Immunoadsorption as a Means for the Purification of Low Molecular Weight Compounds: Isolation of Ecdysteroids from Insects

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Ecdysteroid, Immunoadsorption of Steroids, Antiserum Specificity

Two ecdysteroid-specific antisera, anti-ecdysone-albumin and anti-20-hydroxyecdysone-albumin, were induced in rabbits. The properties of both antisera were tested with a large number of ecdysteroids. The former antiserum was unique in its ability to discriminate between the moultling hormones, ecdysone and 20-hydroxyecdysone, whereas the latter had a broad specificity for ecdysone and several of its metabolites.

\textsuperscript{γ}-globulin fractions of both antisera were covalently linked to Sepharose 4B and used as immunoadsorbents. These were able to bind ecdysteroids from crude extracts of insects. Bound ecdysteroids could be eluted quantitatively from the adsorbents using $3\;\text{m}$ sodium trichloroacetate.

When a mixture of ecdysteroids which contained ecdysone and at least six different ecdysone metabolites were run over a column with anti ecdysone immunoadsorbent only ecdysone and its hydroxysteroids 20- and 26-hydroxy ecdysone were bound to and subsequently eluted from the adsorbent. In contrast a column with anti-20-hydroxyecdysone immunoadsorbent retained most of the different ecdysteroids due to the broader specificity of the antibodies.

These immunoadsorbents provide the potential not only to purify ecdysteroids but also proteins crosslinked to ecdysteroids via photoaffinity labelling. It is well known that immunoadsorption is a powerful tool for the isolation of proteins. The results described here demonstrate that immunoadsorption may also be useful in the isolation of low molecular weight compounds of biological and medical interest.

Introduction

The isolation of specific compounds from natural sources (e.g. homogenates and body fluids) is one of the major technical problems in biochemistry, pharmacology and related fields. For this purpose, affinity chromatography has gained increasing importance. This special type of adsorption chromatography is usually applied to the isolation and purification of macromolecules such as enzymes, plasma proteins, hormone receptors, polynucleotides etc. using small molecules as ligands immobilized to an insoluble support. Very little literature is as yet available on a procedure using this principle conversely, i.e. to isolate small molecules like nucleotides, metabolites of pharmacological agents or steroids by their affinity to macromolecules [1, 2].

Steroid hormones occur in rather low concentrations compared to other biomolecules of low molecular weight. In insects the range is $0.01 – 5\;\text{nmol per g fresh weight}$ [3]. In the course of our studies on the biochemistry of the steroid hormone ecdysone, it was our aim to isolate ecdysteroids (ecdysone plus its metabolites) from the blue blowfly, \textit{Calliphora vicina}, for chemical analysis in order to corroborate results from radiotracer experiments [4]. Because of the limited amount of biological material available — one mature larva has a weight of about $80\;\text{mg}$ — a purification method was needed which combined high selectivity with high efficiency. Since immunoadsorption could be expected to have such properties, this method was developed to isolate ecdysone and related compounds.

Immunoadsorption has several advantages over conventional isolation and purification methods. Because of this reason the method is widely used to purify macromolecules [5]. However, to our knowledge, immunoadsorption has not yet been used to isolate low molecular compounds. Therefore, the aim of our investigation was to isolate ecdysteroids from insects using the immunoadsorption technique and to prove its usefulness.

Abbreviations: ecdysone-CMO, ecdysone-6-carboxymethoxime; HPLC, high performance liquid chromatography; LSC, liquid scintillation counting; NMR, nuclear magnetic resonance; RIA, radioimmuno assay; TLC, thin-layer chromatography.

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of the studies described here was to develop an immunoadsorption technique for the isolation of low molecular weight compounds using ecdysteroids as a representative and interesting example.

The following steps are described in detail:

* preparation of an antigen by linking the low molecular compound, to a protein and production of an antiserum against the hapten-protein conjugate,
* characterization of the binding specificity of the antiserum,
* coupling of the \( \gamma \)-globulin fraction of the antiserum to an insoluble support, and finally,
* evaluation of the conditions for binding and elution of the compound(s) of interest.

**Materials and Methods**

**Reagents**

All reagents were of analytical grade and were obtained from Merck (Darmstadt) except where otherwise stated. Unlabelled steroids were purchased from Simes s.p.a. (Milano, Italy) except 2-deoxyecdysone and 2-deoxy-20-hydroxyecdysone, which were generous gifts from Dr. Horn (Melbourne, Australia), 3-epi-20-hydroxyecdysone from Dr. Rees (Liverpool, England), 3-epiecdysone, 26-hydroxyecdysone and 20,26-dihydroxyecdysone from Drs. Weirich and Thompson (Beltsville, USA), 2,14,22,25-tetradeoxyecdysone and 2,22,25-trisdeoxyecdysone from Dr. Hoffmann (Strasbourg, France). 3-Dehydroecdysone was prepared enzymatically as previously described [6]. [23,24-\(^{3} \text{H}\)]Ecdysone with a specific radioactivity of 68 Ci/mmol was obtained from Zeecon (Palo Alto, USA) and purified prior to use [7]. Radiochemical purity in TLC (see below) was 97\%. Radiolabelled ecdysteroids were produced by incubation of tritiated ecdysone with isolated fat body of the blowfly, *Calliphora vicina*, in vitro (for the details see: [4]).

**Methods**

**Preparation of ecdysone-6-carboxymethoxime (ecdyson-CMO).** — Preparation of ecdysone-CMO followed in general the procedure of Borst and O’Connor [8] and Maroy et al. [9]. Ecdysone was mixed with \([^{3}\text{H}]\text{ecdysone}\) to monitor the reaction process.

The ecdysone-derivative was characterized by analytical TLC (silica gel plate 0.25 mm, type 60 F\(_{254}\), solvent CH\(_2\)OH/CHCl\(_3\), 40/60 (v/v)) and radioscanning: purity 98\%; by ultraviolet spectroscopy; by infrared spectroscopy in KBr (infrared spectrometer type 577, Perkin Elmer, Überlingen); band at 1600 cm\(^{-1}\) (C = N bond); and, by NMR spectroscopy in D\(_2\)pyridine (spectrometer type XL-100, Varian, Darmstadt): signals at 0.726 ppm (singlet, C-18), 1.338/1.331 ppm (doublet, C-21) and 1.372 ppm (singlet, C26/C27).

**Antigen-formation.** — We used the method of Horn et al. [10], to couple the ecdysteroid derivative to bovine serum albumin.

The ratio of steroid to protein in the conjugate was calculated by the ratio of radioactivity (amount of ecdysone) to protein content (Folin’s method).

**Preparation of test-antigen for the capillary test.** — Following the same protocol as in the antigen (= ecdysone-bovine serum albumin conjugate) formation test antigens (ecdysone-rabbit-immunoglobulin and 20-hydroxyecdysone-rabbit-immunoglobulin) were prepared. Ecdysteroid-CMO (5.3 \(\mu\)mol, 0.14 \(\mu\)Ci \(\text{^{1}}\text{H}\)), rabbit immunoglobulin (0.066 \(\mu\)mol) and carbodiimide derivative (263.7 \(\mu\)mol) were incubated in 1.0 ml phosphate-buffered saline (20 \(\text{mM}\) sodium phosphate, pH 7.3, 150 \(\text{mM}\) sodium chloride). A steroid to protein ratio of 6 was achieved with ecdysone and 4 with 20-hydroxyecdysone.

**Immunization of the rabbits and capillary test.** — Two rabbits for each antigen (ecdysone-bovine serum albumin conjugate and 20-hydroxyecdysone-bovine serum albumin conjugate) were subcutaneously immunized each with 2.0 mg ecdysteroid-bovine serum albumin conjugate in 1.0 ml phosphate-buffered saline mixed with 1.0 ml complete Freund’s adjuvant. Thus, 0.2 ml portions were injected at different positions of the back of the animals. Three weeks later the animals were boosted in same way.

Sera were tested by semiquantitative quantitation using glass capillaries (dimension: 80 mm length, 1 mm i. d.). Batches with the highest anti-hapten antibody concentration were pooled and named DUL-1 and DUL-2 (“Dieter-Ursel-Lutz” after the initials of the investigators) for each rabbit. Antisera against 20-hydroxyecdysone-BSA conjugate were named DBL-1 and DBL-2 (“Dieter-Bettina-Lutz”). The antisera with the index 1 had the higher antibody titre and were used throughout this study.

**Characterization of the antisera by radioimmuno assay (RIA).** — The general method used was de-
scribed recently [7]. To separate bound from free hapten in the RIA two different methods were used, ammonium sulphate precipitation [7] or equilibrium dialysis (modification of the method used by De Reggi et al. [19]). RIA measurements were always done in triplicates which were averaged.

Dilution curves of the antisera were measured to obtain optimal dilution factors for further assays. The sera were diluted with borate buffer containing 6% normal rabbit serum (DUL-1 2500-fold, DBL-1 1500-fold). Standard response curves with different ecdysteroids were recorded by variation of the steroid concentration. From the standard response curves the concentrations were taken at which 50% of the radiolabelled ecdysone bound to antibodies was displaced. A comparison of these concentrations between the ecdysteroids tested and ecdysone gave the cross reaction factors. Dissociation constants of the ecdysone antisera complex were derived from Scatchard-plots [12], which were drawn from standard response curves of ecdysone.

Coupling of the γ-globulin fraction to Sepharose 4 B. — Sepharose 4 B (Pharmacia, Freiburg) was activated with CNBr. The subsequent coupling of the γ-globulin fraction followed the method of March et al. [13]. γ-globulin (5 mg) of DUL-1 (DUL-1-Sepharose 4 B) and 9.4 mg γ-globulin of DBL-1 (DBL-1-Sepharose 4 B) obtained by three-fold precipitation in 50% saturated (NH₄)₂SO₄, respectively, were coupled per ml packed activated gel. Coupling efficiency was 95%, with the remaining reactive groups blocked by treatment with ethanolamine. The adsorbents stored at 4 °C in phosphate-buffered saline containing 0.1% NaN₃ (w/v).

Binding of ecdysone to the immunoadsorbent. — A column (0.5 cm i.d.) was filled with 3.3 ml (DUL-1-Sepharose 4 B. Tritiated ecdysone (49 pmol, 3.33 μCi) in 0.5 ml phosphate-buffered saline was passed through the column within 5 min, which was subsequently washed with 40 ml saline. Bound ecdysone was dissociated and eluted from the immunoadsorbent by washing the column with 10 ml 3.0 M sodium trichloroacetate. This eluate was de­salted immediately by gel chromatography on Sephadex G-10 (Pharmacia, column 1.5 cm i.d., bed height 150 cm, solvent CH₃OH/H₂O 50/50 (v/v)). Ecdysone concentrations in the pooled unbound and bound fractions were determined by LSC of the radioactivity. Chromatographic analysis of ecdy-
The antisera could be diluted 2500 fold (DUL-1) and 1500 fold (DBL-1) to bind 50% of labelled steroid ([3H]ecdysone). The specificity of the antisera against ecdysteroids were analysed by measuring the cross-reactivity of different ecdysteroids with ecdysone (Table I). Both antisera were specific for ecdysteroids, i.e. they bound ecdysone and ecdysone-related compounds. Ecdysone precursors like 2,14,22,25-tetradeoxyecdysone and 2,22,25-trideoxyecdysone were virtually not bound. This was also true for certain ecdysteroids with definite structural differences to ecdysone and 20-hydroxyecdysone.

Both antisera had a specificity directed mainly against the side chain. However, the most obvious difference between the two antisera was the extent of specificity for the C-20 structure of ecdysone. 20-Hydroxyecdysone was discriminated with a factor of 47 by DUL-1, whereas this was only 3.0 with DBL-1. The $K_{P_c}$ values for ecdysone were determined in Scatchard plots to be $2.4 \times 10^{-9}$ m (SD = $0.6 \pm 10^{-9}$, $n = 3$) for DUL-1 and $3.2 \times 10^{-9}$ m (SD = $0.6 \pm 10^{-9}$, $n = 3$) for DBL-1.

Preparation and characterization of immunoadsorbents. - γ-Globulin fractions of the antisera were coupled to Sepharose 4 B by CNBr activation. Aliquots of the resulting immunoadsorbents were packed in small columns to analyse the binding characteristics of the material. In the following, experiments with DUL-1 (γ-globulin)-Sepharose 4B will be described in detail. The other immunoadsorbent DBL-1 (γ-globulin)-Sepharose 4B exhibited similar binding characteristics, except in steroid specificity (see below).

Firstly, the ability of DUL-1 (γ-globulin)-Sepharose 4 B to bind ecdysone was tested. For this purpose a solution or tritiated ecdysone (49 pmol, 3.33 µCi for 3.3 ml immunoadsorbent) in phosphate-buffered saline was passed slowly through the column. Subsequently, the column was thoroughly washed with saline. In the flow-through and wash only 1.7% of the label was detected, which was unbound ecdysone. The bound ecdysone (98%) could be eluted completely with 3.0 m sodium trichloroacetate. Immediately after elution the immunoadsorbent had to be washed with phosphate-buffered saline to preserve its binding capacity.

Other common chaotropic reagents like NaCl and NaSCN [15] were also tested in various concentrations. However, they were ineffective in dissociating the steroid from the antibodies. The same was found with lower concentrations of trichloroacetate. In 3.0 m trichloroacetate ecdysone has a limited stability (half-life at room temperature 12 h). Therefore, after its elution from the immunoadsorbent, ecdysone was immediately separated from the salt by gel chromatography. Analysis of the resulting ecdysone by TLC and HPLC, as well as, by LSC

Table I. Specificities of antisera DUL-1 and DBL-1 detected as crossreaction factors of various ecdysteroids with ecdysone in radioimmuno assay. 50% binding of ecdysone was observed at a concentration of 13 nM (= 0.65 pmol in 50 μl; DUL-1) and 24 nM (= 1.2 pmol in 50 μl; DBL-1).

<table>
<thead>
<tr>
<th>Ecdysteroid</th>
<th>Source</th>
<th>Concentration range tested [nM]</th>
<th>Cross reaction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>ecdysone</td>
<td>Simes</td>
<td>$10^{-4}$</td>
<td>1</td>
</tr>
<tr>
<td>2-deoxyecdysone</td>
<td>Melbourne</td>
<td>$10^{-4}$</td>
<td>1.6</td>
</tr>
<tr>
<td>makisterone A</td>
<td>Simes</td>
<td>$10^{-4}$</td>
<td>2.1</td>
</tr>
<tr>
<td>3-epiecdysone</td>
<td>Beltsville</td>
<td>$10^{-4}$</td>
<td>3.5</td>
</tr>
<tr>
<td>3-dehydoecdysone</td>
<td>enzymatic preparation</td>
<td>$10^{-4}$</td>
<td>3.8</td>
</tr>
<tr>
<td>20-hydroxyecdysone</td>
<td>Simes</td>
<td>$10^{-4}$</td>
<td>47</td>
</tr>
<tr>
<td>3-epi-20-hydroxyecdysone</td>
<td>Liverpool</td>
<td>$10^{-4}$</td>
<td>120</td>
</tr>
<tr>
<td>2-deoxy-20-hydroxyecdysone</td>
<td>Melbourne</td>
<td>$10^{-4}$</td>
<td>140</td>
</tr>
<tr>
<td>26-hydroxyecdysone</td>
<td>Beltsville</td>
<td>$10^{-4}$</td>
<td>360</td>
</tr>
<tr>
<td>polypodine B</td>
<td>Simes</td>
<td>$10^{-4}$</td>
<td>200</td>
</tr>
<tr>
<td>20,26-dihydroxyecdysone</td>
<td>Beltsville</td>
<td>$10^{-4}$</td>
<td>560</td>
</tr>
<tr>
<td>muristerone</td>
<td>Simes</td>
<td>$10^{-4}$</td>
<td>620</td>
</tr>
<tr>
<td>inokosterone</td>
<td>Simes</td>
<td>$10^{-4}$</td>
<td>1000</td>
</tr>
<tr>
<td>2,14,22,25-tetradeoxyecdysone</td>
<td>Strasbourg</td>
<td>$10^{-4}$</td>
<td>8200</td>
</tr>
<tr>
<td>2,22,25-trideoxyecdysone</td>
<td>Strasbourg</td>
<td>$10^{-4}$</td>
<td>$\infty$</td>
</tr>
<tr>
<td>ponasterone A</td>
<td>Melbourne</td>
<td>$10^{-4}$</td>
<td>$\infty$</td>
</tr>
<tr>
<td>cyasterone</td>
<td>Simes</td>
<td>$10^{-4}$</td>
<td>$\infty$</td>
</tr>
<tr>
<td>poststerone</td>
<td>Simes</td>
<td>$10^{-4}$</td>
<td>18</td>
</tr>
</tbody>
</table>
Table II. Metabolites of ecdysone from blowfly before and after adsorption to DUL-1 and DBL-1-sepharose 4B. The amount of each compound was expressed in percent of the initial amount of total radiolabelled ecdysteroids (= 100%). The table gives the quantitative evaluation of Fig. 1–5. The sum of each column (A, B or C) does not amount to 100% because minor peaks were omitted.

<table>
<thead>
<tr>
<th>Ecdysteroids</th>
<th>peak</th>
<th>A [%]</th>
<th>B1 [%]</th>
<th>B2 [%]</th>
<th>C1 [%]</th>
<th>C2 [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>High polarity compounds</td>
<td>I</td>
<td>29</td>
<td>16</td>
<td>0</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>20,26-dihydroxyecdysone</td>
<td>III</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>26-hydroxyecdysone</td>
<td>IV</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Peak V substances</td>
<td>V</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>20-hydroxyecdysone</td>
<td>VI</td>
<td>9</td>
<td>0</td>
<td>16</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Ecdysone</td>
<td>VII</td>
<td>20</td>
<td>0</td>
<td>29</td>
<td>0</td>
<td>18</td>
</tr>
</tbody>
</table>

A, Metabolic composition before immunoadsorption (Fig. 1); B1, Metabolites not adsorbed by DUL-1-Sepharose 4B (Fig. 2); B2, Metabolites adsorbed by DUL-1-Sepharose 4B and eluted with sodium trichloroacetate (Fig. 3); C1, Metabolites not adsorbed by DBL-1-Sepharose 4B (Fig. 4); C2, Metabolites adsorbed by DBL-1-Sepharose 4B and eluted with sodium trichloroacetate (Fig. 5).

Fig. 1. TLC separation of radiolabelled ecdysteroids after incubation of tritiated ecdysone with blowfly fat bodies in vitro. The peaks in the order of increasing polarity consist of I: high polarity compounds, III: 20,26-dihydroxyecdysone, IV: 26-hydroxyecdysone, V: inokosterone and other, VI: 20-hydroxyecdysone, VII: ecdysone [4]. The positions of unlabelled reference substances have been marked with α (ecdysone), β (20-hydroxyecdysone), γ (26-hydroxyecdysone) and δ (20,26-dihydroxyecdysone).

Fig. 2. TLC separation of the same radiolabelled ecdysteroids as in Fig. 1 after passage through a column filled with the immunoadsorbent DUL-1-Sepharose 4B.

demonstrated that the steroid was unchanged and that the overall recovery in the immunoadsorption chromatography was greater than 95%.

In order to measure unspecific binding of ecdysone to the immunoadsorption material an analogous adsorbent was prepared with the γ-globulin fraction from serum of a non-immunized rabbit. Under identical conditions as in the preceding experiment the material bound less than 0.1% of labelled ecdysone.

The capacity of the immunoadsorbent was tested by offering an excess of ecdysone (1.17 μmol) which was labelled with some radioactive ecdysone. The steroid was dissolved in phosphate-buffered saline and applied to a column packed with 13.7 ml immunoadsorbent. The amount of ecdysone in the flow-through and wash, as well as, the amount bound and eluted were measured using both parameters, ultraviolet absorption of ecdysone and its radioactivity, with 1.1 nmol steroid per ml packed volume of adsorbent bound and subsequently eluted.
The specificity of the two immunoadsorbents towards different ecdysteroids was tested with radiolabelled metabolites of ecdysone. The metabolites were produced by in vitro incubation of fat body from blowflies with tritiated ecdysone [7] and included the ecdysteroids listed in Table II. An aliquot of the solution containing ecdysteroid in PBS was analysed by TLC and radioscanned (Fig. 1). The relative concentration of single ecdysteroids are given in Table II. Other aliquots of the solution with a total of 21 pmol ecdysteroids were passed in separate experiments through a column filled with DUL-1 or DBL-1-Sepharose 4B. Of the ecdysteroid applied 30% were not bound by DUL-1 and 6% not by DBL-1-Sepharose 4B. TLC analyses revealed that the unbound material from the DUL-1-Sepharose 4B consisted mainly of high polarity compounds, 20,26-dihydroxyecdysone and peak V substances (Fig. 2 and Table II). Unbound material from DBL-1-Sepharose 4B was due to high polarity compounds only (Fig. 4). No ecdysone and 20-hydroxyecdysone could...
be seen in both fractions of unbound ecdysteroids. By elution with 3.0 sodium trichloroacetate the labelled ecdysteroids bound to the immunoadsorbents were recovered (DUL-1: 65% of the applied material, DBL-1: 87%). The ecdysteroids from DUL-1-Sepharose 4B as analysed by TLC and HPLC were mostly 26-hydroxyecdysone, 20-hydroxyecdysone and ecdysone (Fig. 3 and Table II). From DBL-1-Sepharose 4B all of the different metabolites applied were eluted (Fig. 5 and Table II).

This experiment demonstrated that the immunoadsorbents retained the specificities of the antibodies in solution detected by RIA. DUL-1 again was highly specific for ecdysone and bound few of its hydroxylated derivatives only. As in RIA, DBL-1 showed the broader specificity for the entire ecdysteroid family. Furthermore, it was obvious from this and subsequent experiments with the same materials that the immunoadsorbents retained their ability to bind ecdysteroids throughout the entire procedure.

Discussion

Immunoadsorption has been widely employed for purification of antigens and specific antibodies [16]. The results presented here demonstrate that the method can be extended to the purification of small molecules which per se are not antigenic.

For the preparation of the specific antisera ecdysone and 20-hydroxyecdysone were chemically modified. Since ecdysteroids are sensitive to acidic, alkaline and other drastic conditions [17] we tried to avoid any of the above. Thus, it should be stressed that the ecdysteroid-specific antibodies are directed against pure compounds of known structure.

The antisera DUL-1 (anti-ecdysone-albumin) and DBL-1 (anti-20-hydroxyecdysone-albumin) exhibited properties known from similar antisera (for a comparison see: [7,18]). The antibody concentration in the antisera, expressed as dilution factors (DUL-1 = 2500, DBL-1 = 1500), showed the expected values. The antibody specificities were tested with a larger number of ecdysteroids. Obviously both antisera were ecdysteroid specific (Table I). High cross-reaction factors of many ecdysteroids with DUL-1 indicated that this antiserum was ecdysone-specific. In fact, the cross reaction factor of 20-hydroxyecdysone with DUL-1 (= 47) was unique in its size [8]. This antiserum will be useful for the RIA determinations of ecdysone concentrations in biological extracts of insects.

DBL-1 on the other hand exhibited broader specificity for ecdysone and several of its hydroxylated metabolites. This antiserum will be more convenient for the quantitation of ecdysteroid mixtures. However, the intended specificity of DBL-1 for the hapten used for its production (i.e. 20-hydroxyecdysone) was not achieved. In this regard our results confirm the findings of several other laboratories (see [18] and references cited therein). For the lack of specificity for 20-hydroxyecdysone no convincing explanation can be presently given.

Coupling of the antibodies to an insoluble support followed commonly used techniques. The resulting immunoadsorbents prepared from the γ-globulin fractions of antisera DUL-1 and DBL-1 had the following properties:

1) The adsorbents bound ecdysone with a capacity of 1.1 nmol/ml packed adsorbent (DUL-1) and 1.3 nmol/ml (DBL-1). On the basis of bound molecules per ml packed adsorbents the values appear to be low, however, they are similar to the capacity of immunoadsorbents for proteins [19].

2) Nonspecific binding is negligible. This was shown with the immunoadsorbent prepared from nonimmunized rabbit serum, which did not retain steroid.

3) The immunoadsorbents were specific for a definitive steroid structure. Only ecdysone and its hydroxyderivatives 20- and 26-hydroxyecdysone were retained when the DUL-1-Sepharose 4B was tested with a mixture of ecdysteroids which contained ecdysone and at least six different ecdysone metabolites. DBL-1 immunoadsorbent on the other hand had a broader specificity and retained most of the different ecdysteroids. The selectivity of the immunoadsorbents clearly depended on the specificities of the antisera used. For that reason binding properties could be fairly well predicted from the specificities of the antisera in RIA tests for cross reaction factors (Table I).

4) The bound ecdysteroid could be eluted quantitatively by use of a strong chaotropic reagent. When the ecdysteroid was separated from this eluant immediately after elution the structural integrity of the steroid was preserved.

5) The immunoadsorbent was stable and insoluble in the solvents. It could be used repeatedly without loss of affinity and capacity.
The immunoadsorbents for ecdysteroids were developed in order to isolate ecdysone metabolites from non-radiolabelled crude extracts of the blowfly. From the experiment with radiolabelled ecdysteroids it is obvious that DUL-1 immunoadsorbent is too specific for this purpose. DBL-1 immunoadsorbent with its broader specificity seems to be more suitable and will be used for the isolation of unlabelled ecdysteroids from insect material. This work will be reported in the future.

By photoactivation of ecdysteroids these hormones can be linked covalently to macromolecules with affinity for ecdysteroids [20, 21]. Such macromolecules include presumptive transport proteins in the insect haemolymph, intracellular hormone receptors in target organs, as well as, enzymes of ecdysteroid metabolism.

Surprisingly, these ecdysone-protein-complexes are still specifically adsorbed by the immunoadsorbent. They can be dissociated and eluted under appropriate conditions (unpublished results). By these means, for instance, the isolation of sufficient amounts of ecdysteroid receptor will be possible to study its physical properties and to use it for the preparation of antireceptor antibodies.

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