An artificial crystalline DDT-binding polypeptide

Rudolf Moser, Richard M. Thomas and Bernd Gutte

Biochemisches Institut der Universität Zürich, Zürichbergstrasse 4, CH-8028 Zürich, Switzerland

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A hydrophobic 24-residue polypeptide that could potentially form a four-stranded antiparallel β-pleated sheet and bind the insecticide DDT was designed and synthesized. The synthetic peptide aggregated in 1 M acetic acid but was monomeric in aqueous 50% ethanol. In the latter solvent the 24-residue polypeptide and DDT formed a complex with an apparent dissociation constant of $\sim 2 \times 10^{-5}$ M. The DDT binding of an analogue of this peptide possessing the same amino acid residues in a random sequence was more than 2 orders of magnitude and that of bovine serum albumin at least 3 orders of magnitude weaker. The designed polypeptide could be crystallized.

Protein secondary structure prediction *Model building* *Protein design*
Synthetic DDT-binding polypeptide *Crystallization, of artificial polypeptide*

1. INTRODUCTION

The design of several small biologically active polypeptides has been reported recently [1–3]. These artificial polypeptides had minimal or no homology with their natural counterparts. A different approach has been used to design a 63-residue analogue of ribonuclease A [4] and a cyclic hexapeptide analogue of somatostatin [5]. Both analogues represented truncated versions of their parent molecules.

Our work on the design of novel functional proteins began with the synthesis of a 34-residue polypeptide that interacted with nucleic acids [2]. The proposed secondary structure ($\beta\beta\alpha$) of this peptide was partly confirmed by circular dichroism (CD) measurements ([6] and unpublished results). A few crystals were obtained but their quality was poor. It seemed that a polypeptide of 20–30 residues possessing either pure α-helical or pure β-pleated sheet structure might have a more stable conformation and might be easier to crystallize.

In the present work, a DDT-binding polypeptide was designed (fig.1).

Abbreviation: DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane

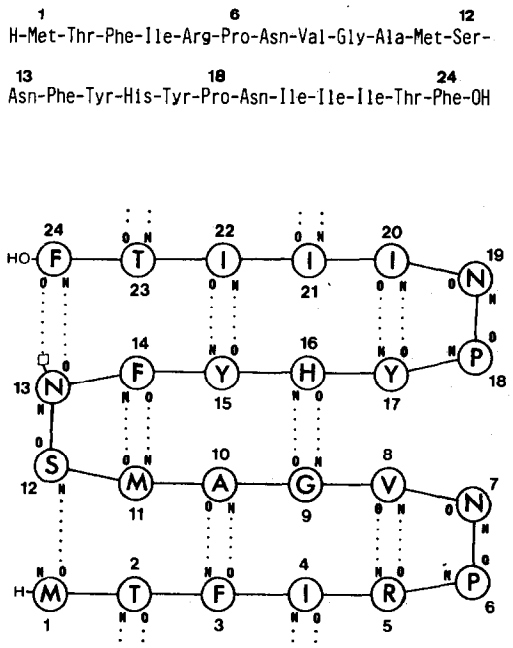

Fig.1. Sequence and proposed secondary structure of the artificial DDT-binding 24-residue polypeptide. Dotted lines indicate hydrogen bonds between NH and CO groups of the backbone and side chains.

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2. EXPERIMENTAL

The designed 24-residue polypeptide was synthesized by the solid phase method [7,8] as in [9]. The HF-cleaved product (88.5 mg) was chromatographed on a Sephadex G-50 column (92 × 2.2 cm) in 1 M CH_3COOH (fig.2A). Only the high relative molecular mass fraction which eluted between 107 and 127 ml had the expected amino acid composition and was obtained in 29% yield (25.6 mg). An aliquot (1.4 mg) of this fraction was applied to a Sephadex LH-60 column (88 × 1.1 cm) and eluted with ethanol/0.05 M NH_4HCO_3 (6:5, v/v) (fig.2B). A semilogarithmic plot of the relative molecular mass against the elution volume showed that the 24-residue polypeptide was monomeric in this solvent.

Needle-shaped crystals were obtained from a solution of the synthetic polypeptide and DDT at a molar ratio of 2:1 in ethanol/0.1 M CH_3COOH (1:1, v/v) (fig.3). The transmission spectrum recorded between 240 and 320 nm in a Universal Microspectrophotometer I unambiguously identified the crystalline material as protein. At present it is not known whether the crystals also contain DDT.

DDT binding was demonstrated by equilibrium dialysis and by gel filtration of the designed 24-residue polypeptide (1.6 mg) on a Sephadex LH-60 column (83 × 1.1 cm) equilibrated with ethanol/0.05 M NH_4HCO_3 (6:5, v/v) containing 0.1 mM DDT [10] (fig.4). There was an increase in the absorbance of the protein fraction at 278 and 237 nm caused by bound DDT. The ratio of the absorbances of the 24-residue polypeptide at 237 and 278 nm was 2.25.

3. RESULTS AND DISCUSSION

3.1. *Design of the DDT-binding 24-residue polypeptide*

DDT is a powerful insecticide which, because of its potential hazards [11], has been banned in many countries. It is a relatively rigid molecule and is insoluble in aqueous buffers. In order to allow interaction with this lipophilic molecule, the 'receptor' must have hydrophobic binding sites. Model building showed that a 24-residue β-sheet consisting of 4 antiparallel strands was large enough to provide a binding locus on each side of the sheet. The amino acid sequence that could produce the proposed structure and a sufficient number of hydrophobic and van der Waals' bonds with the ligand was chosen using the prediction methods of Chou and Fasman [12] and Levitt [13]. Fig.1 shows sequence and proposed secondary structure of the synthetic 24-residue polypeptide. A DDT binding site of high complementarity may be formed by Phe 14, His 16, Ile 21, Met 11, and Ile 4. On the opposite side of the sheet Tyr 15, Tyr 17, Ile 20, Ile 22, Val 8, Arg 5, and Phe 3 may constitute a second but less-favourable binding site. As DDT cannot form hydrogen bonds, the main contributions to the binding of this ligand may come from stacking interactions of the aromatic rings and from van der Waals' contacts.

3.2. *Characterization of the synthetic 24-residue polypeptide*

The amino acid composition of the high-M_r fraction eluting from Sephadex G-50 between 107 and 127 ml (fig.2A) agreed closely with the values expected (48-h hydrolysis): Asp 3.1 (3); Thr 1.8 (2);

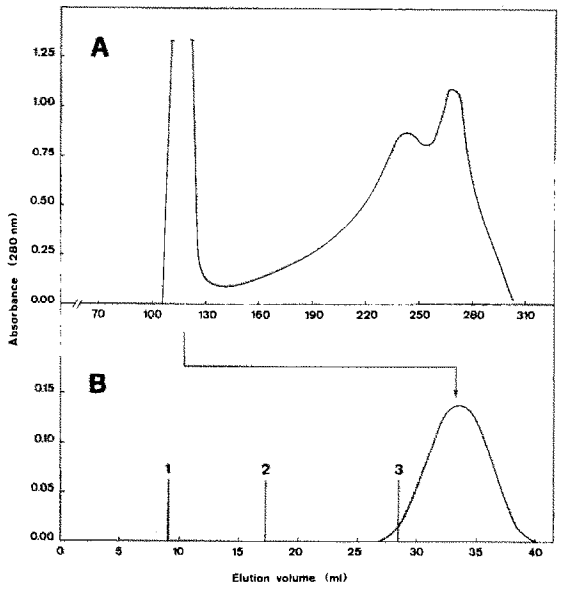

Fig.2. Gel filtration of the synthetic DDT-binding 24-residue polypeptide on Sephadex G-50 in 1 M CH_3COOH (A) and on Sephadex LH-60 in ethanol/0.05 M NH_4HCO_3 (6:5, v/v) (B). Vertical lines marked 1, 2 and 3 indicate the elution volume of bovine serum albumin, ribonuclease A and glucagon.

Ser 0.9 (1); Pro 2.0 (2); Gly 1.0 (1); Ala 1.0 (1); Val 0.9 (1); Met 2.0 (2); Ile 4.1 (4); Tyr 1.7 (2); Phe 3.0 (3); His 0.9 (1); Arg 0.9 (1). The low-M_r fractions were apparently lacking in 1 isoleucine and 1 methionine residue. It was concluded that only material with the correct amino acid composition was capable of forming aggregates that were excluded from both Sephadex G-50 and G-150. The aggregated material was completely converted to the monomeric form in 50% aqueous ethanol as demonstrated by column chromatography (fig.2B) and sedimentation equilibrium measurements in the ultracentrifuge. The N-terminus of this monomer was shown by dansylation to be exclusively methionine. The product was also homogeneous on thin-layer chromatography. It could be crystallized (fig.3) and thus seems to be the first artificial polypeptide obtained in crystalline form.

For reasons of solubility, mixtures of ethanol and aqueous buffers had to be used to measure DDT-polypeptide interactions. As aqueous ethanol is expected to weaken or break hydrophobic bonds, only apparent dissociation constants could be determined. Using the method of Hummel and Dreyer [10], the amount of bound DDT was estimated from the absorbance increase of the protein fractions at 237 nm, a wavelength at which DDT has an absorption maximum ($\epsilon_{237}^{1\,cm}$ = 18620 M^{-1}) (fig.4). The complex of DDT with the designed 24-residue polypeptide had K_d ~2 × 10^{-5} M. This value was verified by gas chromatographic analysis of the amount of DDT contained in the protein fraction as compared with the 0.1 mM DDT background and by equilibrium dialysis measuring the retention of DDT in the dialysis bag in the presence of 24-residue polypeptide. The binding of DDT by the material that lacked 1 isoleucine and 1 methionine residue was ~260-times weaker. No interaction was found between DDT and bovine serum albumin, a carrier of hydrophobic compounds such as fatty acids. There was also no detectable interaction between the designed 24-residue peptide and 2′-CMP as the

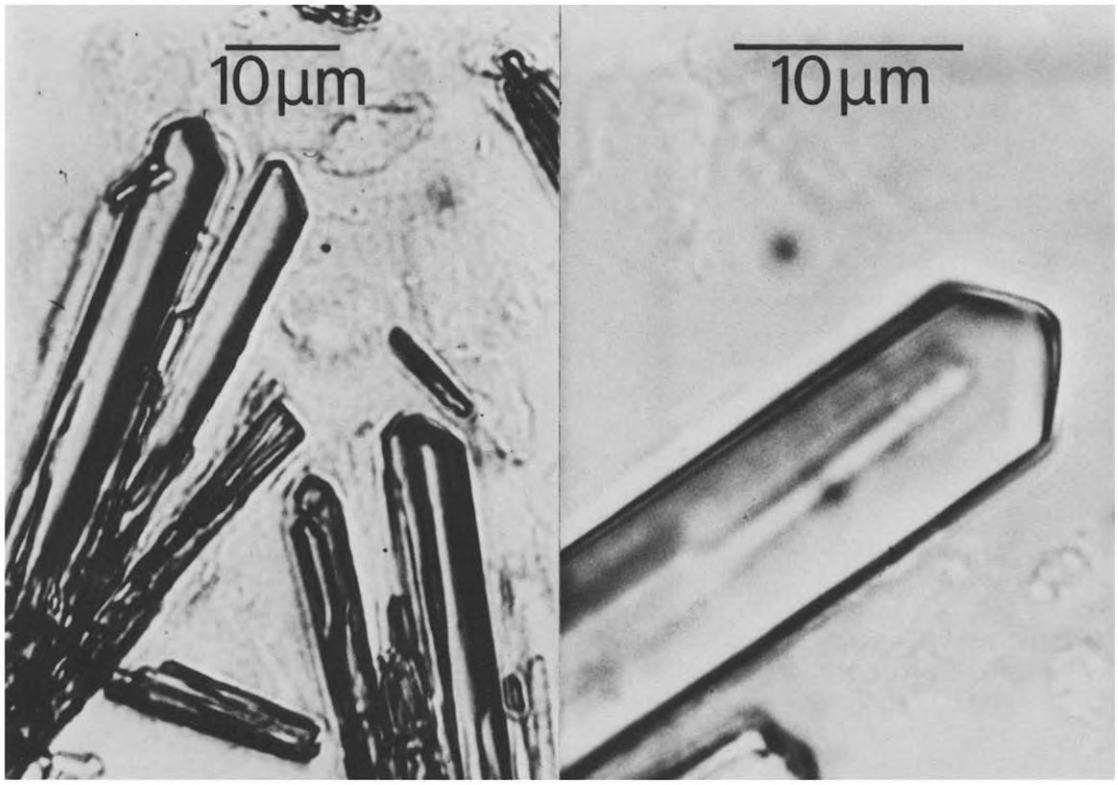

Fig.3. Crystals of the artificial DDT-binding 24-residue polypeptide.

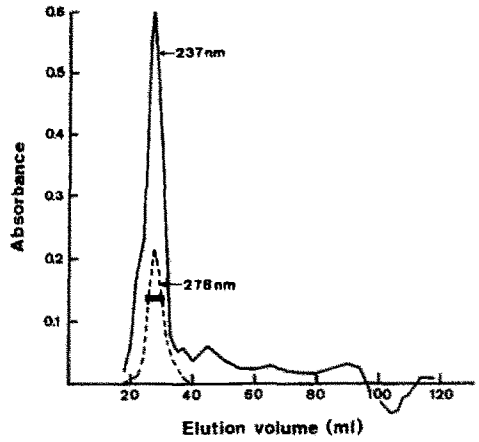

Fig.4. Gel filtration of the designed 24-residue polypeptide on Sephadex LH-60 in ethanol/0.05 M NH_4HCO_3 (6:5, v/v) containing 0.1 mM DDT. The depression in the absorbance of the 0.1 mM DDT background at 237 nm between 95 and 113 ml corresponds to the amount of DDT bound by the peptide.

nucleotide is probably too hydrophilic to bind to the hydrophobic polypeptide.

To test the specificity of DDT binding by the designed 24-residue polypeptide, an analogue possessing the same amino acid residues in a random sequence was prepared. The primary structure of the analogue was as follows:

H-Ser–Arg–Pro–Thr–Ile–Asn–Asn–Ile–
Thr–Tyr–Phe–Val–Pro–Gly–His–Ala–Phe–
Met–Tyr–Met–Asn–Ile–Ile–Phe-OH.

It was found to bind DDT ~140-times less strongly than the designed peptide. Analysis of the secondary structure of the random sequence using the method of Argos et al. [14] predicted an extended conformation for a large portion of the molecule and the weak DDT binding could again be explained by stacking or intercalating interactions with aromatic residues in alternating positions.

The far UV CD spectrum of the designed 24-residue polypeptide in 50% aqueous ethanol indicated the presence of a considerable amount of organized structure. However, a relatively large dichroic band at 224 nm interfered strongly with the quantitative analysis of the spectrum. The near UV CD spectrum of the peptide in the same solvent was characterized by ellipticity minima at 262 nm, 269 nm, and 277 nm. The limited solubility of DDT in 50% aqueous ethanol did not allow titration of the peptide with increasing concentrations of the insecticide.

It seems likely that the conformation of the designed 24-residue polypeptide and the mode of DDT binding will be elucidated only by X-ray diffraction studies of suitable crystals.

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