Specificity of binding of juvenile hormone III to hemolymph proteins of *Leptinotarsa decemlineata* and *Locusta migratoria*

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Summary. Binding specificity of juvenile hormone (JH) III enantiomers and analogs to hemolymph proteins of *Leptinotarsa decemlineata* and *Locusta migratoria* was investigated by competitive displacement tests. The order of binding affinity was 10R-JH-III > 10R, 10S-JH-III >> 10S JH-III > methylfarnesoate for analogs of the epoxide group and diazo-JHA-IV > EFDA for analogs of the methylester. Both the epoxide and ester groups are important for the interaction of JH-III with its binding protein.

Key words. Juvenile hormone III; hormone binding protein; lipophorin; enantioselectivity.

Three different juvenile hormone homologs have been identified in insect hemolymph. The higher homologs (JH-I and JH-II) only occur in Lepidoptera, wheras JH-III seems to be the principal hormone in other species studied so far[1]. In larval hemolymph from a number of Lepidoptera a JH-binding protein of low molecular weight has been characterized, which preferentially binds JH-I and JH-II, and binds JH-III less efficiently[2]. In contrast, a high-molecular-weight binding protein has been discovered in *Leptinotarsa decemlineata*[3], *Locusta migratoria, Periplaneta americana*[4], *Leucophaea maderae*[5] and *Sarcophaga bullata*[6], species known to contain JH-III as the only hormone. These high-molecular-weight binding proteins show high affinity for JH-III and bind JH-I less efficiently. Thus the low-molecular-weight protein seems to be JH-I specific and the high-molecular protein JH-III-specific.

Apart from differences in molecular weight and specificity, some similarity between the two types of binding proteins also exists. Both types of proteins display stereoselectivity, which means that the naturally occurring 10-R enantiomer binds more efficiently than the unnatural 10-S antipode[2,3,7]. However, stereoselectivity of JH-binding in *Locusta migratoria* and *Leptinotarsa decemlineata* was only inferred from an indirect approach. After incubating crude hemolymph with racemic [^{3}H]JH-III, the enantiomer composition of bound and free JH was analyzed by high-performance liquid chromatography after chemical conversion into the corresponding JH-diols and subsequent acylation with (+)-R-α-methoxy-α-trifluoromethylphenylacetic acid chloride[3,7]. However, this approach did not provide quantitative information about the relative affinities of the two enantiomers for the binding protein. Such information can be obtained by competitive displacement with enantiomerically pure 10R and 10S JH-III. This paper describes the affinities of both optical isomers of JH-III for the JH-III-specific binding proteins from the Colorado potato beetle, *Leptinotarsa decemlineata* and the migratory locust, *Locusta migratoria*. These two species were chosen because we found that the two JH-III-specific binding proteins differ essentially in their molecular characteristics. The JH-III specific binding sites in Colorado beetle hemolymph appeared to be associated with the lipophorin fraction[8]. In contrast, JH-III binding occurs to another high-molecular hemolymph protein in locust hemolymph[9].

Hemolymph samples were collected and treated with paraoxon and phenylmethylsulphonyl fluoride as described elsewhere[3]. Suitable amounts of hemolymph, 0.25 µl for the locust and 1.0 µl for the Colorado potato beetle, were incubated in 200 µl TMK-buffer in the presence of 8.000 dpm racemic [^{3}H]JH-III (NEN Chemicals, spec. act. = 11.6 Ci/mmol) and increasing amounts of unlabeled 10R-JH-III, 10S-JH-III (a gift from Dr D. A. Schooley) and racemic (10R/10S) JH-III (Calbiochem., San Diego, USA). After equilibration for 1 h, the JH-protein complex was precipitated with poly (ethylene glycol) (PEG, mol.wt 6000) and counted for radioactivity as previously described[3]. The data were plotted in figure 1. This figure also contains data for methylfarnesoate (a gift from Dr D. A. Schooley) as the unla-

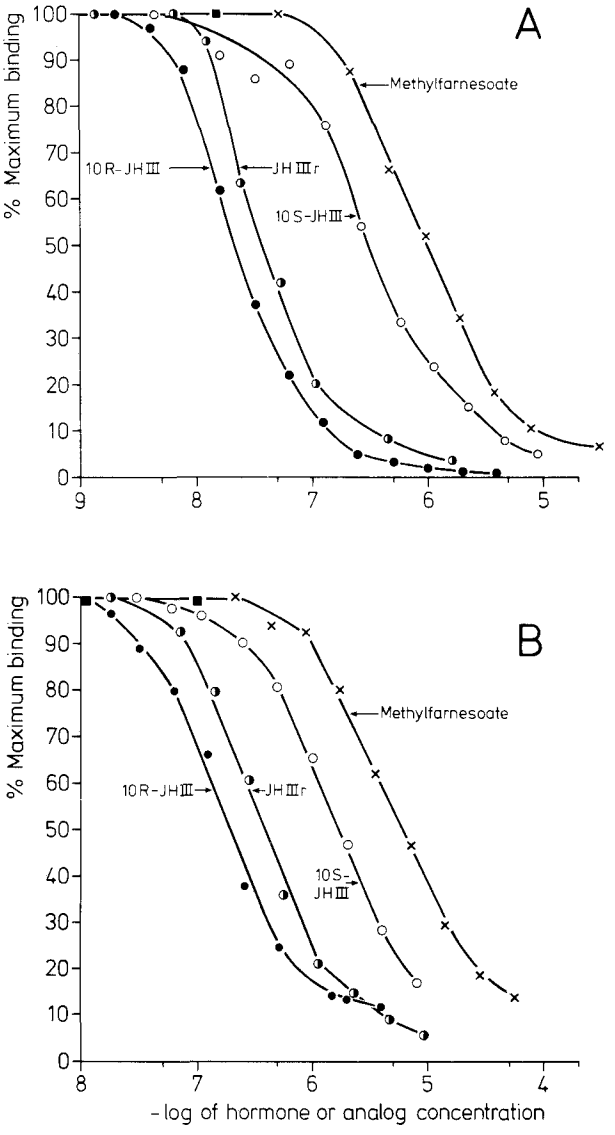

Figure 1. Competitive displacement of racemic [^{3}H]JH-III by unlabeled methylfarnesoate, racemic (r) JH-III, 10R JH-III and 10S JH-III from hemolymph of 5th instar locusts (A) and adult Colorado potato beetle (B). Note that the concentrations of unlabeled ligand are different between A and B.

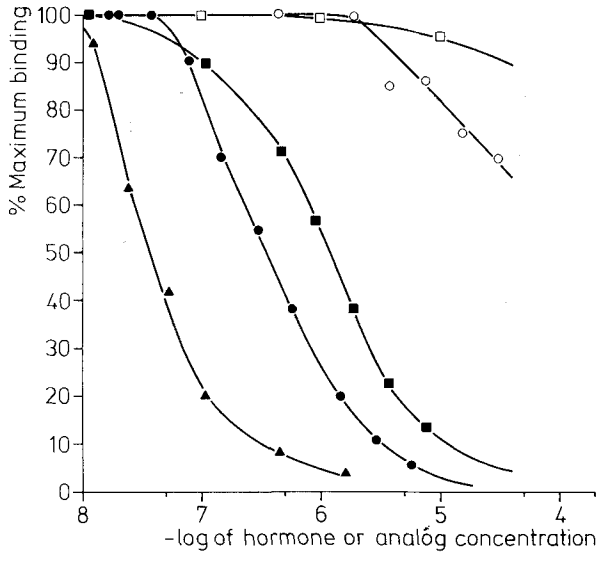

Figure 2. Competitive displacement of racemic [^{3}H]JH-III by unlabeled racemic JH-III (triangles), EFDA (squares) and diazo-JHA-IV (circles) from hemolymph of 5th instar locusts (closed symbols) and adult Colorado potato beetle (open symbols). The displacement curve of racemic JH-III with the hemolymph sample from the Colorado potato beetle has been omitted because it runs too close to one of the other curves (see also fig. 1).

beled ligand. In both species, the order of binding is 10R > 10R,S > > 10S > methylfarnesoate. From these data, apparent dissociation constants (K_d) can be calculated using Scatchard plot analysis. The table summarizes the results of these calculations. The low affinity of the 10S enantiomer is clear from this table. However, the enantiomer preparations used in this study are not completely pure. Each enantiomer contains 8% contamination by the other antipode (Dr D.A. Schooley, pers. commun.). The displacement found by 10S may be due to its 8% contamination with 10R, as also suggested for the low-molecular binding protein of *Manduca sexta*[10]. If this is true, recalculation of the data from figure 1, assuming that only 10R binds and 10S does not, should give similar apparent dissociation constants, irrespective of the enantiomer composition of the mixtures used in these tests. The corrected dissociation constants are also included in the table, which shows that the corrected constants are rather close, suggesting that little competition between 10R and 10S JH-III for the JH-III-specific binding sites occurs. The binding sites apparently have a high stereoselective requirement for the epoxide ring. This can also be concluded from the poor competition by the unepoxidized precursor, methylfarnesoate, for the JH-III-specific binding sites in both species (fig. 1), whereas JH-III diol does not compete at all[3].

Apparent and corrected dissociation contstants (K_d) of different mixtures of JH-III enantiomers calculated form Scatchard plots with hemolymph from Colorado potato beetle and migratory locust

Enantiomer mixture	*Leptinotarsa decemlineata* K_d	K_d, corr	*Locusta migratoria* K_d	K_d, corr
10R (92%R/8%S)	74	9	3.9	2.9
rac. (50%R/50%S)	120	10	6.8	3.3
10S (8%R/92%S)	700	5	14.6	2.4

The K_d values (nmol/l) were obtained by using the total JH concentration, irrespective of the enantiomer composition. The corrected K_d values were calculated by assuming that only 10R binds to the JH-specific binding sites. Hemolymph samples were derived from 4-day-old adult beetles and 5-day-old 5th instar locusts.

Modifications in the structure of the methyl ester function at the other side of the hormone molecule also seem to affect binding. JH-III acid does not bind to hemolymph proteins in the Colorado potato beetle[3]. Moreover, juvenile hormone analogs such as epoxyfarnesyldiazoacetate (EFDA) and diazo-JHA-IV[11] are poor competitors with racemic [^{3}H]JH-III in both insect species (fig. 2).

In conclusion, despite the significant difference between JH-binding proteins from *Manduca sexta, Locusta migratoria* and *Leptinotarsa decemlineata* in their molecular characteristics, their binding properties show some remarkable similarities. In the three types of binding proteins, both the ester and the epoxide groups influence interaction between JH and its binding protein. In *Manduca sexta* the order of binding of the naturally occurring hormones is JH-I > JH-III, whereas in both *Locusta migratoria* and *Leptinotarsa decemlineata* JH-binding proteins are much more specific for JH-III than for JH-I.

Acknowledgments. The gifts of EFDA and diazo-JHA-IV by Dr G.D. Prestwich (Stony Brook, NY) and of 10R, 10S-JH-III and methylfarnesoate by Dr D.A. Schooley (Zoecon Corp., Palo Alto, CA) were highly appreciated. We thank Dr M.A. Khan for critically reading the manuscript and J.C. Rigg for checking the English.

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0014-4754/87/080904-02$1.50 + 0.20/0