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NAD-GLYCOHYDROLASE ACTIVITY IN XENOPUS LAEVIS OOCYTES AND EARLY EMBRYOS

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NAD-glycohydrolase was first detected in Xenopus laevis embryos 20 hours after fertilisation. This activity increased at least 50-fold by 70 hours after fertilisation. Enzyme activity per cell increased 5-fold between 20 and 50 hours. This increase represents a much larger variation in NAD-glycohydrolase activity than has been reported in any other tissue. The NAD-glycohydrolase activity of stage VI oocytes was at least 6-fold greater than that of eggs. Most, or all, of this activity was localised in the follicle cells which make up only a few per cent of the weight of the oocyte.

NAD turnover in vivo has recently been studied in both oocytes and early embryos of Xenopus laevis (Williams, Shall and Ford, manuscript submitted for publication). The NAD in stage V and VI oocytes is very stable, with a half-life of several hundred hours. However, NAD turnover increases sharply after fertilisation, and continues to increase during early development.

The two eukaryotic enzymes which specifically degrade NAD are ADP-ribosyl transferase[2] and NAD glycohydrolase (EC 3.2.2.5). Nuclear ADP-ribosyl transferase is a chromatin-bound enzyme which has been implicated in several aspects of the biochemistry of the nucleus. Involvement in DNA repair has been most convincingly demonstrated (1). In contrast, NAD glycohydrolase is associated with the plasma membrane (2, 3) and possibly also with the endoplasmic reticulum (4). It is possible that the apparent association of some of the enzyme activity with endoplasmic reticulum is due to heterogeneity in the lipid composition of the

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[2] Abbreviations used: ADP-ribose, Adenosine-5'-disphosphoribosyl-5'-ribose NAD Nicotinamide-adenine-dinucleotide.

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plasma membrane fraction wich which the NAD glycohydrolase is associated (5). Indeed, recent reports have suggested that most, or all, of the enzyme activity is expressed at the outer surface of the plasma membrane (5,6). This localization may explain the observation that the turnover of NAD in human D98/AH_2 cells (7) and in Xenopus laevis oocytes and embryos (Williams, Shall and Ford, manuscript submitted for publication) is much slower than would be expected if NAD glycohydrolase were freely able to degrade intracellular NAD. The function of NAD glycohydrolase, which, prior to the studies reported here, had been found in substantial quantities in all eukaryotic tissues examined (8,9) has not yet been defined.

During the development of Xenopus laevis embryos, nuclear ADP-ribosyltransferase activity, measured in isolated nuclei, increases about 50,000-fold by 40 hours (10). However, the principle NAD-degrading activity in embryo homogenates is NAD glycohydrolase (Williams, Shall and Ford, manuscript submitted for publication). In this report we have examined the variation in NAD glycohydrolase activity between stage VI oocytes and 100-hour embryos.

MATERIALS AND METHODS

Xenopus laevis embryos and oocytes

Early embryos were incubated at 22-24°C in 1/10 Barth X solution, as modified by Gurdon (11). Embryos before about stage 23 were dejellied with 2% (w/v) cysteine pH 8 at room temperature. Stages of development were judged visually by reference to Nieuwkoop and Faber (12).

Stage VI oocytes (stages of Dumont (13))were removed from fresh ovaries using fine forceps and kept in modified Barth X. Oocytes were defolliculated either manually using fine forceps or enzymatically using collagenase. Collagenase treatment consisted of incubation of pieces of ovary in 100 mM phosphate buffer pH 6.8 containing 250 µg/ml collagenase at room temperature and collecting the oocytes released after 30 minutes.

Oocytes and embryos were sonicated, on ice, in batches of 5 in 450 ml 50 mM Tris-HCl pH 8.5 using an MSE 150 watt sonicator. Samples were sonicated for a total of 60 seconds with a 30 second break after 30 seconds sonication.

Assay of NAD glycohydrolase activity

The NAD glycohydrolase activities in sonicates of oocytes and embryos were determined by measuring the fraction of unlabelled or tritium-labelled NAD degraded during 30 minutes incubation (Williams, Shall and Ford, manuscript submitted for publication).

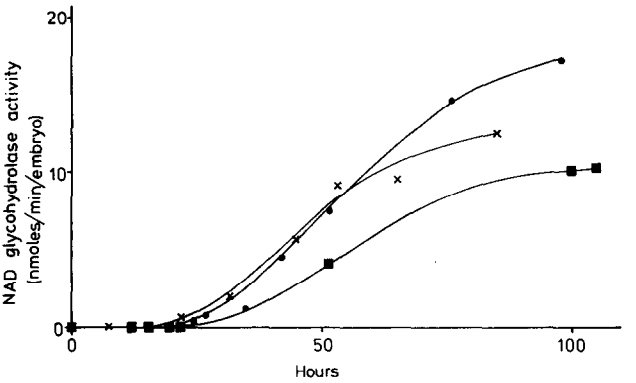

Figure 1 NAD glycohydrolase activity of homogenates of embryos at different stages of development. Batches of embryos were sonicated and their NAD glycohydrolase activity assayed as described in Materials and Methods. The three symbols refer to embryos from separate matings.

RESULTS AND DISCUSSION

NAD glycohydrolase activity during early development

Fig.1 shows the NAD glycohydrolase activities of embryos from three different matings. NAD glycohydrolase activity in embryos less than 20 hours after fertilisation were below the limit of detection of the assay (100 pmol/minute/embryo). NAD glycohydrolase activity increased rapidly between 20 hours and 70 hours after fertilization, which is a period when embryo protein content remains nearly constant (14). The specific activity of the enzyme in embryos less than 20 hours old was therefore less than 6 nmoles of NAD degraded/ hour/ mg wet weight, which is more than three-fold lower than the lowest activity measured in several rat (8) or mouse (9) tissues. In sharp contrast, the specific activity of the enzyme in late embryos was greater than that of any tissue examined in the same reports. NAD glycohydrolase activity therefore increases very dramatically in a short period.

Cell number increases very rapidly during early development (15). The quantity of plasma membrane per embryo therefore also increases substantially, even though embryo protein content is stable. Since NAD glycohydrolase is associated with the plasma membrane, the increase in enzyme activity could be a consequence of the increase in plasma membrane. However, comparison of the enzyme activity per cell in three periods of development (Table 1) shows that NAD glycohydrolase activity increases faster than cell number between 20 and 50 hours, and is nearly

Table 1 NAD glycohydrolase activity per cell in embryos during three developmental periods. NAD glycohydrolase activity was determined as described in Materials and Methods and activities per cell calculated from the measurements of DNA content during development obtained by Dawid (15). Means and standard errors of four batches of embryos are shown in each case.

Developmental Period (hours after fertilization at 22 - 24° C)	NAD glycohydrolase activity per cell (fmoles NAD degraded/minute/cell
15 - 30	3.8 ± 1.0
40 - 50	20.5 ± 3.7
80 - 100	16.6 ± 4.4

constant after 50 hours. The increase in NAD glycohydrolase activity between 20 and 50 hours is therefore greater than the increase in plasma membrane, which is less than the increase in cell number. Average cell size, and therefore average plasma membrane content per cell in fact decreases over this period. The increase in enzyme activity cannot therefore be explained simply by the increase in the organelle with which the enzyme is associated.

NAD glycohydrolase activity of stage VI oocytes

The NAD glycohydrolase activity of whole oocytes was 600 ± 21 pmole NAD degraded / minute / oocyte (S.E.M., n=3). This contrasts with the failure to detect any activity in eggs; the activity in eggs is therefore less than 100 pmole NAD degraded / minute / egg. Since the oocyte loses its follicle cells on maturation, this discrepancy could be explained by localisation of the enzyme in the follicle cells. To investigate this possibility, oocytes were defolliculated either manually or by collagenase treatment, as described in Materials and Methods. Three batches of five manually defolliculated oocytes, three batches of five collagenase defolliculated oocytes and controls were sonicated and incubated in 400 μM NAD, as for the usual NAD glycohydrolase assay, but with incubation time increased to 60 minutes. Table 2 shows that defolliculation by either method reduced NAD degradation by more than 50%. Since follicle cells make up less than 5% of the total weight of the oocyte, the large decrease in activity on defolliculation suggests that NAD glycohydrolase is largely localised in the follicle cells. As removal of follicle cells is usually

Table 2 The effect of defolliculation on the NAD glycohydrolase activity of oocytes. Batches of 5 oocytes, some of which had been defolliculated, were sonicated and incubated in 500 µl 400 µM NAD as described in Materials and Methods. The NAD degraded after 60 minutes incubation was determined. Means and standard errors of three determinations on different batches of oocytes are shown in each case.

Pre-treatment of oocytes	% NAD degraded after 60 mins
No pre-treatment	62.8 ± 21.9 (100)
Manually defolliculated	24.5 ± 4.6 (39)
Collagenase defolliculated	28.7 ± 9.3 (46)

incomplete, especially after manual defolliculation, it is possible that all the enzyme activity is localised in the follicle cells. This interpretation is consistent with the absence of detectable NAD glycohydrolase activity in eggs. An approximate value of 20,000 follicle cells per oocyte can be calculated from the data of Ford and Gurdon (14). If all the NAD glycohydrolase activity of the stage VI oocyte is localised in the follicle cells, the NAD glycohydrolase activity is 30 fmole NAD degraded /minute / cell. This is similar to the activity per cell found in late embryos (Table 1). It appears, therefore, that the NAD glycohydrolase activities of eggs and early embryos are unusually, perhaps uniquely, low. Further investigations of the striking variation in enzyme activity during development should provide valuable information about the role or roles played by NAD glycohydrolase in cell metabolism in general, and in animal development in particular.

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REFERENCES

1. Shall,S., Durkacz, B.W., Gray, D.A., Irwin, J., Lewis, P.J., Perera, M. and Tavassoli, M. (1981) in Mechanisms in Chemical Carcinogenesis (T.Cerruti, ed.) Alan Liss (in press)

2. Amar-Costesec,A. and Beaufoy, H.(1977) Arch.Internat.Physiol.Biochim.85, 949-950

3. Artman,M., and Seeley,R.J. (1979) Arch.Biochem.Biophys. 195, 121-127

4. Diaugustine, R.P., Abe, T. and Voytek, P. (1978). Biochim. Biophys. Acta. 526, 518-530

5. Muller,M. and Schuber, F. (1980) Eur.J. Biochem. 104, 489-500

6. Pekala, P.H. and Anderson, B.M. (1978) J.Biol. Chem. 253, 7453-7459

7. Rechsteiner, M., Hillyard, D. and Olivera, B.M. (1976) Nature 259, 695-698

8. Kakehi,K., Simoyama, M., Tanigawa, J., Asamizu, J. and Ueda, I. (1972) Biochim. Biophys. Acta. 268, 505-517

9. Green,S. and Bodansky, O. (1964) J.Biol. Chem. 239, 2613-2617

10. Farzaneh, F. and Pearson, C.K. (1979) Dev.Biol. 72, 254-265

11. Gurdon, J.B. (1968) J.Embryol.Exp. Morphol. 20, 401-414

12. Nieuwkoop, P.D. and Faber, J. (1956) Normal Table of Xenopus laevis (Daudin), North Holland, Amsterdam.

13. Dumont, J.N. (1972) J.Morphol. 136, 153-179

14. Ford, C.C. and Gurdon, J.B. (1977) J. Embryol.exp. Morph. 37, 203-209

15. Dawid, I.B. (1965) J. Mol. Biol. 20, 581-599