MACROMOLECULE-SMALL MOLECULE INTERACTIONS: A SYNTHETIC POLYMER WITH GREATER AFFINITY THAN SERUM ALBUMIN FOR SMALL MOLECULES

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The binding of small molecules and ions by serum albumin has been of interest for many years not only because of its physiological significance but also as a model of biomacromolecular interactions with substrates and modifiers. Molecularly-oriented investigations (Klotz et al., 1946; Klotz, 1949) early disclosed the number of small molecules bound by serum albumin under different conditions and the energetic quantities accompanying these interactions. Most puzzling, however, was the uniqueness of serum albumin, among soluble proteins, in possessing a varying number of binding sites for small molecules of a given species and in having an affinity for substrates of widely-different structure.

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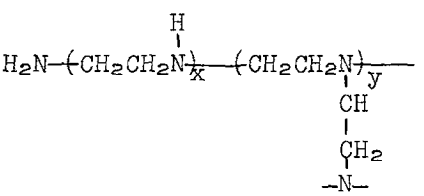
The variation in number of sites with quantity of small molecule could be "due to changes induced in the protein" (Klotz, 1949) by the bound molecule. Similarly the affinity for small molecules with far different structures could be accounted for by attributing "conformational adaptability" (Karush, 1950) to serum albumin.

Both of these suggestions ascribe the special binding properties possessed by serum albumin to a degree of flexibility in this macromolecule. Thus one might expect flexible, water-soluble synthetic polymers with suitable side-chains to show strong affinities for small molecules. In the course of twenty years we have examined the binding ability of polyvinylpyrrolidone, polyvinylpyridine, polylysine, polyacrylamide, polyisopropylacrylamide, polyvinylimidazole, polyvinylmethyloxazolidinone, poly-(vinylmethyloxazolidinone-vinylimidazole), poly(vinylpyrrolidone-vinylimidazole), poly(vinylpyrrolidone-vinylalcohol), poly(vinylpyrrolidone-maleic anhydride), poly-(vinylmethyloxazolidinone-maleic anhydride), and poly(2-dimethylaminoethylmethacrylate-methacrylic acid). Other investigators have also studied similar synthetic polymers (Bennhold and Schubert, 1943; Wunderly, 1950; Strauss and Jackson, 1951; Scholtan, 1953; Saito, 1957; Klotz and Stryker, 1960; Molyneux and Frank, 1961). In our experience no water-soluble polymer binds small molecules with an avidity comparable to serum albumin.

These polymers have high intrinsic viscosities which indicate that the macromolecules are swollen and extended in water. In contrast serum albumin, with an intrinsic-viscosity near 4 (ml/gm) must be relatively compact even

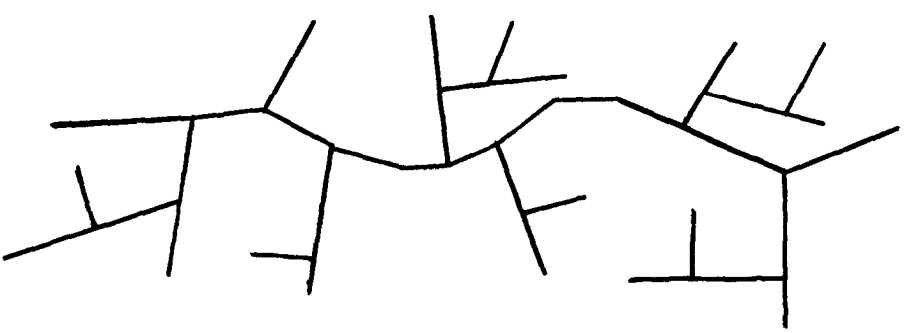
if flexible. A promising approach, therefore, would be to introduce cross-linkages in synthetic polymers to hold the macromolecule in a more compact conformation. Some experiments in this direction are in progress. An alternative approach, from an inverted viewpoint, however, has already produced some striking results.

An interesting polymer constrained to a relatively compact conformation is polyethyleneimine (PEI), which can be prepared by suitable polymerization of ethyleneimine to give a highly-branched rather than a linear macromolecule.[2] A segment of this polymer may be written as



(I)

Approximately 25% of the nitrogens are primary amines, 50% secondary and 25% tertiary.[2] The branching of the polymer may be represented as



(II)

[2]See Dow Chemical Co. bulletin "Montrek® Polyethyleneimine."

Since this polymer contains a high proportion of primary $-NH_2$ groups it is a simple matter to introduce a variety of side chains by forming an amide linkage with an added RCOOH or activated RCOX reagent. Studies of serum albumin-small molecule complexes (Klotz et al., 1946; Klotz, 1949; Karush, 1950) have indicated that apolar interactions play an important role in binding. Therefore, a sample of PEI-6,[2] (average degree of polymerization about 15) with approximately 10% of its residues containing lauroyl side-chains, was examined for binding ability.

Since PEI-6 is too small for equilibrium dialysis measurements, an alternative approach was used. For rapid quantitative measurements, as well as to set aside charge considerations, the binding of a neutral molecule, p-dimethylaminoazobenzene, was measured by increases in its solubility. Table I summarizes some results. It is obvious that lauroylpolyethyleneimine binds molecules more effectively than does serum albumin. Polyethyleneimine (PEI-6) without lauroyl side chains, however, produces essentially no change in solubility of dimethylaminoazobenzene.

PEI-6 has a very low average molecular weight, about 600. Much larger molecular weight polymers are available[2] and these may be even more effective in binding when suitably acylated. Furthermore a wide variety of side chains may be attached to the polyamine backbone. These could even include optically active ones, which should provide a basis for stereochemical specificity in binding.

It has also not escaped our notice that these branched acyl polyethyleneimines, containing apolar binding sites and nucleophilic acid-base groups, provide an environment

TABLE I

Comparison of
Binding of Dimethylaminoazobenzene by Bovine Serum Albumin
and Lauroylpolyethyleneimine at 20°

Macromolecule	Concentration (%)	pH	Buffer Salt	Moles Bound Dye[a] 10^5 g Macromolecule
Lauroylpolyethyleneimine-10[b]	1.0	9.1	Na_2HPO_4	1.1
"	0.5	9.1	Na_2HPO_4	1.0
"	0.1	9.1	Na_2HPO_4	0.9
"	0.5	9.0	(Cl^-)[c]	0.8
"	0.1	8.8	(Cl^-)[c]	0.7
"	0.07	8.8	(Cl^-)[c]	0.6
"	1.0	7.3	HEPES[d]	0.58
"	0.5	7.3	HEPES[d]	0.48
"	0.2	7.3	HEPES[d]	0.33
Bovine serum albumin	1.0	7.3	HEPES[d]	0.21
"	0.5	7.3	HEPES[d]	0.22
"	0.2	7.3	HEPES[d]	0.23
Lauroylpolyethyleneimine-10[b]	1.0	7.3	(Cl^-)[c]	0.61
"	0.5	7.3	(Cl^-)[c]	0.48
"	0.2	7.3	(Cl^-)[c]	0.43
Lauroylpolyethyleneimine-20[e]	1.0	7.3	(Cl^-)[c]	1.6
"	0.5	7.3	(Cl^-)[c]	1.5
"	0.1	7.3	(Cl^-)[c]	1.4
Bovine serum albumin	1.0	7.3	(Na^+)[c]	0.22
"	0.5	7.3	(Na^+)[c]	0.24
"	0.2	7.3	(Na^+)[c]	0.28

[a] Calculated from increase in absorbance at 413 mμ of dissolved dye and an assumed value of 25,000 for its extinction coefficient.

[b] Lauroyl groups on 10% of residues. In bovine serum albumin approximately 40% of residues are apolar, but not as extended as lauroyl.

[c] No buffer present other than polymer itself. pH adjusted with HCl or NaOH.

[d] N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonate.

[e] Lauroyl groups on 20% of residues.

which should affect a number of biochemically-interesting reactions.

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