The Solubility of Insect Juvenile Hormone in Aqueous Solutions and Its Adsorption by Glassware and Plastics

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(Z. Naturforsch. **32 c**, 158–160 [1977]; received November 11, 1976)

Juvenile Hormone Solubility, Juvenile Hormone Adsorption, Juvenile Hormone Binding to Proteins, Insects

The C_{18} juvenile hormone of insects can be dissolved in aqueous solutions up to a concentration of $2.5-3.0\times10^{-5}$ M; changes in pH, buffer composition and ionic strength hardly affect this solubility. The hormone is salted out gradually by increasing ammonium sulphate concentrations. The juvenile hormone is bound to proteins such as bovine serum albumin or goat immunoglobulin G and can be kept in solution by these proteins up to 10^{-3} M. The hormone is strongly absorbed by many commonly used plastic materials but only to a lesser extent by glass and teflon.

Introduction

The juvenile hormone of insects (JH I), the 7-ethyl-3,11-dimethyl-10-epoxy-2,6-tridecadienoic methyl ester [1], is a lipophilic substance which was formerly thought to have a low solubility in aqueous solutions. It has been shown however [2] that JH I is soluble up to 3.4×10^{-5} M and its lower homologues JH II and JH III are even more readily soluble in water [3]. No quantitative study, however, has ever been published on changes in this solubility caused by shifts in pH and ionic strength, neither has the adsorption of JH by laboratory glassware and plastics been investigated so far. During our research on the JH carrier proteins in insect haemolymph we needed such data in our laboratory in order to avoid artifacts and misleading results. We believe that some of these data might be also of interest for other laboratories working in the same field.

Materials and Methods

Tritium-labeled JH I (NEN Dreieichenhain, spec. act. 14 Ci/mmol) was used in all experiments and purified if necessary by preparative TLC on silica gel with benzene : ethyl acetate 4 : 1 as the solvent system. Unlabeled JH I (stereochemically pure) was bought from Ecocontrol (Cambridge, Mass.). All solutions were prepared as follows: the desired amount of JH, dissolved in hexane, was placed in a Corex centrifuge tube, the solvent evaporated with N_2 at room temperature, 1 ml buffer added and the vials shaken at 25 °C for two hours. The solution was then centrifuged at $12500\times g$ for 10 min. Aliquots were mixed with 10 ml of Aquasol (NEN Dreieichenhain) and counted in a Berthold Liquid Scintillation Counter with an efficiency of 29 – 31%.

Ultrasonification treatment was performed by means of a Branson Sonifier with a 3 mm microtip at 40 W for 7.5 seconds. For surface coating the tubes were immersed in a 5% aqueous solution of Carbowax (polyethylene glycol MW 17 – 20000 from Serva Heidelberg) or in a 1% solution of Siliclad (Clay Adams, Parsippany, N.J.) for 5 hours, rinsed with water and dried at 100 °C.

Schneider's medium [4] was obtained from Gibco (Grand Island, N.Y.), Shield's medium was prepared according to [5].

For the measurement of the JH absorption on different surfaces, thin plates (1×1 cm) of the desired material were agitated gently in 10^{-6} M JH solution in 0.02 M Tris/HCl pH 7.5 for various times. After incubation, the platelets were freed from adhering moisture by blotting briefly with filter paper and counted in 5 ml toluene – Scintimix (Zinsser, Frankfurt a. M.).

Results

The juvenile hormone is quickly dissolved in the buffer, as seen in Fig. 1, the maximum concentration being reached 5 min after addition of the buffer, regardless of the final concentration of the hormone. The decrease in the radioactivity in solution during the first two hours seen in the upper curve is due to a slowly increasing adsorption of labeled material by the tube wall (see also Fig. 6). JH I is soluble in 0.02 M Tris/HCl buffer pH 7.5 up to a concentration of $2.5-3.0\times10^{-5}$ M, a value

* Supported by the Deutsche Forschungsgemeinschaft.

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close to that reported by Kramer *et al.*[2]. Ultrasonic treatment before or after the two hour incubation increases the amount of radioactivity in solution both at low and at high hormone concentrations (Fig. 2). This indicates that sonification leads not

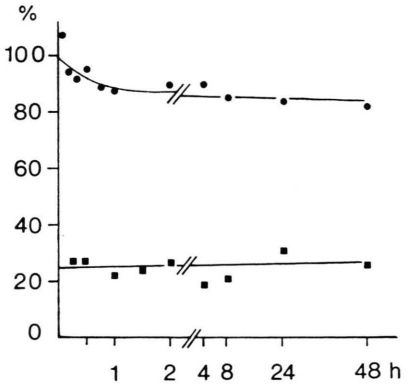

Fig. 1. Dependence of the amount of JH I solubilized in 0.02 M Tris/HCl pH 7.5 on incubation time. Abscissa: Time in hours. Ordinate: Percentage of added JH found in solution. ●–●–● 10^{-6} M JH; ■–■–■ 2×10^{-4} M JH.

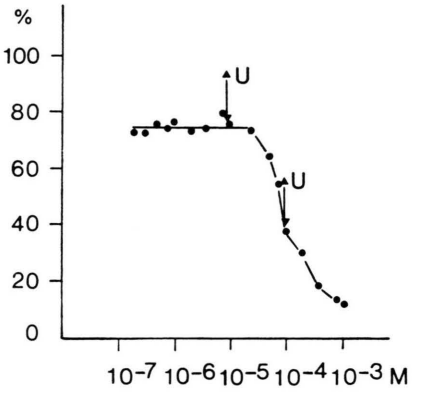

Fig. 2. Percentage of JH I solubilized in 0.02 M Tris/HCl pH 7.5 in relation to the amount added. *U* = effect of ultrasonification on solubility.

only to a rapid and efficient removal of the JH from the glass wall but in addition of the formation of stable and more finely dispersed JH micelles. In order to minimize the adsorption of hormone to the glass surfaces, the tubes were coated with Carbowax or Siliclad. Both treatments reduce the amount of absorbed radioactivity and increase the amount of hormone found in solution by about 5% for concentrations from 10^{-7} to 10^{-5} M. The solubility curve was essentially the same in 0.02 M Tris/HCl from pH 9.0 to 7.2, in 0.02 M Na-phosphate buffer pH 7.0 to 5.0 and in 0.02 M ammonium acetate from 7.5 to 5.0. Addition of 0.5 or 1.0 M NaCl to 0.02 M Tris/HCl has little effect on the solubility, the 1 M concentration shifting the maximum solubility only from $2.5-3.0\times10^{-5}$ M to 1.5×10^{-5} M. $(NH_4)_2SO_4$ however decreases the solubility to 10% at high concentrations (Fig. 3).

The hormone is equally soluble in Schneider's or Shield's medium for *Drosophila* cell culture.

The addition of unspecific proteins to the buffer increases the solubility of JH (Fig. 4) and prevents any absorption of radioactive material to the glass wall. A 5% BSA solution can keep up to 1 mM JH in solution. Goat immunoglobulin G also influences the solubility of JH. The complexes between the hormone and these proteins can be coprecipitated by TCA, but they are not stable during gel filtration

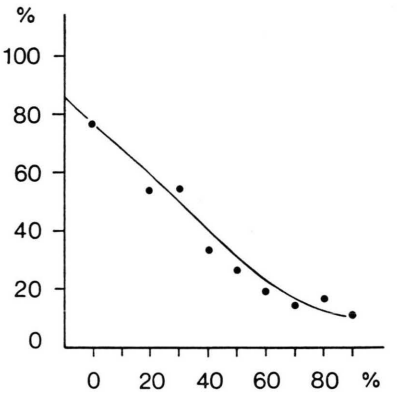

Fig. 3. Effect of increasing concentrations of $(NH_4)_2SO_4$ on JH solubility. Abscissa: % saturation of ammonium sulphate at room temperature; Ordinate: % of JH remaining in solution (initial concentration 10^{-6} M).

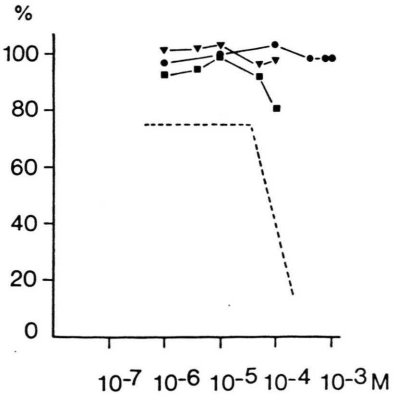

Fig. 4. Effect of various concentrations of bovine serum albumin of JH I solubility. Abscissa: Molar concentration of JH added; Ordinate: % of JH solubilized. ▼–▼–▼ addition of 10% BSA, ●–●–● 5% BSA, ■–■–■ 0.1% BSA.

on Sephadex G 25 (Fig. 5). Only hemoglobin seems to bind a small proportion of the labeled JH I.

As seen from Table I the various materials commonly used for tubes and vials in the laboratory differ widely in their adsorption properties. The lowest values are obtained with surface coated glassware, relatively low adsorption is obtained too with Teflon (polytetrafluoro ethylene) and fairly low values with Plexiglas (polymethacrylate) too. Polyvinylchloride and polystyrene bind remarkable quantities of the labeled material and thus should be avoided in all JH studies. Millipore filters are excellent JH binding material whereas dialysis membranes bind remarkably little hormone. It is interesting that this adsorption of JH from a 10^{-6} M solution to plastics is a relatively slow process, saturation being reached only after 5 hours (Fig. 6).

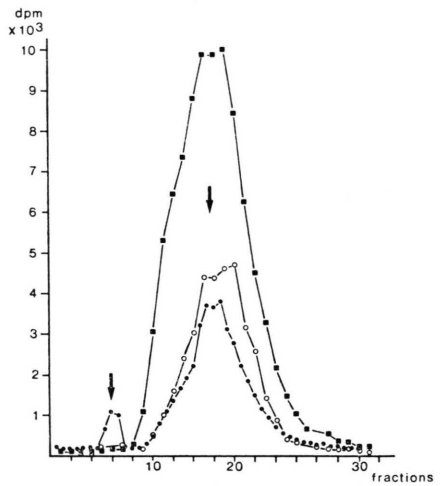

Fig. 5. Gel filtration on Sephadex G25 of a mixture of [³H]JH I and different proteins. Abscissa: Elution volume in ml; Ordinate: dpm of [³H]JH I. ●–●–● JH I and hemoglobin, ○–○–○ JH I and immunoglobulin G, ■–■–■ JH I and bovine serum albumin.

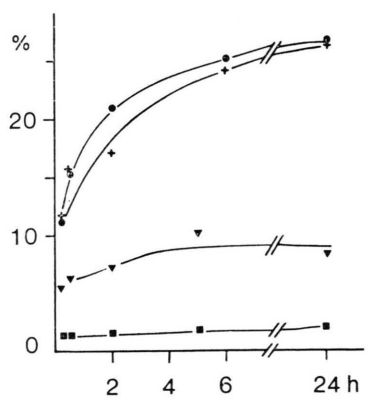

Fig. 6. Time dependance of JH adsorption on various surfaces. Abscissa: time in hours; Ordinate: % of JH I (from 1.5 ml of a 10^{-6} M solution) bound to 1 cm² of surface; ●–●–● polyvinyl chloride, +–+–+ polyethylene, ▼–▼–▼ polyacrylamide, ■–■–■ teflon.

Table I. Adsorption of [³H]JH I on various plastic materials. Plastic sheets of 1 cm² total surface were placed in 1.5 ml of a 10^{-6}M solution of [³H]JH I for 5 hours.

Material	*n*	Adsorption in % of total activity	±σ
Polystyrene	4	43.0	3.9
PVC (polyvinylchloride)	5	26.9	1.8
Polyethylene	4	26.6	1.7
Plexiglas (polymethacrylate)	10	8.5	2.8
Teflon (Polytetrafluoro-ethylene	10	2.2	1.7
Glass	5	1.05	0.1
Glass coated with Carbowax	5	0.46	0.2
Glass coated with Siliclad	3	0.72	0.3
Visking dialysis membrane, untreated	5	0.64	0.2
Millipore filter (PSAC 02510)	2	35.8	—

Discussion

The results show that all biochemical work with JH requires special precautions: definite concentrations of JH in aqueous solutions can be prepared only by dissolving the desired quantity of the hormone by means of a sonifier; solutions above 2×10^{-5} M should be avoided. All work should be done in Carbowax or Silicone treated glassware or teflon vials and tubes; all other plastic material should be omitted. If such material has to be used for special reasons (*e. g.* polystyrene tubes in tissue cultures), it should be kept in mind that the JH concentration in solution may drop during incubation time.

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