Purification and Characterization of the Highly Toxic Lectin Modeccin*

(Received for publication, December 22, 1977)

Sjur Olsnes,‡ Thomas Haylett,§ and Karin Refnes†

From the ‡Norwegian Institute for Cancer Research, Oslo, Norway and the §National Chemical Research Laboratory, Council for Scientific and Industrial Research, Pretoria, Republic of South Africa

Modeccin was extracted from the root of Adenia digitata and purified by gel filtration on Sephadex G-100, ion exchange chromatography on DEAE-cellulose, and affinity chromatography on a column containing immobilized, neuraminidase-treated fetuin. The toxin was eluted from the affinity column with lactose.

Polyacrylamide gel electrophoresis of affinity-purified modeccin in the presence of sodium dodecyl sulfate showed a main protein (M, about 63,000) and two minor bands. After treatment with 2-mercaptoethanol the material moved as two bands corresponding to molecular weights of 28,000 and 38,000.

Upon treatment of modeccin with 2-mercaptoethanol it lost its toxic activity. A considerable part of this activity was recovered after dialysis to remove 2-mercaptoethanol and allow reoxidation to take place.

Sucrose gradient centrifugation of 125I-labeled, affinity-purified modeccin showed that the toxic material moved at the same rate as the major peak of radioactivity. Furthermore, the major part of the radioactivity, as well as the toxic material, were bound to a column containing immobilized concanavalin A and could be eluted with methyl-α-D-mannoside. It thus appears that the toxic material is identical with the major protein eluted with lactose from the affinity column containing immobilized neuraminidase-treated fetuin.

Isoelectric focusing of affinity-purified modeccin revealed the presence of at least four different toxins, most likely toxinoxins.

Immunodiffusion tests and experiments, where the ability of antitoxins to protect HeLa cells against different toxins was measured, showed that modeccin did not cross-react immunologically with abrin and only weakly with ricin. Abrin and ricin showed weak cross-reactions.

Similarities and differences between modeccin, abrin and ricin are discussed.

In a previous report (3) we have presented evidence that modeccin is a lectin and that it acts by inhibiting protein synthesis. In the present paper we present a purification procedure for modeccin and studies on its structure, charge, and immunological properties.

EXPERIMENTAL PROCEDURES

Extraction and Preparation of Crude Modeccin—The entire extraction procedure was performed at 2°C. Roots of A. digitata, harvested in North-Eastern Transvaal, Republic of South Africa, were chopped into small pieces of about 0.5 cm³ each. Chopped material (250 g) was soaked in 500 ml of 0.15 M NaCl overnight and then homogenized for 2 min in a completely filled and sealed Waring Blender jar. Homogenized batches were pooled, left standing overnight, and then squeezed through cheesecloth by wringing. The extract was left standing overnight, the clear supernatant was poured off, and the remaining supernatant was cleared by centrifugation. The combined supernatant was saturated to 90% with respect to ammonium sulfate, and the precipitated protein was recovered by centrifugation, redissolved in a minimum of H₂O, and dialyzed for 2 days against running, distilled water. Finally, the precipitate was removed by low speed centrifugation and the supernatant was lyophilized.

Affinity Chromatography—Sepharose 4B containing covalently bound concanavalin A was obtained from Pharmacia, Uppsala, Sweden. Fetuin-Sepharose was prepared by coupling 10 mg of fetuin (Sigma) to 1 g of cyanogen bromide-activated Sepharose (Pharmacia) according to the procedure given by the company. After washing, half of the fetuin-Sepharose was incubated for 2 h at 37°C with 500 units of neuraminidase (from Vibrio cholerae, Behringwerke, Marburg, Germany) in 2 ml of 0.14 M NaCl, 0.9 mM CaCl₂, 10 mM sodium phosphate (pH 7.0). The material was packed into small columns and washed with several volumes 0.14 M NaCl, 10 mM sodium phosphate (pH 7.4) before use.

The chromatography was carried out by applying the protein in 0.14 M NaCl, 10 mM sodium phosphate (pH 7.4) to the column and then washing with the same buffer. Finally, the column was eluted with the same buffer containing 0.1 M methyl-α-D-mannoside (in the case of concanavalin A-Sepharose 4B) or 0.1 M lactose (in the case of fetuin-Sepharose 4B). The procedure was carried out at room temperature. Protein was measured according to Lowry et al. (4) with bovine serum albumin as a standard.

Cell Culture—HeLa cells maintained in shaker culture were seeded into Linbro tissue culture plates with 16-mm troughs (FB16-24 TC, Linbro Chemicals) as earlier described (3) and increasing amounts of toxin were added to the wells. The next day the medium was removed and replaced by serum-free medium containing one-tenth of the normal concentration of leucine and 21 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.2) instead of bicarbonate; 0.25 μCi of [14C]leucine was added to each well and the incorporation of radioactivity was measured as earlier described (3).

Polyacrylamide Gel Electrophoresis—Protein fractions were made up to contain 5% sodium dodecyl sulfate and, in some cases, 1% 2-mercaptoethanol. The samples were then incubated at 100°C for 5 min and layered onto 7% polyacrylamide gels containing 0.1 M sodium phosphate (pH 7.1) and 0.1% sodium dodecyl sulfate and the electrophoresis was carried out as earlier described (5).

Isoelectric Focusing—The protein sample was mixed into a 0 to 55% sucrose gradient (110 ml) containing 0.2% Ampholine with pH range 3.5 to 10 and 0.8% Ampholine with pH range 5 to 7 (Pharmacia Fine Chemicals, Uppsala, Sweden) and focused at constant voltage (1600V) for 24 h in an LKB 8100 Ampholine column (LKB, Bromma, Sweden).
Purification of the Toxic Lectin Modeccin

PREPARATION OF ANTITOXINS—Anti-abrin and anti-ricin were prepared as earlier described (6). Anti-modeccin was prepared by treating partially purified modeccin (fraction D from Fig. 2) with 1% formaldehyde in 50 mM sodium phosphate (pH 7.5) at room temperature for 3 days. After removing excess formaldehyde by dialysis against 50 mM sodium phosphate (pH 7.5), immunization was initiated by subcutaneous injection of 0.5 mg of protein in complete Freund’s adjuvant, followed by 0.5-mg booster doses (with Freund’s incomplete adjuvant) every 3 weeks. The antisera here used was obtained 16 weeks after the first immunization.

IMMUNODIFFUSION—Agar plates were made from 1% noble agar in 0.14 M NaCl containing 20 mM sodium phosphate (pH 7.1), 20 mM lactose, and 0.02% NaN₃, as earlier described (6).

RESULTS

Purification—Crude modeccin was extracted from Adenia digitata by homogenizing chopped roots in isotonic saline (0.9% NaCl solution). The protein fraction was precipitated with 90% ammonium sulfate, the precipitate was dissolved in water, and, after dialysis against distilled water, it was freeze-dried. One gram of the lyophilized protein was dissolved in buffer and submitted to gel filtration on a Sephadex G-100 column (Fig. 1). The different protein fractions were assayed for toxicity to mice. As shown in Table I, Fraction III proved to be considerably more toxic than the other fractions. This fraction was therefore pooled, and, after dialysis, applied to a DEAE-cellulose column. Part of the material passed through the column, whereas most of the protein was bound and was eluted with a 500-ml linear gradient from 0 to 60 mM NaCl (Fig. 2). The different fractions were tested for toxicity in mice and for their ability to inhibit protein synthesis in HeLa cells, as shown in Fig. 3A. It is clear (Fig. 3A and Table I) that the material in Fraction D was most toxic, and this fraction was therefore pooled, dialyzed against distilled water, and lyophilized.

The material in Fraction D (Fig. 2), when analyzed by polyacrylamide gel electrophoresis, proved to consist of several protein bands (not demonstrated). Due to the small amount of material available, we decided to label part of the material with 125I, in order to better follow the protein during the further purification steps. The labeling was carried out by the lactoperoxidase method (7, 8). Control experiments showed that the labeling of modeccin with 1 iodine atom/protein molecule did not reduce its toxic effect.

Since our earlier data indicated that modeccin binds to cell surfaces and that this binding is increased after neuraminidase treatment of the cells (3), we tried to purify modeccin by affinity chromatography using different immobilized glycoproteins before and after treatment with neuraminidase. The best results were obtained with fetuin. To a Sepharose 4B column containing untreated fetuin (Fig. 4A) the labeled material did not bind, although the elution of part of the material was somewhat delayed. In contrast, part of the radioactive material did bind firmly to a column previously treated with neuraminidase and it could be eluted with lactose (Fig. 4B), but not with methyl-a-D-mannoside (not demonstrated). When this specifically eluted material was dialyzed to remove the lactose and then resubmitted to the column most of the material again became bound (Fig. 4C). In contrast, upon rechromatography of the material which passed through the column, only trace amounts were bound and eluted with lactose (not demonstrated).

The different fractions were then tested for their ability to inhibit protein synthesis in HeLa cells. It is clear (Fig. 3B and Table I) that the fraction eluted with lactose proved to be about 10 to 20 times more toxic than the material applied to the column, whereas the material which did not bind to the column was almost nontoxic.

At room temperature as here used, no toxin was bound to

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Fraction</th>
<th>Yield mg/kg root</th>
<th>LD₅₀/mg protein</th>
<th>Recovery %</th>
<th>Toxicity in mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>(NH₄)₂SO₄ precipitate</td>
<td>Crude modeccin</td>
<td>1000</td>
<td>35</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>I</td>
<td>9</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>45</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>76</td>
<td>143</td>
<td>31.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>800</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DEAE-cellulose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>11.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>3.1</td>
<td>2,000</td>
<td>17.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>36.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-5</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9-10</td>
<td>0.3</td>
<td>20,000</td>
<td>17.1</td>
<td></td>
</tr>
</tbody>
</table>

*Death occurred 2 days after intraperitoneal injection of protein into mice.*
Purification of the Toxic Lectin Modeccin

FIG. 4. Affinity chromatography on Sepharose 4B containing covalently bound fetuin. A, protein from Fig. 2, Fraction D, labeled with \(^{125}\text{I} (70 \text{ ng of protein, 35,000 cpm}) \) was applied to a 1-ml Sepharose 4B column containing about 4 mg of covalently bound fetuin. The column was washed with 0.14 M NaCl, 5 mM sodium phosphate (pH 7.4) and then eluted with 0.1 M lactose in the same buffer. B, a column as in A was treated with neuraminidase as described under “Experimental Procedures.” Two milligrams of protein from Fig. 2, Fraction D, was mixed with some \(^{125}\text{I}-\text{labeled protein (90} \mu\text{g of protein, } 6 \times 10^4 \text{ cpm}) \) and applied to the column which was treated as above. C, part of the material eluted with lactose in B was dialyzed against 0.14 M NaCl, 5 mM sodium phosphate (pH 7.5) and applied to the column once more.

Sepharose 4B alone. However, if the experiment was carried out at 2-4°C varying amounts of toxin were bound and could be eluted with lactose.

Polyacrylamide Gel Electrophoresis—Analysis by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate showed that the material eluted with lactose in Fig. 4B consisted mainly of one protein which moved corresponding to a molecular weight of 63,000. In addition, traces of proteins moving at rates corresponding to molecular weights of about 28,000 and 38,000 were also present. When the material had been treated with 2-mercaptoethanol prior to the electrophoresis, the heavy band disappeared and all material now moved at rates corresponding to 28,000 and 38,000. The data therefore indicate that modeccin consists of two polypeptide chains linked with one or more disulfide bridges. In all cases the bands were somewhat broad and diffuse indicating microheterogeneity of the proteins.

Biological Effects of Treatment with 2-Mercaptoethanol—Modeccin treated with 10 mM 2-mercaptoethanol in 0.14 M NaCl, 10 mM sodium phosphate (pH 7.0) lost its ability...
to inhibit protein synthesis in intact cells, whereas the ability to inhibit protein synthesis in a cell-free system increased about 10-fold (3). Upon dialysis to remove 2-mercaptoethanol and allow reoxidation to take place about 20% of the toxicity was recovered (not demonstrated).

Sucrose Gradient Centrifugation—Since the affinity-purified modeccin did not move as one single band on polyacrylamide gel electrophoresis, the possibility was tested that the toxin might represent only a minor fraction of the total amount of protein eluted with lactose in Fig. 4B. For this purpose we first compared by sucrose gradient centrifugation the sedimentation properties of the radioactive protein with that of the toxic material as earlier described (9). Hemoglobin was used as an internal marker. It was found that the major part of the 125I-labeled protein sedimented slightly more rapidly than hemoglobin and somewhat more slowly than abrin. Two minor peaks of radioactive material sedimented somewhat more slowly than hemoglobin. The amount of each fraction required per ml of cell culture medium to inhibit protein synthesis in HeLa cells to 50% of the control value under conditions as in Fig. 3 (ID50) was measured. The results clearly showed that the toxicity and the main peak of radioactivity followed each other closely.

Affinity to Concanavalin A—Further evidence that the main protein eluted from the neuraminidase-treated fetuin column represents the toxin, was obtained from binding studies to a column containing immobilized concanavalin A. The labeled material eluted with lactose in Fig. 4B, bound almost quantitatively to the column and could be eluted with methyl-

\[ \alpha \text{-d-mannoside}, \text{but not with lactose. When tested on cells in culture the eluted material was as toxic as that applied to the column. The data indicate that modeccin is a glycoprotein containing mannose residues and they further support the view that the major labeled protein eluted with lactose in Fig. 4B, represents the toxin.} \]

Isoelectric Focusing—In spite of the fact that modeccin behaved as an essentially pure protein in gel electrophoresis, sucrose gradient centrifugation, and affinity chromatography, isoelectric focusing revealed the presence of several separate proteins. Thus, the ability to inhibit protein synthesis in HeLa cells was found in four different peaks at positions of the gradient corresponding to pI values of 6.2, 6.6, 6.9, and 7.1.

Immunological Properties—An antiserum to modeccin was prepared by immunizing rabbits with a toxoid formed from partially purified modeccin (Fraction D in Fig. 2) after treatment with formaldehyde. Ouchterlony immunodiffusion tests showed that this antiserum formed several precipitin lines with partially purified modeccin, but only one line with affinity purified modeccin. Anti-abrin and anti-ricin did not form precipitin lines with modeccin and, furthermore, anti-modeccin did not form precipitin lines with abrin and ricin (not shown).

Due to the many similarities between modeccin, abrin, and ricin we decided to test in a more sensitive system if antibodies against one of the toxins were able to neutralize the other toxins. For this purpose we studied the ability of anti-modeccin, anti-abrin, and anti-ricin to neutralize the ability of each of the three toxins to inhibit protein synthesis in HeLa cells. As shown in Table II, 1 μl of antitoxin serum was able to neutralize about 100 ng of modeccin, but only about 0.6 ng of ricin, and virtually no abrin. Similarly, 1 μl of anti-abrin neutralized about 500 ng of abrin, but only about 2.5 ng of ricin, and virtually no modeccin. Anti-ricin (1 μl) neutralized about 550 ng of ricin, about 30 ng of abrin, and virtually no modeccin. It may be concluded that in spite of the similarities in their structure and mechanism of action the three toxins are not immunologically closely related.

**Table II**

<table>
<thead>
<tr>
<th>Antitoxin</th>
<th>Modeccin</th>
<th>Abrin</th>
<th>Ricin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount of toxin inactivated 50% by 1 μl of antiserum*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ng</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-modeccin</td>
<td>100 (± 30)</td>
<td>&lt;0.0006</td>
<td>0.6 (± 0.5)</td>
</tr>
<tr>
<td>Anti-abrin</td>
<td>&lt;0.0003</td>
<td>500 (± 100)</td>
<td>2.5 (± 2.4)</td>
</tr>
<tr>
<td>Anti-ricin</td>
<td>&lt;0.0003</td>
<td>31 (± 18)</td>
<td>550 (± 150)</td>
</tr>
</tbody>
</table>

* Average and range of data obtained with two sets of sera produced in different rabbits.

**DISCUSSION**

Modeccin purified as described here is probably pure in the sense that the material is not contaminated with nontoxic proteins. Most likely the presence of at least four peaks of toxic material in the electrofocusing experiment means that isotoxins are present. In fact, in a variety of plants isolecins differing only slightly in charge and size have been found (10-12). The fact that the bands obtained with polyacrylamide gel electrophoresis were somewhat broad and diffuse in accordance with this interpretation. The small amounts of protein moving in polyacrylamide gels at the same rate as the two constituent peptide chains of the toxin most likely represent dissociated toxin. Possibly, in some of the toxin molecules the two constituent peptide chains are not connected by disulfide bonds, but are held together only by weak interactions which are disrupted by the treatment with sodium dodecyl sulfate.

The fact that modeccin binds to neuraminidase-treated fetuin may give some information on its specificity. The structure of the carbohydrates in fetuin are known in considerable detail (13). Altogether, six oligosaccharide chains are bound to a single peptide chain. 79% in three oligosaccharide chains which are linked to asparagine, and the remaining carbohydrate in three chains which are serine- and threonine-linked. In native fetuin all the carbohydrate chains are terminated with sialic acid. Galactose is the penultimate residue. Ricinus agglutinin has been found to bind to neuraminidase-treated fetuin (14) and, interestingly, the toxins abrin and ricin which are similar to modeccin in their structure and mechanism of action both bind to neuraminidase-treated fetuin with approximately the same strength as to cell surface receptors. Abrin and ricin also bind to pure Sepharose 4B and to a variety of serum glycoproteins to which modeccin does not bind. It is likely that in neuraminidase-treated fetuin abrin and ricin bind to the asparagine-linked carbohydrate chains ending in Galβ(1→4)GlcNAcβ → Man, (15), whereas modeccin possibly binds to the Galβ(1→3)GalNAc → Ser(Thr) sequences.

It has been shown earlier that abrin and ricin are not immunologically closely related (16). The present data show in addition that modeccin is not closely related to abrin and ricin. In spite of this there is a surprising similarity in structure.

and function of these toxins which occur in plants belonging to three different orders.

Acknowledgments—We are indebted to Professor A. Pihl for his critical discussion and to Mrs. Kristin Eiklid, Mrs. Wenche Telle, and Mrs. Lill Naess for excellent technical assistance.

REFERENCES

Purification and characterization of the highly toxic lectin modeccin.
S Olsnes, T Haylett and K Refsnes


Access the most updated version of this article at
http://www.jbc.org/content/253/14/5069.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/253/14/5069.citation.full.html#ref-list-1