NADH DEPENDENT AMINO-TRANSFERASES IN THE TISSUES AND EGGS OF SILKWORM *BOMBYX MORI* L.

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ABSTRACT

L-glutamine: 2-oxoglutarate amino-transferase (reduced NAD oxidising) and L-asparagine: 2-oxoglutarate amino-transferase (reduced NAD oxidising) are detected in the tissues and eggs of silkworm, contradicting the earlier reports on former enzymes exclusive occurrence to prokaryotes. The role of these enzymes in silkworm tissues has been suggested to the fixation of metabolic ammonia for the synthesis of aminoacids in the silk gland.

INTRODUCTION

L-GLUTAMINE: 2-oxoglutarate amino-transferase (reduced $^{NADP}_{NAD}$ oxidising) is restricted to prokaryotic organisms studied by Tempest *et al*[1]. They linked the capacity of fixing atmospheric nitrogen with glutamine pathway of ammonia assimilation. Till to date the asparagine pathway of ammonia utilization is not given much attention in insects. During our study of amino acid metabolism in silkworms we noticed a more or less constant level of glutamic dehydrogenase activity in the extracts of silkgland and eggs and its absence in the extracts of fat body. Glutamine synthetase was detected in the tissue extracts and eggs. We failed to detect the asparagine synthetase activity in silkworm using hydroxylamine as the source of ammonia. Probably glutamine may serve as a amino donor to aspartic acid as in mammals[2]. The presence of glutamine synthetase and absence of glutamic dehydrogenase in the metabolically highly active fat body prompted us to check for the presence of enzymes reported here. During this study we tried with three amino compounds as donors of amide group to 2-oxoglutarate for the formation of glutamate.

MATERIALS AND METHODS

The method of enzyme assays followed was based on colorimetric determination of 2-oxoglutarate[3]. The experimental animals used were those of bivoltine race (KA) and polyvoltine Mysore race of *Bombyx mori.*, maintained in the laboratory. The eggs and tissues are homogenised in distilled water using a Potter-Elvehjem homogeniser. The homogenates were centrifuged at 3500 rpm and the middle clear layer of each supernatant was taken as the source of enzyme.

For the study of L-glutamine: 2-oxoglutarate amino-transferase (GOATase) activity the enzyme was incubated with the substrates for 30 min and the reaction was stopped by the addition of 0.02% dinitrophenylhydrazine. The 2-oxoglutarate utilized is measured colorimetrically at 390 nm using a spectrophotometer (Systronics type 105). The incubation volume was 1.4 ml and contained 47.85 mM of phosphate buffer pH 7.2, 71.4 mM of L-glutamine, 0.357 mM of 2-oxoglutarate, 0.4 mM of NADH-$(Na)_2$. To the controls 2-oxoglutarate was added at the end of incubation. To check for transamination reaction, a control was run in which 2-oxoglutarate, glutamine and phosphate buffer was incubated with the enzyme for 30 min. At the end of incubation NADH was added. We failed to observe transamination activity in the absence of NADH.

L-Asparagine: 2-oxoglutarate amino-transferace (AOATase) activity was similarly determined but the reaction mixture contained 17.85 mM of L-Asparagine. There was a significant reduction in the transamination activity in the absence of NADH (table 3) in the assay medium. Urea upto 0.3 M was also tried as a possible source of amino group for the formation of glutamate from 2-oxoglutarate, but there was no activity. The protein was estimated by the method of Lowry *et al*[4].

RESULTS AND DISCUSSION

The results are shown in tables 1–3. The GOATase activity is rich in the posterior silk gland (table 1) and the AOATase activity in the fat body (table 2); both the enzymes are NADH-dependent (table 3).

The prokaryotes cultured in the ammonia-limited

Table 1 *Distribution of L-glutamine: 2-oxoglutarate amino-transferase (reduced NAD oxidizing) in the tissues of 4th day of 5th instar larvae and chilled diapausing eggs of bivoltine KA race and non-diapausing eggs of polyvoltine Mysore race.*

Enzyme source	Activity†
Posterior silk gland	5.41 ±0.138*
Fat body	3 466 ±0.170
Foregut and midgut	4.425 ±0.240
Chilled diapausing eggs	1.54 ±0.034
Polyvoltine non-diapausing eggs	1.497 ±0.108

† Activity is expressed as nmol of 2-oxoglutarate utilized per minute per mg of protein; * Values are mean ±SD of 6 observations.

Table 2 *Distribution of L-Asparagine: 2-oxoglutarate amino-transferase (reduced NAD oxidizing) in the tissues of 5th day of 5th instar larvae of bivoltine KA race and eggs of polyvoltine non-diapausing Mysore race.*

Enzyme source	Activity†
Posterior silk gland	4.766 ±0.25*
Fat body	19.57 ±0.351
Foregut and midgut	6.206 ±0.34
Polyvoltine non-diapausing eggs	4.536 ±0.062

† Activity is expressed as nmol of 2-oxoglutarate utilized per minute per mg of protein; * Values are mean ±SD of 6 observations.

Table 3 *Distribution of L-Asparagine: 2-oxoglutarate transamination activity (in the absence of NADH in the tissues of 5th day of 5th instar larvae of bivoltine KA race and eggs of polyvoltine nondiapausing Mysore race.*

Enzyme source	Activity†
Posterior silk gland	0.9721 ±0.113*
Fat body	1.877 ±0.123
Foregut and midgut	1.277 ±0.129
Polyvoltine non-diapausing eggs	0.530 ±0.068

† Activity is expressed as nmol of 2-oxoglutarate utilized per minute per mg of protein; * Values are mean ±SD of 3 observations.

media switch on to glutamate synthase pathway of utilizing ammonia from glutamic dehydrogenase pathway which operates only in ammonia rich media[1]. The scheme of enzymic action is given as follows:

1. Glutamate + NH_3 + ATP $\xrightarrow{\text{glutamine synthetase}}$ glutamine + ADP + Pi

2. Glutamine + 2-oxoglutarate

$$+ \begin{matrix}\text{NADPH}\\\text{NADH}\end{matrix} \xrightarrow[\text{synthase}]{\text{glutamate}}$$

2 glutamate + $\begin{matrix}\text{NADP}\\\text{NAD}\end{matrix}$

Glutamine synthetase has a high affinity for ammonia and glutamate synthase has high affinities for both 2-oxoglutarate and glutamine[5]. Thus the organism has efficient mechanism for amino acid synthesis even in ammonia-limiting media.

The presence of glutamine synthetase in the silkworm shows that it has an efficient mechanism of fixation of metabolic ammonia for the synthesis of amino-acids. Greater GOATase activity in the silk gland suggests the importance of the enzyme for the synthesis of amino-acids required for silk production. These animals although regarded as monophagous feed exclusively on mulberry leaves which contain more aspartate than glutamate[6]. The ammonia derived from amino acid metabolism is utilized for the formation of glutamine from glutamic acid. Ito and Arai maintain that both glutamate and aspartate are essential amino acids in silkworm[7] and may not be synthesized in the tissues of the worm. It is proposed that both glutamate and aspartate play a pivotal role in the metabolism of amino acids as donors of amino groups[6].

The production of glutamate from 2-oxoglutarate (derived both from citric acid cycle and transamination of glutamate) with aspargine and glutamine as amino group donors is of paramount importance to the silkworms. As in prokaryotes the glutamine pathway favours amino acid synthesis in silkworms and meets the demand of increased synthesis of amino acids for silk protein production. As the enzymes are not purified we do not know whether the same enzyme has both L-glutamine: 2-oxoglutarate amino-transferase and L-asparagine: 2-oxoglutarate amino-transferase activities. Both glutamine and asparagine pathways are driven by ATP and are irreversible. The net effect seems to be an increased synthesis of amino acids. The occurrence of GOATase activity and its plausible role in the glutamate synthesis in the tissues of silkworm may defy the concept of Ito and Arai[7] who are of the opinion that glutamate is an essential amino acid for the silkworm.

Sum of reactions 1 and 2,

Glutamate + NH_3 + 2-oxoglutarate + ATP

$$+ \begin{matrix}\text{NADPH}\\\text{NADH}\end{matrix} \longrightarrow \quad 2 \text{ glutamate} + \text{ADP} + \text{Pi} + \begin{matrix}\text{NADP}\\\text{NAD}\end{matrix}.$$

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1. Tempest, D. W., Meers, J. L. and Brown, C. M. *The enzymes of glutamine metabolism* (eds) S. Prusiner and E. R. Stadtman, Academic Press, New York and London, p. 167, 1973.
2. Stryer, L., *Biochemistry*, W. H. Freeman and Company, San Francisco, 1981.
3. Bergmeyer, H. U., (ed.) *Methods of enzymatic analysis* Vol. 2., Verlag Chemie Weinheim, Academic Press Inc., New York and London, p. 656, 1974.
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
5. Stadtman, E. R., *The enzymes of glutamine metabolism* (eds) S. Prusiner and E. R. Stadtman, Academic Press, New York and London, 1973, p. 1.
6. Ito, T., *The Silkworm—An important laboratory tool* (ed.) Y. Tazima, Kodansha Ltd., Tokyo, 1978, p. 121.
7. Ito, T. and Arai, N., *J. Insect. Physiol.*, 1967, **13**, 1813.

NEWS

IMPROVEMENT OF FLOOD FORECASTING SYSTEMS IN INDIA

Important steps have been taken in implementing the project 'Improvement of flood-forecasting systems in India'. The project has been extended up to June 1984 with more study tours, fellowships in flood-forecasting systems, radar techniques for flood forecasting and the development of a snow hydrology programme. A three-month group training course on hydrological models was completed last March under the supervision of Mr. S. Cooper (USA), chief technical adviser of the project. There were 20 participants from Indian Central Water Commission. A follow-up course is being organized with more emphasis on the operational side. Eight fellowships were completed in 1983 and nine are planned for 1984.

Sites for the network of river-flow and precipitation stations have been selected and building work completed. The computer has been installed and automatic telemetering is in progress. Work on appraising several mathematical models has been initiated and a computer specialist, Mr. N. Jensen (Norway) has assisted the chief technical adviser in this field. Snow hydrology equipment was received and installed during the last quarter of 1983 in the upper Yamuna River basin. (*WMO Bulletin, Vol. 33, No. 1, January 1984, p. 64*)

HIGH VOLUME AIR SAMPLER

Control Engineering Manufactures and Markets through Control Engineering Services, WA, a high-volume air sampler used extensively throughout Australia and exported for use by the World Health Organisation. It is designed to be suitable for use in extreme variations of temperature (70°C) in remote locations, as well as monitoring air pollution in cities and industrial localities. The recent addition to accessories of a cascade filter unit enables it to be used for monitoring inhalable particulates smaller than 15 micrometres. In the automatic mode, flow may be maintained from 10 to 100, $\pm$ 1, cubic metres per hour.

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