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SELF-ASSEMBLY OF STREPTAVIDIN/BISBIOTIN MONOLAYERS AND MULTILAYERS

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Abstract A range of different bifunctional bisbiotin ligands have been synthesised and their ability to form polymers with the proteins avidin and streptavidin investigated. Polymers were characterised by gel chromatography and transmission electron microscopy.

INTRODUCTION

The goal of fabricating electronic circuits on a molecular scale depends on the development of techniques for assembling well-ordered molecular arrays and small molecular aggregates. In addition to the more conventional approaches based on LB deposition and chemisorption, we are investigating the use of affinity polymerisation as a self-assembling technique. The method utilises the strong affinity of some proteins for their complementary ligand and in a number of recent publications[1,2] we have reported on the formation of linear polymers as well as the assembly of built-up multilayers (Figure 1) using a bisbiotin ligand to bind molecules of streptavidin (and avidin). Structures of the type shown in Figure 1(b) are of particular interest since electrically and/or optically active moieties can be incorporated into the bisbiotin ligand. However, the introduction of these additional moieties may adversely affect ligand/protein binding.

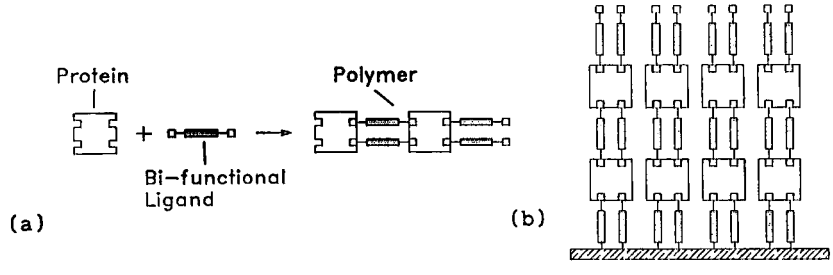


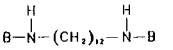
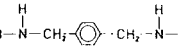
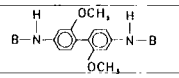
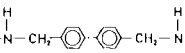
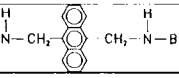
FIGURE 1 Diagrammatic representation of a polymer chain (a) and multilayer (b).

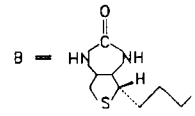
Accordingly, we have begun a systematic investigation of the influence of a range of different incorporated functional groups on the ability of the bisbiotin ligand to undergo affinity polymerisation with streptavidin and avidin.

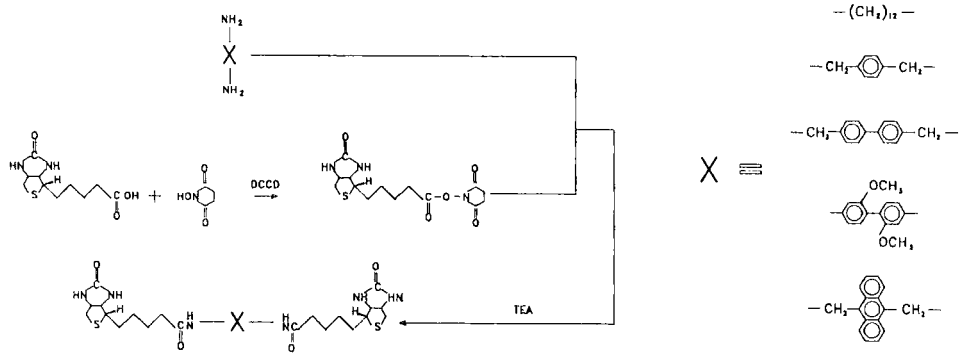
EXPERIMENTAL

The bisbiotin ligands shown in Table 1 were synthesised following Scheme 1. Biotinyl-N-succinimide ester (BNHS) was prepared by coupling *d*-biotin with N-hydroxysuccinimide using dicyclohexylcarbodiimide (DCCD) according to Wilchek and Bayer[3]. Reaction of BNHS (2 equiv.) with a previously synthesised diamine (1 equiv.) in DMF containing triethylamine (TEA) afforded a crystalline product after evaporation of DMF, work-up and recrystallisation.

TABLE 1 Ligands synthesised in this work

	Ligand (B-X-B)	m.p.
1,12-bisbiotindiamidododecane (DDBB)		243-245°C
1,4-bis(biotinamidomethyl)benzene (BBB)		257-258°C
2,2'-dimethoxy-4,4'-bis(biotinamido) biphenyl (DMBB)		262-265°C
4,4'-bis(biotinamidomethyl) biphenyl (BPBB)		255-260°C
9,10-bis(biotinamidomethyl) anthracene (ABB)		>300°C





SCHEME 1

Protein-ligand (1:1) polymers were formed in solution by titrating an aliquot of the bisbiotin ligand dissolved in DMF (0.1mg/ml) with protein dissolved in 100mM Tris (pH 8.0). The DMF concentration in the final solution was always less than 10% v/v.

Initial attempts to quantify the binding of bisbiotin ligands to both avidin and streptavidin were made using the dye titration assay reported by Green *et al*[4]. The method relies on the displacement of the dye 2-(4'-hydroxyazobenzene)-benzoic acid (HABA) from the protein binding site by biotin ligands. The resultant decrease in the 500 nm absorption band of the dye-protein complex should provide a direct measure of the biotin binding stoichiometry. However, we have found that owing to competitive binding between HABA and DMF confirmatory evidence for affinity polymerisation is required from other techniques such as gel filtration chromatography and transmission electron microscopy (TEM).

Chromatography was carried out with Sephadex G-100 columns (1 cm x 20 cm) run at room temperature, fractions being collected by hand. The exclusion limit of the column was approximately 100,000 Da. An aliquot of the separated polymer fraction was then applied to a prepared TEM grid[5], stained and viewed in a Philips E301G TEM.

RESULTS AND DISCUSSION

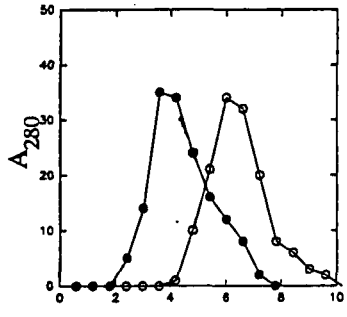
Comparison of the gel filtration profile of streptavidin with that of a 1:1 molar mixture of streptavidin and biphenylbisbiotin (BPBB) in Figure 2a shows clearly that the protein has polymerised. This should be contrasted with Figure 2b which shows that anthracene bisbiotin (ABB) is unable to polymerise the protein (avidin in this case), the "polymer" eluting in the same fraction as the protein monomer, and benzene bisbiotin (BBB) which seems to convert about 10% of the protein to a polymer (Figure 2c). A summary of the gel filtration experiments is given in Table 2.

The amount of ABB bound to the protein can be deduced from the intensity of the characteristic absorbance for anthracene at approximately 265nm. (A red shift, *ca.* 5nm, occurs upon binding to the protein). Assuming that the extinction coefficient for the anthracene moiety remains unchanged after binding then for the ABB/avidin fraction shown in Figure 2b the results suggest that one out of four binding sites was occupied by the bisbiotin ligand.

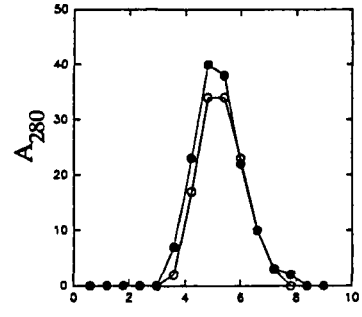
Clearly, both the length of the bisbiotin ligand and the depth of the protein binding site are important factors in polymer formation. Avidin has a deeper binding site than streptavidin and does not form polymers with BBB whereas streptavidin does. However, steric hindrance

TABLE 2 Polymer forming properties of various bisbiotin ligands in 1:1 mixtures with avidin and streptavidin

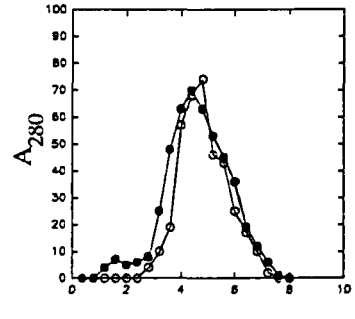
	DDBB	BBB	BPBB	DMBB	ABB
Length of ligand (nm)	3.9	2.8	3.7	3.0	2.8
Avidin	Yes	No	Yes	Yes	No
Streptavidin	No	10%	Yes	Yes	No



(a)



(b)



(c)

FIGURE 2 Gel filtration profile of streptavidin/biphenyl bisbiotin (a), avidin/anthracene bisbiotin (b) and streptavidin/benzene bisbiotin (c) (-●- polymer, -o- monomeric protein)

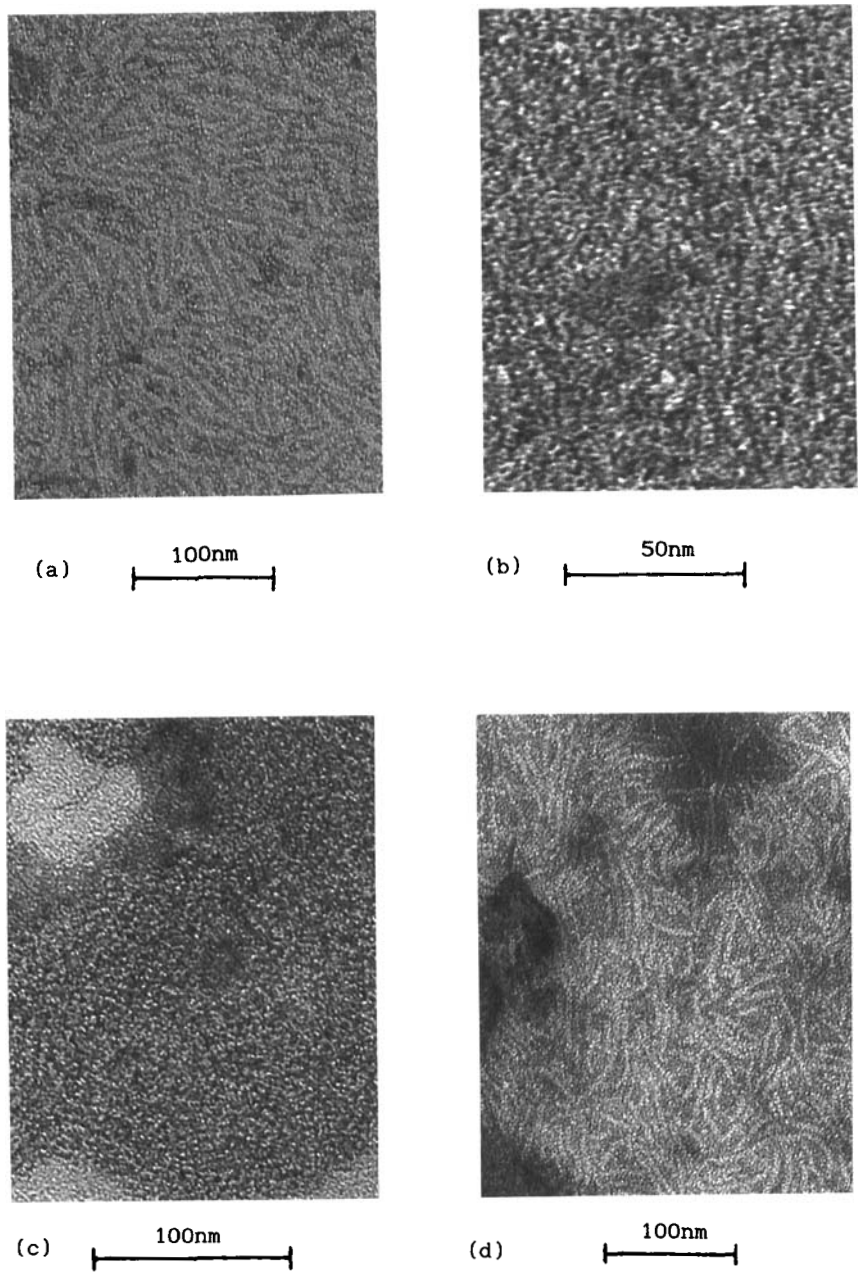


FIGURE 3 Transmission Electron Micrographs of (a) Streptavidin/BBB, (b) Avidin/DMBB, (c) Avidin/BPBB and (d) Avidin/DDBB

may also be a governing factor in polymer formation since partial polymerisation occurs with BBB but not with ABB.

Transmission electron micrographs of some of the polymers is shown in Figure 3. It can clearly be seen that the short ligand containing aromatic residues produce short straight polymers but polymers made from the dodecane ligand are more flexible owing to the flexibility of the ligand.

CONCLUSION

A range of bisbiotin ligands have been synthesised and used to form polymers with streptavidin and avidin. Quantitative binding of an anthracene ligand was determined spectrophotometrically. Polymers were characterised by gel filtration chromatography and transmission electron microscopy. The nature of the bifunctional ligand was found to be important in determining the final polymer properties.

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