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Toxicological Significance of Silicon-protein Interaction

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ABSTRACT

In order to understand the molecular mechanism of the toxicity of *Si* containing particulate air pollutants, the interaction between silicate anion and proteins was studied. On the basis of molecular sieving profile, the presence of a protein fraction capable of binding silicic acid was detected in rat lung and serum. The binding is firm being able to withstand dialysis, *Si*-binding by Bovine Serum Albumin (BSA) follows stoichiometric principles indicating true chemical reaction in terms of effects of pH, temperature and period of incubation. Fluorescence spectrum of the BSA-*Si* complex decreased with an increase in *Si* concentration. Effect of *Si*-binding on trypsin activity against albumin showed that proteins other than albumin could also interact with *Si*-trypsin containing silica showed distinctly low catalytic activity against native BSA. When both the substrate and enzyme contained bound *Si*, the activity further reduced by 36 per cent as compared to both pure trypsin and pure BSA, clearly indicating that binding of *Si* with substrate or enzyme proteins can adversely effect the biological activity. Complexing with proteins is likely to play a role in pathogenesis of pneumoconiosis, elimination of dusts, formation of silicate stones in plants and animals, and possibly in the reported role of *Si* in nutrition, cardiovascular diseases and ageing.

1. INTRODUCTION

It is a paradox that silicon (*Si*) and its compounds which account for over a fifth of earthly mass and which are intimately associated with the origin of life and evolution of species and also in physical sustenance of life[1,2] have become alarmingly hazardous xenobiotics.[3] Ever since the industrial revolution, health hazards caused by silicon containing particulate air pollutants in mining and ore processing occupations, have always been an alarming threat to the workers.[4] In fact, lung diseases due to dusts (pneumoconiosis) are the most serious among occupational and environmental

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diseases. Dusts of free silica such as quartz and silicates, e.g., asbestos, talc and mica are known to produce diverse toxic effects which are very well documented.[5] However, inspite of several theories and a large number of experimental and clinical studies,[6] the exact molecular mechanism responsible for the pathomorphological and physiological lesions is not yet clear. There is considerable evidence pointing out that silicic acid dissolved from the dusts could be a pathogenic factor, as postulated in the solubility theory of King and its follow up studies. Nevertheless, in terms of today's knowledge of bioinorganic chemistry and cell biology, the manner in which silicon acts as cellular organelle and molecular loci is not clear. The major reason for the lacunae in this field is the comparative inertness of silica in biological systems and the inherent methodological difficulties.

Apart from the interest of silicon biochemistry in environmental toxicology, this field has assumed significance in diatom physiology[8,9] and plant nutrition.[10,11] Moreover, in recent times, possible roles of silicon in collagen formation, as a trace nutrient[12-14] in cardiovascular diseases[15,16] in ageing[17,18] and in origin and evolution of life and exobiology[11] have been implicated. Silica is also ubiquitously present in several animal tissues.[11,19] Thus, the role of silicon in comparative biochemistry, in health and disease is an important, yet not well documented area of biochemical, biomedical and environmental sciences.[20]

Holt and Went[21] showed that binding with polysilicic acid gave rigidity to laminarin fibres. The pH pattern of *Si*-polysaccharide binding indicated esterification or chelation, rather than H-bonds. It was suggested[22] that the normal role of acid mucopolysaccharides in colagen deposition can be irreversibly taken up by silicic acid in silicosis. *Si* deficient diatoms get a coating of glucosides. Also, in silicotic patients considerable alterations in glycoproteins take place.[11] Thus, protein-*Si* interaction is a major aspect of *Si* biochemistry.

Si can interact with proteins through adsorption, coagulation and actual binding.[23] Leather gets tanned by polysilicic acid and albumin gets coagulated with colloidal-*Si*. Holt[24] reported that polysilicic acid cross linked with proteins at several points, preventing further uncoiling. Inspite of a large number of preliminary reports, the biochemical nature and significance of protein-*Si* interaction is still unclear. Also, evidence is limited regarding the physiological implications of these interactions. Therefore, using bovine serum albumin as a model protein, binding of *Si* has been studied. In this paper the possible bioinorganic significance of protein-*Si* binding in toxicity of quartz has also been explored.

2. MATERIALS AND METHODS

2.1 Chemicals

The various biochemicals were obtained either from Boehringer, BDH Anal R or E. Merck extrapure. UICC standard chrysotile asbestos from West Germany and quartz dust with particle below 5 μm were from the same sources as used earlier in Industrial Toxicology Research Centre, Lucknow. Silicic acid solution was prepared according to Rahman et al.[25]

2.2 Animals

Male albino rats weighing 160-180 g drawn from Industrial Toxicology Research Centre Animal Colony, maintained on Hindustan Lever pellet diet under standard conditions, were used.

Autoclaved suspensions of quartz dust (10 mg per ml) in normal saline were intratracheally instilled in rats, the trachea were exposed by blunt dissection. After administering the dust, the wound was sutured. Animals were killed after 15 days, lungs and blood collected and processed.

2.3 Preparation of Subcellular Fractions of Lung

Subcellular fractionation of the 5 per cent W/V homogenate in 0.25M sucrose was done to separate mitochondria and microsomes according to Mustafa.[26] The nuclear fraction was removed by centrifuging the homogenate at 700 × g for 10 min and the resulting supernatant was spun at 9000 × g for 10 min to sediment mitochondrial pellet. All centrifugation steps were performed in refrigerated centrifuges, Janetzki models K_{24} and K_{70}.

2.4 Assay of BSA-*Si* Interaction

One volume of 10 per cent trichloroacetic acid (TCA) was added. At this stage, in the control tubes silicic acid was added to account for any unspecific co-precipitation of *Si* with the proteins. The precipitate after five washings with 5 ml 5 per cent TCA was dissolved in 2.5 ml fresh 0.05N NaOH. Using aliquots below 50 μg protein, to avoid turbidity, *Si* was estimated.

2.5 Fluorescene Studies of BSA-*Si* Complex

BSA-*Si* complex was measured at 340 nm, excitation 280, using an Aminco Boman SPF 500 Spectroflurometer.

2.6 Binding of Silicic Acid by Lung and Serum Proteins

Rats were given intratracheal silica (10mg) for 7 days, after which the post-mitochondrial supernatants of the lungs were saturated with 90 per cent ammonium sulphate with gentle and constant stirring at 2-6°C. This was dialysed against water for 12 hours after which dialysis against 0.025M Tris buffer (pH 7.4) was done. The dialysed sample centrifuged and 5 ml of this sample containing 15 mg protein was applied to a 3 × 80 Sephadex G-75 column (Pharmacia) which was pre-equilibrated with 0.025M Tris buffer pH 7.4 Void volume and column volume was pre-determined from exclusion of blue dextran and potassium dichromate. The elution profile of protein along with silica and carbohydrate contents were followed. Fractions of 5 ml each were collected at the flow rate of 25 ml/hr. Simultaneously, the same experiment was performed with a set of control animals too.

2.7 Effect of *Si*-BSA Binding on the Tryptic Activity

A three ml (5 mg/ml) solution of BSA was taken in a dialysis bag and dialysed against a solution of *Si* (200 μg/ml). At different time intervals 2 ml of the solution from the beaker was taken out and *Si* estimated. After 8 hrs of dialysis the bag was opened and volume measured. *Si* and protein were also estimated in the contents of the bag. Similarly, 5 mg/ml trypsin was taken in the dialysis bag and dialysed against *Si* solution.

Tryptic activity measured in the following set of experiments :

(a) BSA (5 mg/ml untreated) + tris buffer and trypsin (5 mg/ml untreated).
(b) BSA (5 mg/ml from dialysis bag) + tris buffer + trypsin (5 mg/ml untreated).
(c) BSA (5 mg/ml) + tris buffer + trypsin (from dialysis bag).
(d) BSA (from dialysis bag) + tris buffer + trypsin (from dialysis bag).

All these were allowed to incubate at 37°C for 20 min. The reaction in all the sets stopped with 2 ml of 10 per cent TCA mixed thoroughly, allowed to stand for 30 min at room temperature and centrifuged for 20 min at 3,000 g. The tryptic activity of supernatant fluid was measured following increase in O.D. of the supernatant at 280 nm.

2.8 Estimations

Silica was estimated by the silicomolybdate method of King.[27] Carbohydrate content was determined by the phenol sulphuric acid method of Montgomery.[28] Protein content was determined by the procedure of Lowry *et al.*[29]

3. RESULTS

3.1 Effect of *Si* on Heat Denaturation of BSA

The data for turbidity studies of 2 mg/ml solution of BSA and BSA-*Si* complex after incubating for different time periods at 100°C pH 7.4 are given in Fig. 1. It follows that binding with *Si* accords complete protection to albumin against denaturation. Heat denaturation of 60°C was also likewise retarded considerably by *Si* binding. The beneficial effect of *Si* was dose dependent, 150 μg/ml giving complete protection for 2.0 mg/ml BSA.

3.2 Optimum pH of *Si*-BSA Interaction

The extent of binding was very low at highly acidic and alkaline pH values, as tested for optimim pH in veronal, buffer, the optimum being in the neutral range pH (6.8).

3.3 Time Course of the *Si*-BSA Interaction

Si binding increased with time more or less linearly, reaching maximum at 150 min. Further incubation at 37°C did not cause any change.

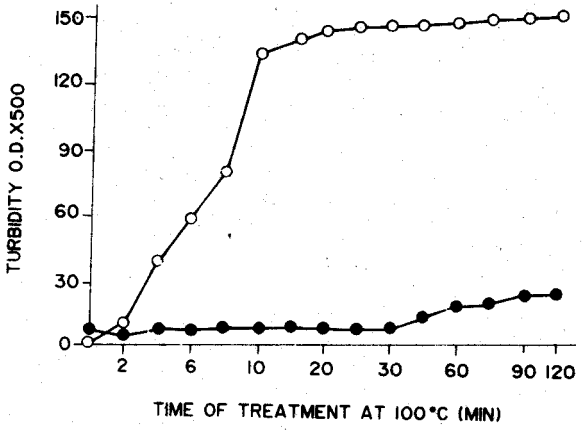

Figure 1. Effect of Si on heat denaturation of BSA. pH 7.4, Si-BSA (-●-), BSA (-o-).

3.4 Temperature Optimum of *Si*-BSA Interaction

The magnitude of silicon binding by BSA increased slowly with enhancing temperature of incubation till 20°C and then more rapidly. The optimum temperature was 43°C beyond which there was a decline. At 60°C, the activity was only 53 per cent of the optimum, while at 37°C it was over 90 per cent.

3.5 Fluorescence Studies on BSA-*Si* Complex

There was no deviation in the fluorescence spectrum of the BSA-*Si* complex as compared to native BSA. The peak of both was at 340 mμ (Fig.2). Effect of *Si* on

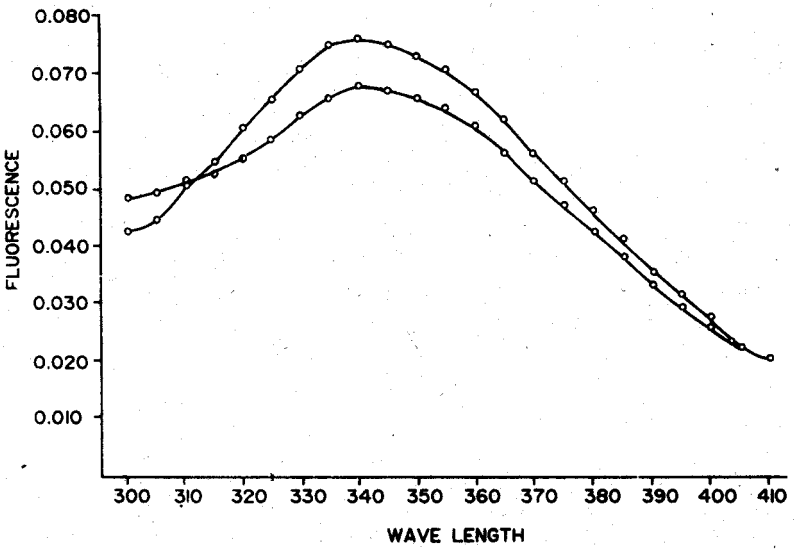

Figure 2. Fluorescence spectrum of BSA (-o-) and Si bound BSA (-●-).

the fluorescence of different concentrations of BSA does not vary much at lower concentrations, but when the concentration of BSA was not changed and silica varied, it was noticed that fluorescence decreased with an increase in *Si* concentration.

3.6 Binding of Silicic Acid by Lung and Serum Proteins

The elution profile of protein along with silica and carbohydrate contents were followed after applying 5 ml of lung post-mitochondrial fraction (15 mg protein) and serum (20 mg protein). Several protein peaks, some of them containing carbohydrates emerged. The protein pattern of both post-mitochondrial supernatant and serum showed only minor changes in the experimental (Figs. 3 and 4) as compared to the

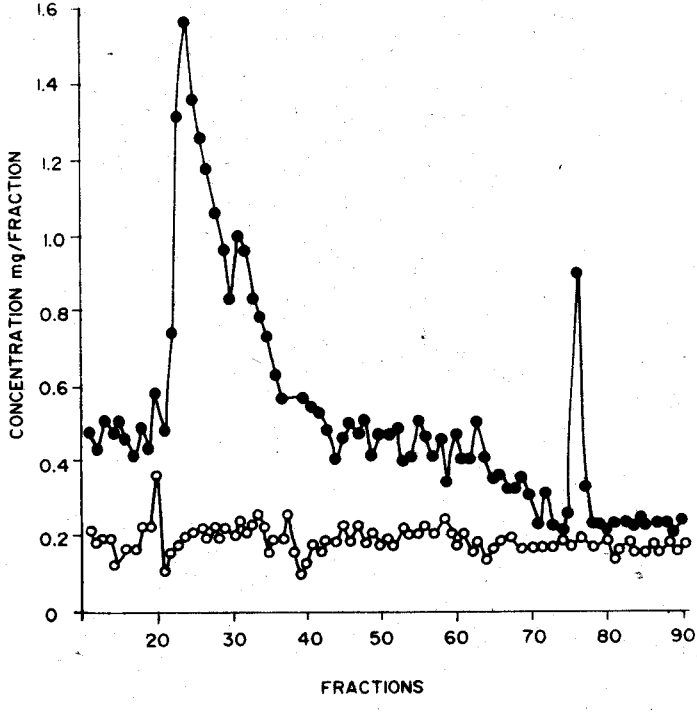

Figure 3. Sephadex G-75 column chromatographic profile of protein (-●-) and carbohydrate (-o-) of ammonium sulphate fractionated preparation of post–mitochondrial supernatant of quartz dust exposed lung.

respective controls. The major silica fractions in PMF, (Fig.5) were numbers 79 and 88 containing 0.22 mg protein and 0.1 mg *Si*. Similarly, for serum, the major silica fractions were numbers 63, 75 and 86 containing 0.2-0.4 mg protein and 0.13-0.14 mg *Si*. The *Si* containing fractions also showed the presence of carbohydrate.

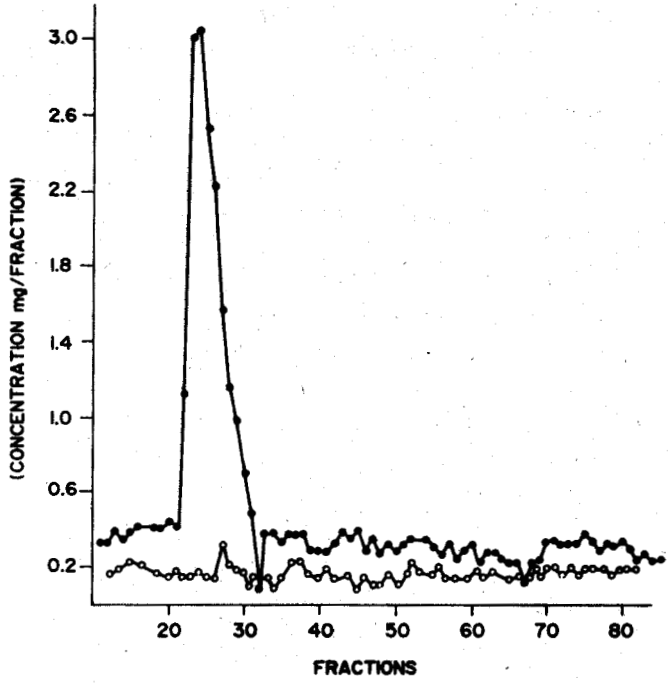

Figure 4. Sephadex G-75 column chromatographic profile for protein (-●-) and carbohydrate (-o-) of serum of Si exposed albino rat.

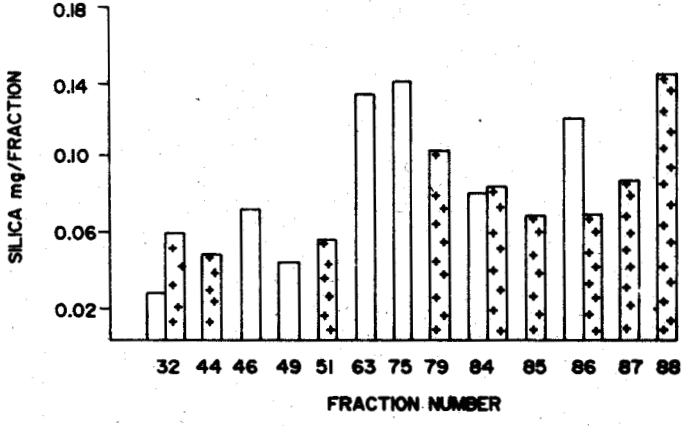

Figure 5. Sephadex G-75 column chromatographic profile for Si concentration of serum □ and ammonium sulphate fractionated preparation of post-mitochondrial supernatant ⊞ of quartz dust exposed rat lung. Only the fractions containing Si have been considered in the graph for comparison.

3.7 Distribution of Silica in Serum and Lung Post-mitochondrial Fractions

Results of *in vitro* studies for protein bound silica in the blood and lung of animals exposed to quartz dust indicated that *Si* could get bound to serum proteins. It was of interest to see that such a phenomenon took place *in vivo* too, in animals exposed to silica containing dust. Silica in serum and lung cytosol protein from quartz treated

and normal lung is given in Table 1. Significant amount of *Si* was found in the experimental animals both in serum and lung post-mitochondrial supernatant as

Table 1. Distribution of *Si* in blood serum and lung post-mitochondrial fraction of individual experimental animals

Si in serum (μg/ml)	*Si* in post-mitochondrial fraction (μg/g fresh weight)
267.72	23.15
299.36	17.60
245.12	26.30
274.35	20.74

In none of the control animals *Si* could be detected.

compared to control animals. Thus, the silicic acid could get dissolved in body fluids from the dust and get bound to proteins. It is also likely that protein binding is one of the factors involved in the clearance of dust from the lungs.

3.8 Effect of Silicon Binding on the Tryptic Activity against Albumin

The data recorded in the Tables 2 and 3 show that when BSA was replaced by a same amount of BSA-*Si* complex, the activity was reduced by about 30 per cent.

Table 2. Activity of trypsin on BSA and BSA-*Si* complex

Incubation mixture	Activity in trypsin (units/mg protein)
BSA + Trypsin	12.50×10^2
* BSA-*Si* + Trypsin	8.65×10^2
BSA + Trypsin-*Si*	8.41×10^2
* BSA-*Si* + Trypsin-Si	7.93×10^2

The experiment was repeated 3 times and representative data expressed here.

* BSA-*Si* : – BSA dialysed against silicic acid till the equilibrium was achieved.

** Trypsin-*Si* : – Trypsin dialysed against silicic acid till the equilibrium was achieved.

(0.124 g atom Si bound to 1 g mole of BSA was obtained after dialysis).

Table 3. *Si* content in BSA and trypsin after equilibrium dialysis

μg/mg protein	g atom Si/g mole
BSA 260	0.124
Trypsin 268	0.411

Under this condition, 0.124 g atom *Si* was found to bind with 1 mole BSA. When trypsin was subjected to dialysis against *Si,* 0.411 g atoms got bound to the enzyme protein. Trypsin + *Si* when used instead of native trypsin, the activity against trypsin alone showed only negligible effect. When both the substrate and enzyme contained bound *Si,* the activity was further reduced by 3 per cent as compared to both pure trypsin and pure BSA. Thus, the binding with *Si* reduced the efficacy of albumin to serve as a substrate for tryptic activity. Form this, it follows that di-basic amino acids of BSA are involved in the binding of silica. Since *Si* is bound to trypsin also, it is likely that other proteins can also bind with silica. Trypsin containing silica showed distinctly lower catalytic activity against native BSA. The amino acids involved in the active centre of trypsin are likely to be influenced by *Si* binding. Further, when both the substrate and enzyme contained *Si,* activity was even lower, clearly indicating that binding of *Si* with substrate or enzyme proteins can adversely effect the biological activity.

4. DISCUSSION

The above observations regarding protein silicon interaction along with earlier work on the role of silicic acid in the toxic effects of silicon containing dusts, Misra[30,31] *et al.* assume biochemical significance in the understanding of the environmental biochemistry of silicon. Regarding the mechanism of action of silicic acid on collagen fomation, Cagliotti[32] *et al.* suggested that collagen fibres could be made *in vitro* by interacting gelatin solution with orthosilicic acid, but not with poymerised silicic acid. Holt[24] considered that polymerisation could take place on collagen, if pH is around 6.5 and if OH^- groups of *Si* not linked to collagen are free and if local concentration of *Si* is not high. Knake and Peter[33] on the basis of *Si* studies[31] on rats concluded that orientation of collagen precursor into collagen fibres takes place after *Si* adsorption and polymerization leading to stabilization. *Si*-protein interaction during early dust toxicity is indicated in the present study. Binding of *Si* could also be involved in the inhibition of enzymes[34] by silicic acid and its transport across membranes.[9] Binding with proteins could also be involved in the effects of silicon compounds in cosmetic medicine and on the toxic effects of organo silicon compounds. Besides being a pathogenic mechanism for silicosis and silicatosis, protein *Si* binding may also be involved in the essential role of *Si* as a micro-nutrient for some mammals, for collagen formation in normal[13] and abnormal conditions,[35] in diatom physiology[8,9] and plant nutrition.[10] Decrease in *Si* of blood vessels on ageing and in cardiovascular disease vis-a-vis its nutritional status[15,36] also assumes significance. Therefore, the possible existence of "receptor" molecules for silicon may be of significance evolutionary biochemistry, environmental physiology and occupational diseases. A role for *Si* compounds in origin of life, biochemical evolution of species and exobiology also is a possibility.[37] The localization of *Si* receptor sites in lungs, especially with respect to membrane damage and collagenesis as indicated in this work awaits further study.

Si binding by BSA follows stoichiometric principles indicating true chemical reaction in terms of effects of pH, temperature and period of incubation. The binding is firm being able to withstand trichloroacetic acid and organic solvents and dialysis as well as it is not dissociated on ion exchange columns or molecular seiving. It is

also stable at room temperature during the gel filtration run. However, the alterations in solubility of albumin as evident from the salting out and Cohn fractionation profile along with the altered electrophoresis and anion exchange and cation exchange profile (unpublished results) suggest chemical binding through ionic groups. Further, albumin is protected from thermal denaturation by silicic acid which presumably acts in a manner similar to N-acetyl tryptophan. The involvement of tryptophan residue also is likely from the decrease in fluorescence without altering the spectrum. It is also interesting that binding of *Si*, reduces the ability of BSA to function as a substrate of trypsin action indicating that the structure-activity relation requisite for the enzyme, mainly the vicinity of basic amino acids are affected by the binding. This also explains our observation that isolated BSA-*Si* complex is dissociated only on prolonged tryptic digestion as compared to native BSA. Further, the capacity to bind *Si* was shown by trypsin molecule also, so that proteins other than albumin could also interact with *Si*.

The present results indicate the existence of *Si* binding protein in lung and serum. Besides clearance through lymphatic system and coughing, silicon containing dusts could dissolve in lung fluids, bind with lung proteins and get transported, and eventually dissociated and eliminated in urine. It may be pointed out that *Si* is a normal constituent of urine in persons exposed through *Si* as dust or as food component.[38] The universal distribution of *Si* in different tissues,[39] silicon calculi in farm animals[40] and phytolith formation in certain plants[41] also suggest possible translocation of *Si* in living systems. Further post-mortem of a fatal case of renal failure and proteinurea in a sand blaster revealed severe damage to the sialoproteins of the glomerular capillary epithelial cells with high concentration of non-particulate silicon.[42] How far protein bound *Si* is an index to exposure to noxious and innocent fugitive dusts and related to the progress of the disease is another exciting possibility. It could also be worthwhile to explore whether agents capable of suppressing *Si*-protein binding are useful in reducing the toxic effects *in vitro* and *in vivo*. Thus bioinorganic chemistry of *Si*, especially interaction with proteins is an important aspect of biomedical and environmental schemes.

5. SUMMARY

In order to explore the molecular mechanisms of the effects of silicon containing dusts on biological systems, especially on the macromolecule like biotransformation enzymes, interaction between *Si* and proteins was studied in detail.

On the basis of molecular sieving profile, the presence of a protein fraction capable of binding silicic acid has been detected in rat lung. In silica dust treated animal serum also, evidence was obtained for *Si*-binding proteins. Using experiments with serum albumin, silicic acid-BSA protein interaction was characterized. The fluorescence of albumin was decreased by *Si* interaction. *Si* binding also reduced the tryptic activity on BSA, involving binding with both enzyme and substrate proteins. Evidence is presented for the chemical nature of the binding involving specific groups and the stoichiometry is established. Significant alterations in the chemical and physical properties of protein due to *Si*-binding were evident. The importance of *Si*-protein interaction in environmental biochemistry and biological effects of *Si* compounds is discussed and the bioinorganic mechanisms involved suggested.

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