Electron microscopic investigations on the structure of lectin I from *Viscum album* L.

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Molecules of mistletoe lectin I (ML I) were investigated electron microscopically by negative staining with uranyl salts. An equilibrium was found between monomers and dimers at the used protein concentration. ML I monomers show a nearly triangular or rounded profile with dimensions of 80 × 90 Å. ML I dimers are rod-like particles composed of two triangular parts which are dislocated against each other. In another view the dimers appear thinner without a pronounced substructure. The dimensions of the dimers are 175 × 80 × 60 Å.

Viscum album *lectin* *Electron microscopy* *Negative staining*

1. INTRODUCTION

Lectins are proteins or glycoproteins with binding sites for special carbohydrates [1–3]. The lectin I from mistletoe (*Viscum album* L.) has a specificity for D-galactose. It consists of two kinds of polypeptide chains with M_r values of 29000 and 34000, respectively [4]. They correspond to the A and B chains of ricin and abrin. In each case one pair of these chains is connected by disulphide bond(s) forming the ML I monomer with an M_r of about 60000 [5]. At higher protein concentration ML I dimers are formed. In analogy to ricin and abrin the ML I mediated inhibition of protein synthesis on ribosomal level [6] is effected by the A chain [7,8]. This mechanism is probably included in antitumor activity of mistletoe preparations postulated by several authors [9].

Up to now only electron microscopic investigations on the structure of abrus agglutinin crystals [10] and anti-A_{HP} [11] have been published to our knowledge. We here report on the structure of negatively stained lectin I molecules from *Viscum album* L.

2. MATERIALS AND METHODS

2.1. *Preparation of lectin I from* Viscum album *L.*

ML I was isolated from ground plant material from mistletoe grown on the locust tree (*Robinia pseudoacacia*) using affinity chromatography with acid-treated agarose as carrier [4].

2.2. *Electron microscopy*

Freeze-dried samples of ML I were dissolved in 0.9% NaCl or 5 mM Tris–HCl, 200 mM KCl, 2 mM $MgCl_2$, pH 7.4 (TKM buffer) to a concentration of 4–40 μg protein/ml. Negative staining was carried out with 1% uranyl acetate or formiate (pH not corrected) as in [12] in the sandwich modification described in [13]: 300 mesh coppergrids covered with a holey carbon film were used as supports. Micrographs were taken with a Philips EM 400T at a magnification of × 100000. The electron microscope magnification was checked with a carbon grating replica (Fullham Inc., Schenectady) not to vary above 2% in the used magnification range. Measurements on the micrographs were performed at a magnification of × 500000 with a scale magnifier (Mautner) with a subdivision of 0.1 mm.

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3. RESULTS AND DISCUSSION

ML I molecules are visualized after negative staining with uranyl acetate or formiate as two different image types: a, as rod-like images; b, as triangular or rounded images with about half of the size of the rod-like ones (fig.1). These two image types were reproducibly found under the conditions described in section 2. They could represent two projections of a rod-like three-dimensional object; i.e., the rounded images could be interpreted as an end-on view of rod-like particles. On the other hand, the two image types could be monomer and dimer forms of the ML I molecule. In order to elucidate this question different concentrations of lectin I and the influence of sodium dodecyl sulfate (SDS) have been investigated. Variation of the ML I concentration leads to a variation of the relation of rod-like to rounded images. At a concentration of 40 μg protein per ml, 66% rod-like and 34% rounded images have been observed. Lowering of the ML I concentration is accompanied by an increase in the amount of rounded images. At a protein concentration of 4 μg protein per ml, 52% rod-like and 48% rounded particles have been found. These results reflect the adjustment of an association-dissociation equilibrium between the monomer and dimer form of ML I at a given protein concentration (see also [8]). Therefore, the rounded images should mainly represent monomers and the rod-like ones dimers of the ML I molecule.

The same conclusion can be drawn from the behaviour of ML I molecules after treatment with

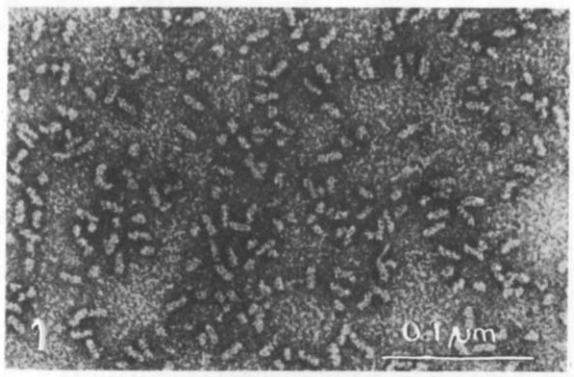

Fig.1. Electron micrograph of negatively stained lectin I from *Viscum album*. × 200000.

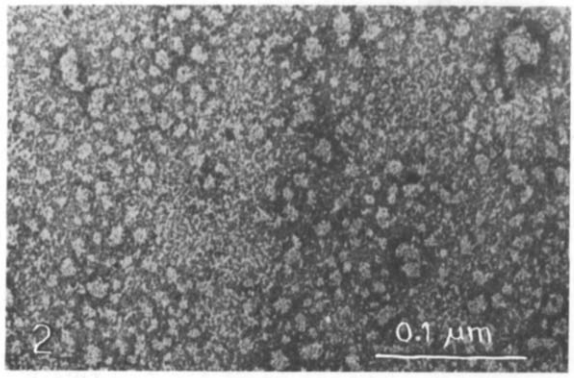

Fig.2. Electron micrograph of negatively stained lectin I from *Viscum album* after treatment with SDS (see text). × 200000.

SDS. ML I was dissolved in 0.9% NaCl containing 0.1% SDS and kept at 65°C for 5 min. After that the sample was diluted 1:50 with 0.9% NaCl because negative staining was impossible using a higher SDS concentration. Fig.2 shows that after this procedure no rod-like particles are found, but rounded particles of different sizes (with a diameter ranging from <50 Å up to >200 Å) and large aggregates of material. The same result was obtained with ML I treated with SDS in distilled water or TKM buffer. This gives rise to the assumption that the ML I dimers are split by SDS not only into the monomers but in some cases also into the constituent A and B chains that reaggregate in an irregular manner at the low SDS concentration necessary for negative staining. Moreover, this result points to the fact that not all A and B chains of the ML I monomers are connected by disulfide bonds as originally assumed. This is also obvious from SDS-polyacrylamide gel electrophoresis experiments which show two lightly stained protein bands at M_r 29000 and 34000 in addition to the main band at M_r 60000 (not shown). Similar results have been obtained in [8].

To compare the size and structure of ML I with the known structure of a protein of similar M_r this material has been mixed with IgG molecules from rabbits and negatively stained. Fig.3a shows a field of dimeric and monomeric ML I as well as IgG molecules. Selected images of these three types of protein molecules are demonstrated in fig.3b–d. From these micrographs it is clearly visible that the size of the investigated proteins lies in the same

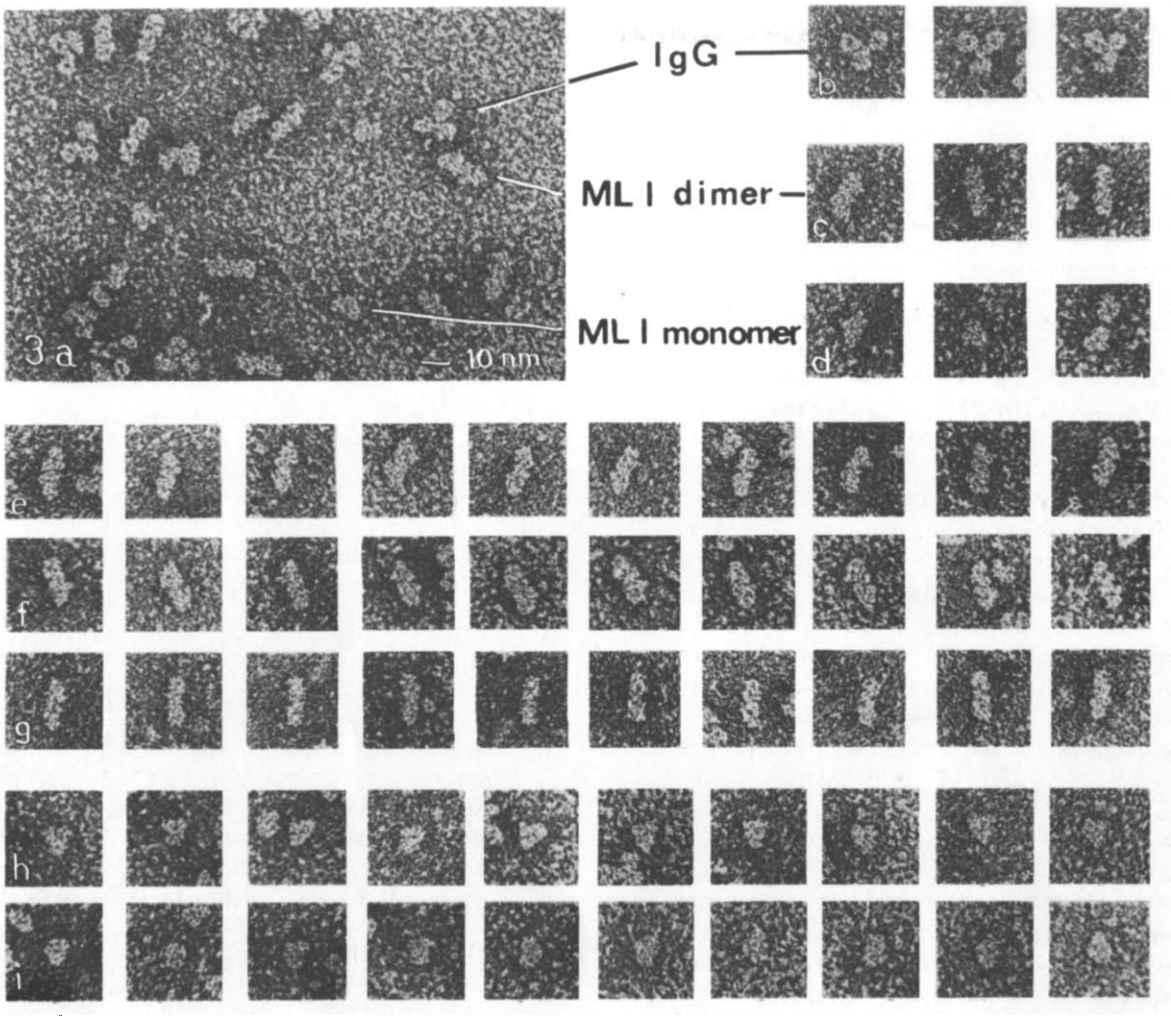

Fig.3. Electron micrographs of a negatively stained mixture of lectin I from *Viscum album* and IgG from rabbits. × 400000. (a) General view, (b–i) selected images.

range of order. The dimensions of one Fab arm (M_r ~50000) have been determined to be about 70 × 90 Å. These are higher values than those described in [12] but they are in good agreement with dimensions determined in [14] and [15] for IgG in antigen antibody complexes after negative staining. The dimensions of the ML I monomers (M_r ~60000) were estimated to be about 80 × 90 Å. The length of the ML I dimer (M_r ~120000) amounts to about 175 Å and the width to about 80 Å. The exact values of the measurements are given in table 1.

A closer inspection of the rod-like images reveals a subdivision of these images into two nearly triangular parts (fig.3c,e,f). If one orientates the line of attachment between the monomers in horizontal direction, it becomes obvious that in most cases the monomers are shifted against each other by about one third of the length of the attachment side. The upper triangle can be displaced to the right (fig.3c,e) or the left (fig.3f). These images probably reflect the existence of two views of the ML I dimer after rotation through an angle of 180° around the longitudinal axis of the particle. Sometimes a small dark notch is seen in the middle, or slightly eccentrically located in the triangular parts of the dimers (the two frames on the left in fig.3e,f). Besides, a horizontal staining

Table 1

Measurement of the size of negatively stained ML I and IgG molecules

	Length [Å]	Width [Å]	Number of measured images
ML I dimers			
Images as in fig.3e	178 ± 14	76 ± 9[a]	32
		84 ± 7[b]	32
Images as in fig.3f	168 ± 4	82 ± 8[a]	19
		83 ± 7[b]	19
Images as in fig.3g	166 ± 8	61 ± 5	20
ML I monomers			
Triangular profiles (fig.3h)	91 ± 10	84 ± 14	53
Rounded profiles (fig.3i)	96 ± 11	82 ± 12	19
IgG from rabbit (see fig.3b)			
Fab arm	89 ± 6	67 ± 8	186
Fc fragment	77 ± 8	71 ± 10	93

Value for the upper [a], and lower [b] triangle

line in some cases subdivides the triangular profiles (the two frames on the right in fig.3e,f). It is likely that this subdivision reflects the composition of the monomers of one A- and one B-chain. Less frequently rod-like images with a width of only 60 Å are observed (fig.3g) that do not show the substructure described above. It is supposed that they represent an approximately orthogonal view to those shown in fig.3e,f.

Selected images of monomers of ML I are shown in fig.3h,i. They possess a nearly triangular (fig.3h) or rounded profile (fig.3i). Substructures are rarely observed in these images. Occasionally a small dark notch is found (the two frames on the left in fig.3h) as described for the dimers.

The results described give, for the first time, an idea of the fine structure of single lectin molecules. The measured dimensions are in good agreement with those determined for abrus agglutinin [8], but significantly higher than those given for an animal lectin of comparable M_r [10].

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