Proc. Indian natn. Sci. Acad. **B48** No. 4 pp. 476–480 (1982)

Turnover of Proteins in Haemolymph of the Desert Locust, *Schistocerca gregaria* Forsk*.

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(*Received 8 April 1982*)

Turnover of ^{14}C-labelled haemolymph proteins (HP) and ^{131}I-labelled human serum albumin has been investigated in the haemolymph of the adult desert locust, *Schistocerca gregaria* Forsk. HP was found to have half-life of 6.40–6.87 and 2.98–3.91 days based on protein radioactivity, and specific activity criteria, respectively in locusts. The half-life of ^{131} I-human serum albumin was observed to be in the range of 32.73–40.77 min. The physiological significance of turnover of proteins in the desert locust is discussed.

Key Words: Turnover, Haemolymph proteins, Human serum albumin, Desert locust, *Schistocerca gregaria* Forsk.

Introduction

A Survey of various reviews (Chen 1971, 1978, Thomson 1975, Wyatt & Pan 1978), show that there is still a considerable lack of information on turnover of haemolymph proteins (HP) in insects. Limited work carried out so far suggest that larval proteins are degraded at varying rates before being incorporated into adult proteins during insect development (Agrell 1964, Chen & Levenbook 1966, Dinamarca & Levenbook 1966, Williams & Birt 1972). Further the evidence has been accumulating to suggest that the rate of turnover of various HPs differs significantly in various insect species (Boyd & Mitchell 1966, Tobe & Loughton 1966, 1970, Maynard Smith et al. 1970).

Although three major groups of HP have been identified as occurring in the desert locust *Schistocerca gregaria* (Kulkarni & Mehrotra 1970), only recently it was demonstrated that ^{14}C-leucine injected in haemolymph gets readily incorporated in HP (Nakat 1974). The present work attempts to study the rate of locusts' own HP and extraneous protein, such as human serum albumin since there is not much information available in this regard.

Materials and Methods

Experimental Animals

Insects used in the study were seven-day-old adults of the desert locust, *S. gregaria*, belonging to gregarious phase. These were obtained from a laboratory culture maintained according to Mehrotra and Rao (1966).

*Part of Ph.D. thesis submitted by the first author

Adults emerging within 24 hr period were collected and used for these studies so as to maintain uniformity in age as far as possible. Seven-days-old adults were used because it is known that by this time, adult characteristics get fixed in the locust (Norris 1957).

Chemicals

^{14}C-(U)l-leucine (290 mCi/mmol) and ^{131}I-human serum albumin (21 μCi/mg) (HSA) were procured from the Isotope Division, Bhabha Atomic Research Centre, Trombay.

All other chemicals used were analytical grade reagents of highest purity.

Preparation of ^{14}C-labelled HP

The method of preparation of ^{14}C-labelled HP was essentially same as that of Tobe and Loughton (1969); 4 μCi of ^{14}C-leucine was injected per locust through the 5th intersegmental membrane of the abdomen. The locusts were bled 48 hr after injection of labelled leucine and haemolymph collected by the method of Kulkarni and Mehrotra (1970). The haemolymph was pooled and centrifuged *ca.* 4000 rpm for 5 min to remove haemocytes as well as suspending particles. The labelled haemolymph was dialysed against sugar to remove free ^{14}C-leucine. The labelled haemolymph when subjected to gel chromatography on Sephadex G 200 was found to have its radioactivity mainly associated with the fraction having molecular weight of 480,000 daltons and above. More than 80% of radioactivity and 84% of proteins were recovered in this fraction. This fraction corresponded to F 1 fraction and part of F 2 fraction of Kulkarni and Mehrotra (1970). The specific activity of proteins in the haemolymph sample ranged between 23699 and 24351 dpm/mg protein. The Trichloroacetic acid (TCA) precipitable fraction of haemolymph was taken to represent proteins. All these operations were carried out at 4–8°C. The labelled HP was stored at −5°C until used. The storage period was kept to a minimum so as to avoid melanization of haemolymph and possible degradation of HP.

Experimental Procedure

Adult locusts were injected with ^{14}C-labelled HP and ^{131}I-labelledH SA at pleurosternal region of the 5th inter-segmental membrane of abdomen. Injections were performed with the help of an alcohol sterilized 27 gauge needle attached to 1 ml tuberculin syringe operated with microapplicator (M1025, ISCO, USA). The male locusts were injected with 15.5 μl of labelled haemolymph containing 7488 dpm TCA precipitable activity in 0.31 mg proteins, whereas the female locusts were injected with 25 μl of labelled haemolymph containing 16115 dpm TCA precipitable activity in 0.68 mg proteins. The volume of ^{14}C-labelled haemolymph sample injected was about 7% of the total haemolymph volume of the locusts. For experiments with ^{131}I-HSA, 5 μl of labelled serum albumin (specific activity 1.05 μCi/50 μg protein) was injected in the adult locust.

At various time intervals, the locusts were bled and haemolymph collected. Samples (5 or 10 μl) of haemolymph were processed for assaying the total and TCA precipitable activity.

Processing of Haemolymph Samples

The method of sample processing was essentially that of Mans and Novelli (1961) as modified by Tobe and Davey (1975). The haemolymph samples (5 or 10 μl) were spotted on Whatman No. 42 filter paper discs of 2 cm diameter and air-dried. For counting of total radioactivity, air-dried discs were transferred directly to scintillation vials. Estimation of TCA precipitable activity was done on the filter paper discs which were treated with 10 μl of 20% freshly prepared cold TCA solution and air-dried. These discs were again

treated with 2 ml of ice-cold 10 per cent TCA for 45 min at 4–8°C; transferred to 5% TCA for 15 min at room temperature. After TCA treatment, the discs were suspended in ether-ethanol mixture (5 : 2 v/v) for 30 min and then transferred to ether for 15 min. Finally, they were washed with ether and air-dried before transferring to scintillation vials for assaying TCA precipitable activity.

Assay of Radioactivity

Processed samples on filter paper discs were assayed for their radioactivity using 5 ml of toluene-based scintillation cocktail. The scintillation cocktail was prepared according to Mans and Novelli (1961); the cocktail contained 4 g PPO, 100 mg POPOP in 1 L toluene.

Besides ^{14}C-labelled samples, ^{131}I-labelled samples were also estimated by liquid scintillation spectrometry (Polesky & Seligson 1965). The radioactivity was measured by using Packard Tricarb Liquid Scintillation Spectrometer (Model 3255).

Usual corrections for background and quenching were made.

Estimation of HP

Proteins present in the haemolymph samples were estimated by the method of Lowry et al. (1951) using bovine serum albumin fraction V (Sigma) as a standard. All optical density measurements were made by using ECIL–GS 865 spectrophotometer at 610 nm.

Results

It is evident from figure 1 A and B that the loss of HP and HSA from the haemolymph followed a first-order kinetics. That this contention was true can be seen from the fact that even when the data were analysed by two different criteria, i.e., total TCA precipitable activity and specific activity of the proteins present in the haemolymph, it showed

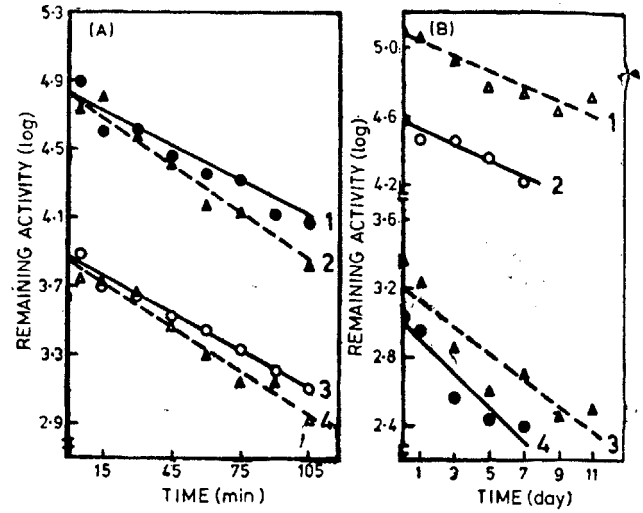

Figure 1. A, B, A, Loss of human serum albumin in male (1 & 3) and female (2 & 4). locusts 1 and 2 specific activity, 3 and 4 TCA precipitable activity. **B,** Loss of HP—legend as in Figure. 1 A.

the linear relationship with time, thus confirming the first-order kinetics for the loss of HP and HSA Further confirmation that the first-order kinetics was followed, was established by subjecting the data to least square fitting by a computer programme. The correlation coefficient was always above 0.9 in all the cases analysed.

Loss of HP

From the data presented in the figure 1, it would be seen that on the basis of TCA precipitable activity, the half-life ($t_{0.5}$) of HP was found to be 6.4 days in male and 6.87 days in female locusts. Using criterion of specific activity of proteins present in the haemolymph, the half-life of HP was found to be 2.98 days in male and 3.91 days in female locusts. Thus, it appears that there are statistically no differences in the turnover rate of HP within sexes. However, analysis of data based on the two criteria showed significant differences. The half-life values of HP based on specific activity was nearly half of that based on TCA precipitable activity. It was to be about 3.5 days on the basis of

specific activity of HP and 6.5 days on the basis of total TCA precipitable activity.

Loss of Human Serum Albumin

Figure 1 B represents the loss of ^{131}I-labelled human serum albumin in the haemolymph of locusts. The half-life of albumin was found to be 40.77 min in male and 32.73 min in female locusts on the basis of specific activity whereas it was 40.77 min in male and 35.38 min in female locusts on the basis of TCA precipitable data. The differences within sexes and those based on specific/TCA precipitable activity data appears to be insignificant.

Table 1 *Turnover constants of haemolymph proteins and human serum albumin in the adult desert locust*

	Haemolymph proteins		Human serum albumin	
	Half-life (days)	Turnover time (days)	Half life (min)	Turnover time (min)
On the basis of specific activity				
Male	2.98	4.30	40.77	58.71
Female	3.91	5.63	32.73	47.13
On the basis of TCA precipitable activity				
Male	6.40	9.22	40.77	58.71
Female	6.80	9.90	35.38	50.95

Discussion

Loss of ^{14}C-labelled HP

Turnover constants of the HP and HSA are presented in table 1. Turnover of ^{14}C-labelled HP in the present study was determined by an indirect method (Traver 1954). The turnover time of HP was calculated as 1.44 times the half-life (Zilversmit 1960). The results on half-life of HP show that there exists significant differences in two criteria, i e., total TCA precipitable activity and the specific activity of the proteins. The half-life values calculated on the basis of specific activity reflects both the synthesis of new HP as well as its sequestration to various tissues/organs. The half-life values based on TCA precipitable activity are perhaps a true reflection of sequestration of HP to various tissues/organs of the body.

The turnover time in male locust was found to be 4.3 days based on specific activity data. Considering pool concentration of 47.92 mg protein/ml of haemolymph, the rate of loss of protein from haemolymph was found to be 11.14 mg/ml/day.

The turnover time in female locust was found to be 5.63 days on the basis of specific activity. Considering pool concentration of 71.2 mg protein/ml of haemolymph, the rate of loss of protein was found to be 12.64 mg/ml/day.

Loss of ^{131}I-labelled Human Serum Albumin

The rapid decrease in activity both specific and TCA precipitable activity in relation to time shows that human serum albumin was lost at a much faster rate than HP in the haemolymph of the desert locusts. The faster rate of loss of albumin perhaps might be due to its lower molecular weight (69,000) in comparison to high molecular weight HP (480,000) present in the locust (Kulkarni & Mehrotra 1970). These findings, however, differ from those of Boyd and Mitchell (1966) who observed that *Drosophila* was unable to degrade non-homologous protein 'hemoglobin' at a rate at which it was able to degrade its own HP, thereby showing that turnover is specific phenomenon in that species.

Acknowledgement

One of us (GTG) is grateful to the Indian Council of Agricultural Research for the grant of Senior Research Fellowship during doctoral programme.

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