disposition of the naphthalene to the surface of silicate oxygen atoms. The surface occupied by the organic cation with this disposition is close to that available by exchange cation.

Therefore it may be concluded that upon treating montmorillonite with aqueous solution of propranolol hydrochloride the propranolol-ammonium cations are adsorbed into the interlayer space of the silicate, giving rise to the formation of a definite complex of 17·31 Å basal spacing corresponding to the intercalation of a monomolecular cation layer. The formation of this complex is independent of the pH of the solution, within a pH margin of 3·0–8·0, but does depend on the concentration of the solution. The adsorption mechanism is one of cation exchange and the maximum amount adsorbed is 78 mequiv/100 g.

These results are considered a valid base for the initiation of desorption studies in vitro and, later in vivo.

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Entrapment of proteins as disulphide cross-linked thiolated macromolecules within cross-linked dextran ("Sephadex") gels

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The entrapment of two proteins, α-chymotrypsin and bovine serum albumin (BSA), within cross-linked dextran ("Sephadex") gels as their disulphide cross-linked thiolated macromolecules is described. This type of preparation could have a use in medicine as a depot for the slow release of a protein drug (Mahbouba et al 1974).

Methods and results

Thiolated BSA (10·4–13·8 SH group mol^{-1}) was prepared from crystalline albumin (Koch-Light) by the silver ion-imidazole catalysed reaction with *N*-acetyl homocysteine thiolactone (AHTL) (Mahbouba et al 1974) the reaction being complete in 10 min. Thiolated α-chymotrypsin (see below) from α-chymotrypsin (thrice recrystallized; Koch-Light) contained 3–5·5 SH groups mol^{-1}.

The molecular size of the macromolecules formed by ferricyanide oxidation (see below) of thiolated BSA (1·4–13 thiol groups mol^{-1}) was largely independent of the thiol titre and dependent on the concentration of thiolated BSA present in contrast to cross-linking of other thiolated proteins (Mahbouba & Smith 1977). Low concentrations (0·36–0·43% w/v) with 1·35–9·5 thiol groups mol^{-1} gave n (aggregation number) = 1·0–2·0; intermediate concentrations (0·9–1·4) with 3–10·8 thiol groups mol^{-1} gave n = 1·1–5·8; high concentrations (1·8–3·1 (limiting solubility)) with 4·8–12·8 thiol groups mol^{-1} gave n = 2·2–11·1.

Cross-linked dextran (Sephadex G-100, Pharmacia; 0·5 g dry weight) was soaked with a solution of phosphate buffer (3·5 ml, 0·1 M) pH 8·0 containing thiolated α-chymotrypsin (25–100 mg) for 3 days at 4 °C. Potassium ferricyanide solution (1·8%, 1 ml diluted with water to the volume required for complete swelling of the gel) was then added and after storage (4 °C) for a further day to ensure oxidation of the thiolated α-chymotrypsin and complete swelling of the gel, the mixture was placed in a column (30 × 1·5 cm i.d.) and the excess reagent and the untrapped disulphide cross-linked thiolated protein washed out. The protein fraction was separated from the reagent using a column (45 × 2·5 cm i.d.) of cross-linked dextran (G-25) in phosphate buffer (0·1 M) pH 8·0, the concentration of the protein fraction determined (Lowry et al 1951) and the approximate amount of protein entrapped in the gel (33–40%; Table 1) calculated from the difference between the initial amount used and the excess protein washed out from the column. The molecular size of the untrapped macromolecule (n = 10–11) was measured by light-scattering (Mahbouba et al 1974) to indicate the approximate size

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Table 1. Entrapment of disulphide cross-linked thiolated proteins within cross-linked dextran gels.

Thiolated protein	Gel grade (0·5 g dry weight)	Wt. of thiolated protein (mg)	Thiol group, mol^{-1}	Initial volume for soaking (ml)	Entrapment yield (%)	Aggregation (n)
α-Chymotrypsin	G-100	25	5·5	3·0	32	
		50	4·3	3·0	40	
		60	3·5	3·0	30	
		60	4·0	3·0	42	
		60	3·8	3·0	33	
		60	3·9	3·0	33	
		60	5·5	3·0	42	
		66	4·3	3·0	38	
		98	3·0	3·0	38	
		100	5·5	3·0	40	
BSA	G-75	25	‡	5·0	28	7·0
		25		3·5	16	14·5
		25		6·5	20	5·0
	G-100	25		8·0	8	6·2
		25		4·5	20	10·5
		50		8·0	7	5·1
		60		3·5	33	46·6
		60		3·5	25	72·8†
	G-200	25		12·5	12	6·8

† potassium ferricyanide added without dilution.
‡ within the range 10–12 SH group mol^{-1}.

of the macromolecules inside the gel. Similar yields were obtained using a partially pre-swollen gel.

Thiolated BSA was similarly entrapped in cross-linked dextran gels ("Sephadex" G-75, G-100 and G-200) to the extent of 7–33% (Table 1) of the initial protein present.

Microscopic examination of both of the disulphide cross-linked thiolated protein-dextran conjugates stained with Coomassie Blue-acetic acid showed that most of the spherical beads contained aggregates of stained protein although a few were coloured throughout, suggesting that the macromolecules had been precipitated or covalently bound to the dextran structure. Attempts to release the immobilized protein from the BSA-dextran conjugate by either the action of dextranase (E.C. 3.2.1.11) (Janson & Porath 1966), or ultrasonic disintegration (Elpiner 1964), both of which destroyed the bead structure, were unsuccessful.

However, addition of α-chymotrypsin (2 mg, 4×10^{-4} M) to BSA-conjugate (520 mg) in phosphate buffer (20 ml, 0·1 M) pH 8 with incubation at 37 °C for 1 h followed by separation of the unbound protein in the usual manner gave a water soluble digest containing 68% of the protein content of the gel. Furthermore, addition of dithiothreitol (DTT; 15·4 mg, 5×10^{-3} M) to the conjugate (260 mg) in phosphate buffer (20 ml), followed by incubation at 20 °C for 1 h, released 30–40% of the immobilized protein.

Discussion

Thiolated α-chymotrypsin and thiolated BSA have been incorporated in cross-linked dextran beads as the disulphide cross-linked thiolated protein macromolecule, to the extent of 33–40% and 7–33% respectively of the initial protein titre. Microscopic examination of both conjugates followed by degradative experiments on the BSA-conjugate suggested that the macromolecules were covalently bound to the cross-linked dextran, a process which could initially occur due to grafting of the thiolated protein to radical sites (Imoto et al 1965) on the dextran formed by the action of the ferricyanide (Kolthoff & Meehan 1953), followed by disulphide coupling with other thiolated protein molecules to produce the aggregate. α-chymotrypsin and DTT, at high concentration, were capable of reacting with the BSA-conjugates' protein indicating that the physical form of the BSA-conjugate permitted covalent bond exchange reactions.

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