Isolation and structure of a novel charged member of the red-pigment-concentrating hormone–adipokinetic hormone family of peptides isolated from the corpora cardiaca of the blowfly *Phormia terraenovae* (Diptera)

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A hypertrehalosaemic neuropeptide from the corpora cardiaca of the blowfly *Phormia terraenovae* has been isolated by reversed-phase h.p.l.c., and its primary structure was determined by pulsed-liquid phase sequencing employing Edman chemistry after enzymically deblocking the *N*-terminal pyroglutamate residue. The *C*-terminus was also blocked, as indicated by the lack of digestion when the peptide was incubated with carboxypeptidase A. The octapeptide has the sequence pGlu-Leu-Thr-Phe-Ser-Pro-Asp-Trp-NH₂ and is clearly defined as a novel member of the RPCH/AKH (red-pigment-concentrating hormone/adipokinetic hormone) family of peptides. It is the first charged member of this family to be found. The synthetic peptide causes an increase in the haemolymph carbohydrate concentration in a dose-dependent fashion in blowflies and therefore is named '*Phormia terraenovae* hypertrehalosaemic hormone' (Pht-HrTH). In addition, receptors in the fat-body of the American cockroach (*Periplaneta americana*) recognize the peptide, resulting in carbohydrate elevation in the blood. However, fat-body receptors of the migratory locust (*Locusta migratoria*) do not recognize this charged molecule, and thus no lipid mobilization is observed in this species.

INTRODUCTION

In insects, the corpora cardiaca are major neurohaemal organs, which not only store and release neurosecretory products synthesized in the brain, but also contain intrinsic glandular cells producing a variety of bioactive factors affecting, for example, metabolic processes. Many of these factors appear to be peptides involved in the regulation of carbohydrate release, mostly that of trehalose, the major blood sugar of insects [1,2]. The presence of such hypertrehalosaemic factors is inferred in a wide range of insect species [1,3] in which glycogenolysis, phosphorylase activation or the increase of carbohydrates in the haemolymph occur in response to injections of extracts of their own corpora cardiaca, or in which extracts of their corpora cardiaca are hypertrehalosaemic in cockroaches [1,4,5]. Hypertrehalosaemic peptides have been fully characterized in certain cockroaches (*Periplaneta americana*, *Blaberus discoidalis* and *Nauphoeta cinerea*) [6–10], stick insects (*Sipylloidea sipylus* and *Carasius morosus*) [11,12], and a moth (*Heliothis zea*) [13]. All these peptides are structurally similar and belong to the so-called 'red-pigment-concentrating hormone/adipokinetic hormone family' of peptides (RPCH/AKH family). They are characterized by being uncharged, *N*- and *C*-terminally blocked, and possessing a phenylalanine residue at position 4 and a tryptophan residue at position 8 [2,14]. Although it is well known that blowflies use almost exclusively carbohydrates for flight activity [15,16], previous studies on the blowflies *Calliphora erythrocephala* and *Phormia regina* have only shown the existence of a hypertrehalosaemic substance, and no progress in its characterization has been reported [17–19].

Recently, our own research provided evidence that the corpus cardiacum of the blowfly *Phormia terraenovae* contains a factor that causes activation of abdominal glycogen phosphorylase and an increase of blood trehalose levels in this insect as well as in the cockroach *Periplaneta americana* [20]. In addition, release of hypertrehalosaemic material was shown by collecting and purifying blood from stressed blowflies and subsequently injecting the peptide fraction into acceptor cockroaches [20]. The present work was performed to isolate the hypertrehalosaemic compound from *P. terraenovae* and to elucidate its primary structure.

MATERIALS AND METHODS

Insects

Blowflies (*Phormia terraenovae*) were reared as described previously [21]. Adult (4–7-day-old) males were used throughout the present study. Adult (15–25-day-old) male migratory locusts (*Locusta migratoria*) were purchased from a commercial dealer, and adult male American cockroaches (*Periplaneta americana*) were gifts from the Department of Zoology, University of Regensburg, Regensburg, Germany, or from Fa. Thompson, Düsseldorf, Germany. The latter two species were maintained in our insectary in Düsseldorf at 25 °C with a 14 h-dark/10 h light cycle and were fed as described previously [5].

Isolation of blowfly hypertrehalosaemic peptide (Pht-HrTH)

Corpora cardiaca/corpora allata complexes from *P. terraenovae* were collected by dissection as outlined previously

Abbreviations used: RPCH, red-pigment-concentrating hormone; AKH, adipokinetic hormone; Pht-HrTH, *Phormia terraenovae* hypertrehalosaemic hormone; RP-, reversed-phase; PITC, phenyl isothiocyanate; PTC, phenylthiocarbonyl; PTH, phenylthiohydantoin; Pea-CAH-I, *Periplaneta americana* hypertrehalosaemic hormone.

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[20], and the preparation of methanolic extracts was as in [22]. These extracts were applied to a Nucleosil 100 C₁₈ column for reversed-phase (RP-) h.p.l.c. in batches of 100 glands by using the equipment described previously [23]. The active fraction was collected manually, and the combined material from a total of 700 corpora cardiaca was used for the determination of the amino acid composition and for primary-structure elucidation.

Amino acid composition

An h.p.l.c.-purified aliquot (equivalent to 200 corpora cardiaca) of the active fraction was dried *in vacuo* (Savant Speed Vac concentrator) and hydrolysed with 5.7 M-HCl for 24 h. Precolumn derivatization with phenyl isothiocyanate (PITC) and detection of the phenylthiocarbamyl (PTC) amino acids at 254 nm after separation of the derivatives by RP-h.p.l.c. (Spherisorb ODS II; 3 μ material; column size 125 mm $\times$ 4.6 mm) were essentially as described in [24]. The presence of tryptophan was deduced from spectrophotometric data. The active peak material was monitored with a fluorimeter (excitation at 276 nm; emission at 350 nm; Shimadzu fluorescence spectromonitor RF-530).

Enzymic deblocking and pulsed-liquid phase sequencing

The dried blowfly hypertrehalosaemic material (equivalent to 400 corpora cardiaca) was enzymically deblocked using L-pyroglutamate aminopeptidase as described previously [25]. The deblocked peptide was separated from the intact molecule by RP-h.p.l.c. (for conditions see Fig. 2), manually collected, dried by vacuum centrifugation, and used for pulsed-liquid sequencing.

Automated Edman degradation was performed with a pulsed-liquid phase sequencer (model 477A; Applied Biosystems, Foster City, CA, U.S.A.) connected to an on-line phenylthiohydantoin (PTH) amino acid analyser (model 120A; Applied Biosystems). Sequencing reagents and solvents were from Applied Biosystems. Sequencing and PTH analysis were carried out with standard programs.

C-Terminal analysis

An aliquot of the h.p.l.c.-purified blowfly peptide (equivalent to 100 corpora cardiaca) was treated with carboxypeptidase A as described in [8]. H.p.l.c. equipment and solvents were the same as in [23], but the gradient ran from 30 to 56% B in 20 min.

Synthetic peptide

The synthetic blowfly peptide was a gift from Dr. M. H. Schaffer (University of Texas, Dallas, TX, U.S.A.). This peptide is now commercially available from Novabiochem G.m.b.H. (Sandhausen, Germany). Synthetic Pea-CAH-I, the hypertrehalosaemic hormone of the American cockroach, came from Peninsula Laboratories (Belmont, CA, U.S.A.).

Bioassays

Haemolymph sampling, injection of material into adult blowflies and determination of their haemolymph carbohydrate and lipid concentrations are described elsewhere [20]. The bioassays in cockroaches and locusts were performed as described in [26].

RESULTS

Peptide purification

A crude methanolic extract of 100 glands from *P. terraenovae* was fractionated on an analytical RP-h.p.l.c. column in a gradient mode using trifluoroacetic acid as ion-pairing reagent. This method proved to be successful in isolating a fraction with a

Fig. 1. Isolation of blowfly hypertrehalosaemic factor

The chromatogram shows the separation of a methanolic extract from 100 corpora cardiaca. The analysis was performed on a Nucleosil C₁₈ column, which was eluted with a linear gradient of 0.11% trifluoroacetic acid (solvent A) and 0.1% trifluoroacetic acid in 60% acetonitrile (solvent B). The gradient ran from 20 to 80% B within 60 min at a flow rate of 1 ml/min. The elution was monitored at 214 nm. Fractions (1 ml each) were collected, dried *in vacuo*, dissolved in 100 μ l of distilled water, and an aliquot (10 μ l) was injected into at least six cockroaches. An increase in blood carbohydrates was found only in fraction 43 (indicated by ●).

retention time of 42.5 min that elevated carbohydrates in cockroaches and corresponded to a distinct absorbance peak (Fig. 1); no other fractions were active in this bioassay, including those fractions which showed very prominent absorbance peaks (16 min; between 45 and 48 min; Fig. 1).

Amino acid composition and primary-structure data

The results of the amino acid analysis after hydrolysis in HCl of an aliquot of the h.p.l.c.-purified material are summarized in Table 1. The presence of seven amino acids was shown. An additional tryptophan residue was indicated by spectrophotometric data; chromatography of another batch of corpora cardiaca also showed a distinct peak at 42.5 min when monitored with a fluorimeter (results not shown). Fluorescence at 276 nm indicates the presence of either tyrosine or tryptophan; but tyrosine, which is not destroyed during acid hydrolysis, as is tryptophan, was not detected by amino acid analysis (cf. Table 1).

Another aliquot of the h.p.l.c.-purified blowfly peptide was incubated with pyroglutamate aminopeptidase and subjected to RP-h.p.l.c. (Fig. 2). Complete digestion was reached after 60 min, and a new absorbance peak with a retention time of 4.4 min less than that of the undigested peptide was observed. This peak yielded the sequence given in Table 2. The last amino acid was detected in cycle 7 (tryptophan), and the next two gave no degradation product. Together with the *N*-terminal pyroglutamate residue, which was split off by enzymic digestion, this sequence accounts for all amino acids found in the amino acid analysis and by the fluorimetric data.

Incubation of an aliquot of the h.p.l.c.-purified blowfly peptide with carboxypeptidase A in the presence of Leu-enkephalin resulted in no change in retention time for the blowfly peptide when re-chromatographed, whereas Leu-enkephalin was cleaved and a new absorbance peak was observed, indicating that the C-terminus of the blowfly peptide was blocked (results not shown).

Table 1. Amino acid composition of the blowfly hypertrehalosaemic peptide after total hydrolysis

H.p.l.c.-purified bioactive material from 200 corpora cardiaca was hydrolysed with 5.7 M-HCl for 24 h and, after derivatization, the PTC amino acids were quantified by h.p.l.c. Values are given as molar ratios with respect to leucine (= 1).

Amino acid residue	Composition
Asx	0.97
Glx	1.06
Ser	0.90
Thr	0.94
Pro	1.10
Leu	= 1
Phe	0.96

Table 2. Amino acid sequence of blowfly hypertrehalosaemic peptide by automated Edman degradation

The *N*-terminal pyroglutamate residue of purified hypertrehalosaemic peptide from 400 corpora cardiaca was cleaved off by pyroglutamate aminopeptidase, and the deblocked peptide was analysed.

Amino acid	Recovery (pmol)
Leu ¹	56
Thr ²	21
Phe ³	50
Ser ⁴	11
Pro ⁵	36
Asp ⁵	13
Trp ⁷	11
— ⁸	—
— ⁹	—

Fig. 2. Enzymic deblocking of blowfly hypertrehalosaemic peptide

The bioactive material from 400 corpora cardiaca previously purified by RP-h.p.l.c. was incubated with pyroglutamate aminopeptidase for 60 min. After digestion, the material was fractionated on a Nucleosil C₁₈ column with the solvents described in Fig. 1. The linear gradient ran from 33 to 58% B during 25 min at a flow rate of 1 ml/min. The fraction labelled 'Pht-HrTH' had hypertrehalosaemic activity in cockroaches (indicated by ●), whereas the fraction with the new peptide (labelled 'Pht-HrTH (-pGlu)') had no activity.

Comparison of natural and synthetic peptide

A peptide with all the structural characteristics that we had determined for the blowfly peptide has recently been isolated from *Drosophila melanogaster* (the accompanying paper [27]) and the synthetic peptide, C-terminally blocked with an amide, was made available to us by this group. This synthetic octapeptide had the same chromatographic behaviour in our h.p.l.c. system as the natural blowfly peptide and was co-eluted as a single absorbance peak when co-injected (results not shown).

The synthetic blowfly peptide was tested in three different bioassays. It had no hyperlipaemic activity in locusts, despite using a dose as high as 50 pmol for injections (Table 3). On the other hand, haemolymph carbohydrates in the cockroach were elevated significantly when a low dose of 1 pmol was injected. The maximal effect, however, was reached after injection of 5 pmol, and 10 and 50 pmol had no greater effect. This maximal response was only about 65% of that achieved by injecting the cockroach hypertrehalosaemic hormone I (Pea-CAH-I; Table 3). Con-specific injection of the synthetic blowfly peptide into *P. terraenovae* caused a dose-dependent increase in the blood carbohydrate concentration, but failed to elicit an increase of haemolymph lipid levels (Figs. 3a and 3b).

DISCUSSION

Using essentially one step of RP-h.p.l.c. we successfully isolated a peptide from corpora cardiaca of the blowfly *P. terraenovae* sufficiently pure for structure elucidation. A previous attempt to isolate such corpora-cardiaca peptides from another group of dipterans, namely the Tabanidae, needed a combination of three RP-h.p.l.c. steps to obtain a pure preparation [28]. Brief enzymic digestion of our peptide with pyroglutamate aminopeptidase yielded a deblocked peptide which was accessible for sequence analysis by Edman degradation. Sequential degradation showed all the amino acids expected from amino acid analysis and revealed an octapeptide. Treatment with carboxypeptidase A showed that the C-terminus was blocked; all other members of the RPCH/AKH family have a primary amide at the C-terminus, which is consistent with a neurohormonal function, since it protects such peptides from haemolymph-borne carboxypeptidase. Amidation at the C-terminus for the blowfly peptide was established by synthesis. Thus the full structure characterizes this peptide unequivocally as a novel octapeptide member of the RPCH/AKH family: it is C- and N-terminally blocked by an amide and a pyroglutamate residue respectively, and has phenylalanine and tryptophan residues at positions 4 and 8 respectively [14]. However, the molecule is unique in having a charged residue, i.e. aspartic acid, at position 7. All other members known so far have an asparagine, serine or glycine residue at position 7 [14].

The negative charge at position 7 does argue that the conformation of the new peptide may be quite different when compared with the other, uncharged, family members. This, then, should also influence the receptor of the blowfly. Our bioassay data show clearly that receptors in the blowfly recognize

Table 3. Adipokinetic and hypertrehalosaemic activity of blowfly hypertrehalosaemic peptide in locusts and cockroaches respectively

Insects were injected as indicated. Total haemolymph lipid concentration in adult male locusts and total haemolymph carbohydrate concentration in adult male cockroaches are expressed as mg/ml of blood, and results are means $\pm$ S.D. The significance of the difference between values before and after injection is indicated by $^aP < 0.01$ and $^bP > 0.001$ using paired t tests.

Treatment	Acceptor insect...	<i>Locusta migratoria</i>				<i>Periplaneta americana</i>			
		Blood lipids (mg/ml)			<i>n</i>	Blood carbohydrates (mg/ml)			<i>n</i>
		0 min	90 min	Difference		0 min	120 min	Difference	
Control (10 μ l of distilled water)		6	11.0 $\pm$ 3.5	13.5 $\pm$ 2.8	2.5 $\pm$ 1.6	6	13.8 $\pm$ 1.9	16.4 $\pm$ 2.6	2.6 $\pm$ 2.0
Synthetic blowfly peptide									
1 pmol	—	—	—	—	7	13.5 $\pm$ 2.3	20.2 $\pm$ 3.6	6.7 $\pm$ 2.2 ^a	
5 pmol	—	—	—	—	7	13.5 $\pm$ 2.2	29.7 $\pm$ 2.8	16.2 $\pm$ 2.7 ^b	
10 pmol	8	11.9 $\pm$ 5.2	12.9 $\pm$ 4.7	1.0 $\pm$ 1.4	7	12.5 $\pm$ 2.2	30.1 $\pm$ 3.4	17.6 $\pm$ 2.9 ^b	
20 pmol	8	9.0 $\pm$ 3.3	11.2 $\pm$ 4.3	2.2 $\pm$ 2.7	—	—	—	—	
50 pmol	5	10.5 $\pm$ 2.4	13.3 $\pm$ 4.2	2.8 $\pm$ 2.4	4	12.9 $\pm$ 2.7	30.0 $\pm$ 1.9	17.1 $\pm$ 0.7 ^b	
Synthetic Pea-CAH-I (10 pmol)	—	—	—	—	7	13.3 $\pm$ 3.2	40.4 $\pm$ 7.2	27.1 $\pm$ 5.4 ^b

Fig. 3. Effect of synthetic blowfly hypertrehalosaemic peptide (Pht-HrTH) on blood carbohydrate and lipid levels in *P. terraenovae*

Blowflies were injected as follows: □, distilled water; ■, 0.75 pmol of Pht-HrTH; ▨, 1.5 pmol of Pht-HrTH; ▩, 3 pmol of Pht-HrTH. Total haemolymph carbohydrate (a) and lipid (b) concentrations in 4-day-old adult blowflies are expressed as mg/ml of blood and are means $\pm$ S.D. ($n = 11$). A significant difference from control values is indicated by $^*P < 0.05$ and $^{**}P < 0.001$.

the molecule and that the interaction leads to an elevated haemolymph carbohydrate concentration. The locust, however, has quite specialized fat-body receptors, as judged from previous structure-activity studies using naturally occurring members of the RPCH/AKH family and certain analogues to induce lipid mobilization [29]. This is supported by the present investigation: no lipid release is seen when the charged blowfly peptide is injected into locusts at a dose up to 50 pmol. All other members of this family produced at least 50 % of the maximal adipokinetic response in locusts with this dose [29]. The structural requirements of the fat-body receptors in the American cockroach are thought to be much less specific than those of the locusts [29]. This statement appears to be supported by the present results; the blowfly peptide is recognized by the cockroach's receptors, but apparently it does not fit completely, because only 60 % of the maximal hypertrehalosaemic response is produced. However,

it should be borne in mind that this statement is made without taking into consideration, for example, differential access to the receptor or accelerated breakdown of the blowfly peptide by proteinases in the cockroach's haemolymph. Nevertheless, it is tempting to speculate that our results argue in favour of the concept of co-evolution of receptor and ligand.

The physiological role of the blowfly peptide is the mobilization of carbohydrates during flight. From previous studies it appears that *P. terraenovae*, like other blowflies, uses carbohydrates as the sole fuel for flight [30,31]. After 1 h of flight the glycogen content of the fat-body is significantly decreased and the haemolymph carbohydrate concentration is elevated [20]. Furthermore, a hypertrehalosaemic factor is released into the haemolymph of stressed flies [20]. It seems reasonable to suggest that this compound is the newly discovered blowfly peptide characterized in the present study. Therefore, we suggest the name '*Phormia terraenovae* hypertrehalosaemic hormone' (Pht-HrTH), consistent with our proposed nomenclature for members of the RPCH/AKH family of peptides [32].

The blowfly peptide shows the most striking resemblance (80 %) of its primary structure to the octapeptide sequenced from species of the Tabanidae (pGlu-Leu-Thr-Phe-Thr-Pro-Gly-Trp-NH₂) [28,33] and the hypertrehalosaemic hormone II of the American cockroach (pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH₂) [6–9]. Interestingly, the novel peptide shares its N-terminus (1–5) with the hypertrehalosaemic decapeptide isolated and sequenced from the corpora cardiaca of a lepidopteran species, *Heliothis zea* (corn earworm moth) (pGlu-Leu-Thr-Phe-Ser-Ser-Gly-Trp-Gly-Asn-NH₂) [13].

Finally, it is noteworthy that, within the order Diptera, the species *P. terraenovae* (family Calliphoridae; the present study) and the fruitfly *Drosophila melanogaster* (family Drosophilidae; [27]), both belonging to the infraorder Cyclorhapha, contain one identical octapeptide, whereas members of the family Tabanidae, belonging to the infraorder Brachycera, contain an octa- and a deca-peptide with different primary structures [28,33]. Family specificity has also been observed in the order Coleoptera (beetles) [34].

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REFERENCES

1. Goldsworthy, G. J. & Gäde, G. (1983) in *Endocrinology of Insects* (Downer, R. G. H. & Laufer, H., eds.), pp. 109–119, Alan Liss, New York
2. Wheeler, C. H., Gäde, G., Goldsworthy, G. J. (1988) *Soc. Exp. Biol. Semin. Ser.* **33**, 141–157
3. Steele, J. E. (1983) in *Endocrinology of Insects* (Downer, R. G. H. & Laufer, H., eds.), pp. 427–439, Alan Liss, New York
4. Gäde, G. (1984) *J. Insect Physiol.* **30**, 729–736
5. Gäde, G. & Scheid, M. (1986) *Physiol. Entomol.* **11**, 145–157
6. Scarborough, R. M., Jamieson, G. C., Kalish, F., Kramer, S. J., McEnroe, G. A., Miller, C. A. & Schooley, D. A. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5575–5579
7. Witten, J. L., Schaffer, M. H., O'Shea, M., Cook, J. C., Hemling, M. E. & Rinehart, K. L., Jr. (1984) *Biochem. Biophys. Res. Commun.* **124**, 350–358
8. Siegert, K. J. & Mordue, W. (1986) *Physiol. Entomol.* **11**, 205–211
9. Hayes, T. K., Keeley, L. L. & Knight, D. W. (1986) *Biochem. Biophys. Res. Commun.* **140**, 674–678
10. Gäde, G. & Rinehart, K. L., Jr. (1986) *Biochem. Biophys. Res. Commun.* **141**, 774–781
11. Gäde, G. & Rinehart, K. L., Jr. (1987) *Biol. Chem. Hoppe-Seyler* **368**, 67–75
12. Gäde, G. (1989) *Physiol. Entomol.* **14**, 405–418
13. Jaffe, H., Raina, A. K., Riley, C. T., Fraser, B. A., Bird, T. G., Tseng, C.-M., Zhang, Y.-S. & Hayes, D. K. (1988) *Biochem. Biophys. Res. Commun.* **155**, 344–350
14. Gäde, G. (1990) *J. Insect Physiol.* **36**, 1–12
15. Sacktor, B. (1970) *Adv. Insect Physiol.* **7**, 267–347
16. Wheeler, C. H. (1989) In *Insect Flight* (Goldsworthy, G. J. & Wheeler, C. H., eds.), pp. 273–303, CRC Press, Boca Raton
17. Friedman, S. (1967) *J. Insect Physiol.* **13**, 397–405
18. Normann, T. C. & Duve, H. (1969) *Gen. Comp. Endocrinol.* **12**, 449–459
19. Vejbjerg, K. & Normann, T. C. (1974) *J. Insect Physiol.* **20**, 1189–1192
20. Wilps, H. & Gäde, G. (1990) *J. Insect Physiol.*, in the press
21. Collatz, K.-G. & Wilps, H. (1985) *Exp. Gerontol.* **20**, 347–357
22. Gäde, G., Goldsworthy, G. J., Kegel, G. & Keller, R. (1984) *Hoppe-Seyler's Z. Physiol. Chem.* **365**, 393–398
23. Gäde, G. (1985) *Biol. Chem. Hoppe-Seyler* **366**, 195–199
24. Heinrichson, R. L. & Meredith, S. C. (1984) *Anal. Biochem.* **136**, 65–74
25. Gäde, G., Hilbich, C., Beyreuther, K. & Rinehart, K. L. (1988) *Peptides* **9**, 681–688
26. Gäde, G. (1980) *J. Insect Physiol.* **26**, 351–360
27. Schaffer, M. H., Noyes, B. E., Slaughter, C. A., Thorne, G. C. & Gaskell, S. J. (1990) *Biochem. J.* **269**, 315–320
28. Jaffe, H., Raina, A. K., Fraser, B. A., Keim, P., Rao, K. R., Zhang, Y.-S., Lancaster, J. L. & Hayes, D. K. (1988) *Biochem. Biophys. Res. Commun.* **151**, 656–663
29. Gäde, G. (1990) *Physiol. Entomol.*, in the press
30. Wilps, H. & Collatz, K.-G. (1986) *Comp. Biochem. Physiol.* **83B**, 435–440
31. Wilps, H. & Collatz, K.-G. (1986) *Comp. Biochem. Physiol.* **84A**, 325–330
32. Raina, A. K. & Gäde, G. (1988) *Insect Biochem.* **18**, 785–787
33. Jaffe, H., Raina, A. K., Riley, C. T., Fraser, B. A., Nachman, R. J., Vogel, V. W., Zhang, Y.-S. & Hayes, D. K. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8161–8164
34. Gäde, G. (1989) *J. Comp. Physiol. B* **159**, 589–596

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