and E₈. (Figure 2). The appearance of sharper and narrower esterase zones seems to be due to the more efficient sieving by much smaller gel pores in 7½% gel compared with the gel pores of 5% gel. In addition to sharper resolution, the carboxylesterase E₈ (which had remained near the origin in 5% gel formulation) moved freely through the courser 3% gel portion and was concentrated at the boundary of the 7½% gel portion. In addition to the improved resolution, quantitative differences were apparent on the gel between esterases of the normal and resistant strain. Supernatants of susceptible and resistant strains, and of the same concentration were separated on the same gel. The quantitative differences observed were found repetitive and thus in my opinion fairly reliable. Of the 10 carboxylesterase zones in the normal strain, 8 esterases were found in the SKA strain. The 2 esterase (E₈ and E₉) which could not be resolved visually, perhaps occur in greatly reduced quantity and thus did not resolve from the closely occurring and highly active esterase E₈. This is further suggested by a rather broader esterase zone E₈ in the densitometric tracing of the resistant strain (Figure 2). Of the observed 8 esterases in the resistant strain all, with the exception of E₄ (second from the anode) showed a reduced level of esterase activity, as can be seen from the densitometric tracings. This is in agreement with the fact that the resistant strain showed one quarter hydrolysis of ethylbutyrate compared with the susceptible strain. The 'low esterase' phenomenon therefore appeared in the SKA strain, not due to the absence of a single and most active anodic band as reported earlier but due to the absence of 2 esterases and reduction in the activity level of all the other esterases with the exception of one. E₄, E₂ and E₉, (Figure 2). The appearance of sharper and narrower bands in the resistant strain was not due to an additional phosphatase enzyme, but is due to an increased production of one of the esterases already present in the susceptible housefly. Clearly more work is required to elucidate the precise involvement of this enzyme.

**Zusammenfassung.** Die Carboxyesterase-Isozyme von 2 Musca domestica-Stämmen wurden analysiert: Die Esteraseaktivität im organophosphat-resistenten Stamm ist bedeutend geringer als im suszeptiblen Stamm. Hingegen ist eines der 10 Isozyme mit Phosphataseaktivität im resistenten Stamm aktiver oder in höherer Konzentration vorhanden als im suszeptiblen Stamm, was die Annahme einer direkten Resistenz-Beziehung nahelegt.

S. Ahmad

**Department of Entomology and Economic Zoology,** Rutgers University, The State University of New Jersey, New Brunswick (New Jersey 08903, USA), 1 October 1973.

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**Human Placental Alkaline Phosphatase, an Inhibitor of Hemagglutination by PR8-Influenza A Virus**

Human placental alkaline phosphatase (EC 3.1.3.1) is a sialoglycoenzyme containing terminal sialic acid moieties. The enzyme preparation, especially its heavy molecular weight variants have been reported to inhibit hemagglutination by Toolan’s H₁ virus isolated from a human neoplasm (HEp₂). The present work was undertaken to determine if purified placental alkaline phosphatase preparations containing heavy molecular weight B variants can inhibit hemagglutination by the well-characterized PR8-influenza A virus and also to ascertain the effects of altering alkaline phosphatase by treatment with proteolytic enzymes, neuraminidase, oxidizing agents, for example sodium metaperiodate and precipitation of the enzyme with specific antiserum on its hemagglutination inhibition (HA-I) titer.

**Materials and methods.** PR8-influenza A virus was a standard preparation supplied by the Viral and Rickettsial Registry and Distribution Center of the American Type Culture Collection, Rockville, Md. 1 hemagglutinating unit is that amount of virus which causes agglutination of 0.2 ml of 0.4% (v/v) adult chicken erythrocytes (Microbiological Associates, Bethesda, Md.). Hemagglutination (HA) and hemagglutination inhibition (HA-I) titrations were carried out as reported in previous communications. The method for purifying alkaline phosphatase was similar to that of Ghosh and Fishman. Alkaline phosphatase activity of enzyme preparations was assayed as described in an earlier publication. Specific activity of alkaline phosphatase is expressed in μmoles of

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shows a chromatogram obtained during purification of

10 B. LEVINSON, D. PEPPER and G. N. K. GHOSH and R. P. Cox, Enzyme

ment with sodium meta-periodate also caused a sub-

stantial decrease in the HA-I titer of the enzyme (Table II).

Treatment of alkaline phosphatase preparations with 1 of

the carbohydrate moities in the glycoprotein by treat-

ment with neuraminidase also led to a reduction in the HA-I titer.

The later fractions (33 to 50) contain low molecular

weight variants A6 and exhibit considerably less or no

HA-I activity. Typical hemagglutination patterns of

HA-I titration decreased with increasing neuraminidase

334 Specialia

and Cox14.

Results and discussions. Table I shows specific alkaline

phosphatase activities and HA-I titers during purification

of an alkaline phosphatase preparation derived from a

single human placenta. The results indicate that as the

purity of alkaline phosphatase increases there is a rise in

the specific HA-I activity. The most active HA-I fractions

from Sephadex G-200 gels with the heavy molecular

weight isoenzymes had a specific enzyme activity of 164

units and a HA-I titer of 136,000 per mg protein. Figure 1

presents a chromatogram obtained during purification of

human placental alkaline phosphatase on Sephadex

G-200 gel. The peaks of enzyme activities of early Sephadex

G-200 eluates contain the heavy molecular weight (B)

variants8 and are fairly superimposable on those of HA-I

titers and sialic acid content. The earliest fractions, 28 to

32 in Figure 1 have the heavy molecular weight, slow

moving isoenzymes B4 and demonstrate high HA-I activities.

The later fractions (33 to 50) contain low molecular

weight variants A6 and exhibit considerably less or no

HA-I activity. Typical hemagglutination patterns of

PR8-influenza A virus before and after incubation with

human placental alkaline phosphatase preparations are

presented in Figure 2.

The release of sialic acid residues from the enzyme

glycoprotein by incubation with Vibrio cholerae neur-
amidase led to a reduction of HA-I titer (Table II). The

HA-I titer decreased with increasing neuraminidase

concentration. The oxidation of the cis-hydroxyl groups of

the carbohydrate moities in the glycoprotein by treat-

ment with sodium meta-periodate also caused a sub-

stantial decrease in the HA-I titer of the enzyme (Table II).

Treatment of alkaline phosphatase preparations with 1 of

Table I. Enzyme activities and HA-I titers of placental alkaline phosphatase preparations during purification

<table>
<thead>
<tr>
<th>Steps in purification</th>
<th>Specific alkaline phosphatase activity (μmoles phenol/min/mg protein)</th>
<th>Specific hemagglutination inhibition titer of alkaline phosphatase (HA-I titer/mg protein x 10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant of placental homogenate</td>
<td>0.3</td>
<td>4</td>
</tr>
<tr>
<td>n-Butanol extract of homogenate</td>
<td>9.3</td>
<td>38</td>
</tr>
<tr>
<td>30-60% Ammonium sulfate fraction</td>
<td>23.0</td>
<td>67</td>
</tr>
<tr>
<td>Sephadex G-200 eluate</td>
<td>164.0</td>
<td>136</td>
</tr>
</tbody>
</table>

* Enzyme preparations were derived from a single placenta but purification was carried out in several batches. b HA-I titers were determined with 8 hemagglutinating units of PR8 influenza A virus in 25 mM Tris-HCl buffer, pH 8.4 containing 150 mM saline.

Fig. 1. Enzyme activity, hemagglutination inhibition titers and sialic acid content of human placental alkaline phosphatase fractions obtained by Sephadex G-200 gel filtration. Alkaline phosphatase (AP) activity (Å) is expressed in μmoles phenol per ml per min. Sialic acid (0) is given in μmoles per ml as determined after hydrolysis with 100 mM sulfuric acid at 80°C for 1 h. HA-I titer(0.1 ml (10⁻³) was measured using 4 HA units of virus and is expressed as the reciprocal of the highest dilution of the enzyme completely inhibiting agglutination of adult chicken erythrocytes. The 60% ammonium sulfate fraction used for gel filtration was prepared by the precipitation method as previously described8.

Table II. Effects of neuraminidase, sodium periodate, chymotrypsin, trypsin and papain on HA-I titer of placental alkaline phosphatase

<table>
<thead>
<tr>
<th>Reagent</th>
<th>HA-I Titer of alkaline phosphatase preparations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Neuraminidase (0.05 U)</td>
<td>30</td>
</tr>
<tr>
<td>Neuraminidase (0.5 U)</td>
<td>12</td>
</tr>
<tr>
<td>Neuraminidase (5 U)</td>
<td>3</td>
</tr>
<tr>
<td>Sodium periodate (75 mM)</td>
<td>12</td>
</tr>
<tr>
<td>Sodium periodate (75 mM)</td>
<td>3</td>
</tr>
<tr>
<td>Chymotrypsin (0.125 mg/ml)</td>
<td>25</td>
</tr>
<tr>
<td>Chymotrypsin (2.0 mg/ml)</td>
<td>0</td>
</tr>
<tr>
<td>Trypsin (0.5 mg/ml)</td>
<td>25</td>
</tr>
<tr>
<td>Trypsin (2.0 mg/ml)</td>
<td>24</td>
</tr>
<tr>
<td>Papain (0.125 mg/ml)</td>
<td>0</td>
</tr>
<tr>
<td>Papain (2.0 mg/ml)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Specific activity 140 units per mg protein.