SYNCHRONOUS APPEARANCE OF ADENINE NUCLEOTIDE TRANSLOCASE ACTIVITY AND OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA FROM FLIGHT-MUSCLE OF THE DEVELOPING SHEEP BLOWFLY, *LUCILIA CUPRINA*

Freda A. DOY, Arlene A. DADAY and Fyfe L. BYGRAVE
Department of Biochemistry, Faculty of Science, Australian National University, Canberra, A.C.T., Australia

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1. Introduction

The metamorphosing insect provides an experimentally availing system for studying mitochondrial development [1–4]. Recently we showed that respiration in coupled mitochondria isolated from flight-muscle of the blowfly *Lucilia cuprina* increases from near-minimal rates immediately prior to adult emergence to near-maximal rates within 2–4 hr after emergence [5]. The development of this complex activity over such a short period of time provides an excellent framework around which one can study many facets of mitochondrial biogenesis. The system is particularly suited for examining the development of metabolite transport systems located in mitochondria. In this regard we have described the development of the Ca^{2+} transport system in flight-muscle mitochondria [5].

We report here on the ability of *Lucilia* mitochondria to translocate adenine nucleotides. It is shown that the general properties of adenine nucleotide translocation in adult insect mitochondria are similar to those of mammalian mitochondria and, more importantly, that the development of maximal rates of adenine nucleotide translocation in *Lucilia* mitochondria parallels that of oxidative phosphorylation.

2. Experimental

The source and maintenance of fly cultures as well as the isolation of tightly-coupled mitochondria from their flight-muscle were as described previously [5].

Measurement of adenine nucleotide translocation: in the majority of experiments AdN* translocation was measured by the forward-exchange technique [6] as described elsewhere [7]. Incubation media contained 150 mM KCl, 2 mM Hepes-Tris (pH 7.4) and 0.3 to 0.4 mg of mitochondrial protein in a total vol of 0.25 ml. Mitochondria were pre-incubated for 60 sec prior to the addition of AdN (containing 0.5 μCi [^{3}H] AdN). Reactions were carried out at 0°C, terminated by the addition of 50 μM atractyloside and the mixture rapidly centrifuged (Eppendorf microfuge, 0.75 min). The pellets were washed with 200 μl 150 mM KCl plus 2 mM Hepes-Tris (pH 7.4) and then dissolved in 100 μl soluene and counted for radioactivity [8].
Adenine nucleotides were determined [9,10] after extraction from the mitochondria with $HClO_4$ and KOH.

The exchangeable AdN pool was determined as described by Pfaff and Klingenberg [6]. All materials were obtained from sources previously indicated [7].

Each experiment was carried out at least twice and in some cases three times. Immediately prior to each translocase assay, the mitochondria were tested for their ability to carry out controlled respiratory 'jumps' [5]. Those which exhibited any slight loss of control were discarded.

3. Results and discussion

3.1. *General properties of adenine nucleotide translocation in mitochondria from Lucilia flight-muscle.*

Adenine nucleotide translocation in mitochondria from a range of mammalian tissues (reviewed in [11])

* *Abbreviations*: AdN, adenine nucleotide. CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

and more recently from several strains of yeast [12–14] is becoming well characterised. Various features of this system are common to all mitochondria which so far have been examined. These include the very rapid rate of translocation, the discrimination in coupled-mitochondria between rates of ADP and ATP translocation, the high affinity and specificity for these nucleotides and inhibition of translocation by specific compounds such as atractyloside. Furthermore, in some species of mitochondria, translocation of ATP can be modified by changes in the ionic environment, especially by Ca^{2+} [7,15–17].

Data in fig.1 outline the general properties of AdN translocation in *Lucilia* mitochondria. The rates of translocation of both nucleotides are very rapid even when measured at 0°C (fig.1A). Exchange of ADP is virtually complete after about 15 sec. As with mammalian mitochondria [6] ADP is translocated more rapidly than ATP.

The AdN translocase in insect mitochondria is saturated by low concentrations of AdN similar to those which saturate this activity in other mitochondria [6,7 18,19]. Half-saturation values for ADP and ATP are approx. 20 and 40 μM, respectively (fig.1B).

The potent inhibition by atractyloside of AdN translocation in the mitochondria of insect flight-muscle is shown in fig.1C. It should be noted that a high concentration of K^+ is present in the incubation system used. This ion markedly increases the potency of inhibitor [15,20]. The values obtained for the K_i under these conditions (approx. 1 μM) are similar to those found with rat liver and beef heart mitochondria. The data of fig.1D show that also like mammalian mitochondria, uncouplers of oxidative phosphorylation stimulate the rate of ATP translocation in insect mitochondria to values approaching those obtained with ADP.

The influence of Ca^{2+} on ATP translocation was studied (data not shown). In contrast to rat liver and beef heart mitochondria, where the ion stimulates [7,15] and ascites tumour mitochondria where the ion inhibits ATP translocation [17], Ca^{2+} had no significant effect on the translocation of this nucleotide in the insect system.

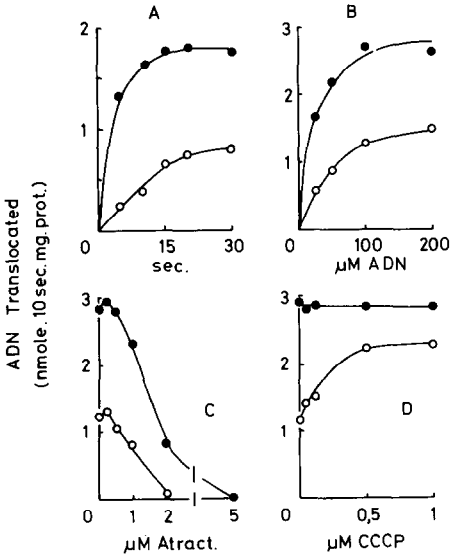

Fig.1. Properties of adenine nucleotide translocation in mitochondria from flight-muscle of *Lucilia*. Translocation was determined (see Experimental) in a medium consisting of 150 mM KCl, 2 mM Hepes–Tris (pH 7.4) and 0.3 to 0.4 mg mitochondrial protein from 1-day-old flies. Except for fig.1A the reaction time was 10 sec and for fig.1B the concentration of nucleotides were 100 μM. Data in fig.1A are expressed as nmoles adenine nucleotide translocated per mg protein. (●), ADP; (○), ATP.

3.2. *Relation of adenine nucleotide translocase activity to mitochondrial development*

As mentioned in the Introduction we have established that maximal rates of respiration in flight-muscle mitochondria are entirely dependent on the age of the fly from which the mitochondria are isolated [5]. Since the availability of exogenous AdN can control the rate at which oxidative phosphorylation proceeds (see [21]) it was of interest to see if any changes in ability of the mitochondria to translocate AdN accompanied the change in their ability to carry out coupled-respiration.

The data in fig.2 show that mitochondria isolated from flight-muscle of the emerging fly exhibit very low rates of ADP and ATP translocation. These mitochondria are intact and functional as judged by their ability to carry out coupled-respiration (see [5] and Experimental). Almost complete ability to maximally translocate both ADP and ATP is acquired by the flight-muscle mitochondria within only several hours of adult emergence. The attainment of maximal rates of translocation thus parallels the attainment of maximal rates of coupled-respiration in these mitochondria (cf. [5]).

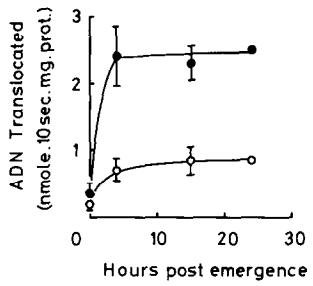

Fig.2. Ability of mitochondria obtained from different stages of adult development to translocate adenine nucleotide. Mitochondria were obtained from flight-muscle of adult flies at the times shown. Immediately after their isolation their ability to translocate 50 μM ADP (•) or ATP (○) was determined (see Experimental). Each point represents the mean value from two separate experiments.

Since the rate of AdN translocation is related to the intramitochondrial content of AdN, it was important to estimate these at various stages of mitochondrial development. Data in table 1 show that mitochondria from newly-emerged flies contain significant amounts of ADP, ATP and AMP. Those from 4-hr-old and 24-hr-old flies contain slightly greater proportions of ADP and ATP and much greater amounts of AMP. Indeed the amounts of AMP present relative to ADP and ATP, are similar to those found in mitochondria from blow-fly flight-muscle of *Calliphora* [22]; the age of the fly was not specified in that study.

The other important feature of the data in table 1 is that only about 10% of the ADP and ATP in mitochondria of newly-emerged flies is readily exchangeable. In the mitochondria of older flies a much larger proportion is exchangeable. The values obtained in this study also agree well with those of Danks and Chappell [22].

4. Conclusions

The synchronous appearance of AdN translocase activity with that of oxidative phosphorylation has now been seen in mitochondria isolated from at least three different developing systems. Lauquin and Vignais [14] observed that growth conditions which repress the development of aerobic metabolism in mitochondria from *Candida utilis* also lead to decreased rates of ADP translocation. They concluded (see also [12,13,23]) that the functioning of the AdN translocator is dependent to large degree on membrane components provided by the mitochondrial protein-synthesing system. Nakazawa et al. [24] examined energy-transducing activities in rat liver mitochondria during development and found that atractyloside-sensitive translocation was low in foetal mitochondria and increased significantly in post-natal mitochondria.

For some time we have advocated that lipid-protein interactions play an important role in the operation of the AdN translocator [7,16,25] a view now shared by Lauquin and Vignais [14] and by Scherer and Klingenberg [26]. Whether the increased activity in translocation seen with mitochondrial development is a reflection of changes in lipid-protein interactions during such development (see also [14]) or reflects the change in the exchangeable AdN pool (table 1)

Table 1
Total and exchangeable adenine nucleotide content in flight-muscle mitochondria obtained from different stages of adult development

Age of mitochondria	ADP	ATP	AMP	% Exchangeable	
	(nmoles/mg protein)			ADP	ATP
Newly-emerged[a]	2.77 ± 0.59	2.33 ± 0.63	1.31 ± 0.53	12	10
4-hr-old[b]	3.9	3.2	2.05	50	25
24-hr-old[a]	3.57 ± 0.49	2.7 ± 0.64	4.0 ± 1.37	50	40

[a] Average of 3 determinations ± Standard Deviation
[b] One determination
Adenine nucleotide content and exchangeable pool sizes were determined (see Experimental) in mitochondria from flight-muscle of flies at the ages indicated.

remains to be established. The finding that in the insect flight-muscle phospholipid synthesis is maximal at emergence and rapidly declines thereafter [27] is inclined to enhance the former of these two possibilities.

References

[1] Brosemer, R. W., Vogell, W. and Bucher, T. L. (1963) Biochem. Z. 338, 854–910.
[2] Bucher, T. L. (1965) Symp. Biochem. Soc. 25, 15–28.
[3] Birt, L. M. (1970) in: Automony and Biogenesis of Mitochondria and Chloroplasts (Boardman, N. K., Linnane, A. W. and Smillie, R. M. eds.) pp. 130–139, North-Holland, Amsterdam.
[4] Lennie, R .W. and Birt, L. M. (1967) Biochem. J. 102, 338–350.
[5] Bygrave, F. L., Daday, A. A. and Doy, F. A. (1975) Biochem. J. 146, 601–608.
[6] Pfaff, E. and Klingenberg, M. (1968) Eur. J. Biochem. 6, 66–79.
[7] Spencer, T. L. and Bygrave, F. L. (1972) Biochem. J. 129, 355–365.
[8] Reed, K. C. and Bygrave, F. L. (1974) Biochem. J. 140, 143–155.
[9] Lamprecht, W. and Trautscheld, I. (1965) in: Methods in Enzymatic Analysis (Bergmeyer, H. U. ed.) p. 543, Academic Press, New York.
[10] Adam, H. M. (1965) in: Methods in Enzymatic Analysis (Bergmeyer, H. U., ed.) p. 573, Academic Press, New York.
[11] Klingenberg, M., Wulf, R., Heldt, H. W. and Pfaff, E. (1969) FEBS Symposium 17, 59–77.
[12] Kolarov, J., Subik, J. and Kovac, L. (1972) Biochim. Biophys. Acta 267, 465–478.
[13] Haslam, J. M., Perkins, M. and Linnane, A. W. (1973) Biochem. J. 134, 935–947.
[14] Lauquin, G. and Vignais, P. V. (1973) Biochim. Biophys. Acta 305, 534–556.
[15] Meisner, H. (1971) Biochemistry 10, 3485–3491.
[16] Spencer, T. L. and Bygrave, F. L. (1974) Biochem. J. 140, 413–422.
[17] Thorne, R. F. W. and Bygrave, F. L. (1974) FEBS Lett. 41, 118–121.
[18] Winkler, H. H., Bygrave, F. L. and Lehninger, A. L. (1968) J. Biol. Chem. 243, 20–28.
[19] Duee, E. D. and Vignais, P. V. (1969) J. Biol. Chem. 244, 3920–3931.
[20] Spencer, T. L. and Bygrave, F. L. unpublished observations.
[21] Heldt, H. W. and Pfaffe, E. (1969) Eur. J. Biochem. 10, 494–500.
[22] Danks, S. M. and Chappell, J. B. (1974) Biochem. J. 142, 353–358.
[23] Groot, G. S. P., Kovac, L. and Schatz, G. (1971) Proc. Natl. Acad. Sci, (U.S.) 68, 308–311.
[24] Nakazawa, T., Asami, K., Suzuki, H. and Yukawa, O. (1973) J. Biochem. (Tokyo) 73, 397–406.
[25] Spencer, T. L. and Bygrave, F. L. (1971) Biochem. Biophys. Res. Commun. 43, 1290–1295.
[26] Scherer, B. and Klingenberg, M. (1974) Biochemistry 13, 161–170.
[27] Williams, M. L., Bygrave, F. L. and Birt, L. M. (1974) Insect Biochem. 4, 161–172.