0	0.0	0.0

Depurination of DNA inthe presence of varibus concentrations of silicic acid was done by heating at 70 °C for 5 minutes in citrate buffer pH 3.5. S1 nuclease reaction mixture in 1 ml contained 400 µg native, denatured or depurinated DNA, 0.1 M acetate buffer pH 4.5, 1 mM $ZnSO_4$ and 100 units of S1 nuclease. The incubation was done at 40 ° C for 2 hours and the reaction stopped by adding 0.2 ml of 10 mg/ml bovine serum albumin and 1 ml cold 14% perchloric acid. Alkaline hydrolysis of various samples was carried out as described in the text using similar amounts of DNA.

speculate at this stage that the protective action of silicic acid on depurination of DNA in vivo may slow down the excision repair of chemically-induced damage in DNA (Weiss and Grossman, 1987), thereby altering the overall cellular processes which may be playing some role in asbestos-mediated carcinogenic responses. Further studies are suggested in this direction to support the aforesaid hypothesis.

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