Retarded decline in poly-ADPR content and poly-ADPR synthetase activity in chicken dystrophic muscle

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The activity of poly-ADPR synthetase declines just after hatching in normal chick muscle nuclei However, in dystrophic chick muscle nuclei it decreases 5 weeks after hatching, A delayed decrease in the amount of poly-ADPR is also observed in dystrophic chick muscle nuclei These observations suggest that dystrophic muscle follows an abnormal developmental program

Poly-ADPR *Muscular dystrophy* *Muscle development*

1. INTRODUCTION

A failure of normal development in dystrophic muscle has been observed by many investigators [1–5]. For example, immature isozyme patterns of lactic dehydrogenase [1–3], myosin [4], and tropomyosin [5] in dystrophic muscle have been documented.

Among many factors which influence the development and differentiation of embryonic cells, we focused our attention on cellular poly-ADPR levels and the activity of poly-ADPR synthetase of developing muscle. Poly-ADP-ribosylation is one of the post-translational modifications of nuclear proteins in eukaryotes [6] and has been suggested to be involved in the regulation of various nuclear activities, such as DNA synthesis, histone function, cell differentiation and DNA repair [7–11]. Concerning cell differentiation, it has been pointed out that the activity of poly-ADPR synthetase decreases during the differentiation of muscle cells [9] and erythroleukemia cells [10].

We describe here the changes in poly-ADPR levels and the activity of poly-ADPR synthetase in both normal and dystrophic chicken muscle during development.

Abbreviations ADPR, adenosine diphosphate ribose, PR-AMP, 2′-(5′′-phosphoribosyl)-5′-AMP

2. MATERIALS AND METHODS

2.1. *Animals and materials*

Fertilized eggs of dystrophic chickens (New Hampshire strain, line 413) and normal chickens (New Hampshire strain, line 412) were obtained from the Muscular Dystrophy Research Group of Japan.

Snake venom phosphodiesterase was purchased from Worthington. Dihydroxyboryl polyacrylamide resin (Affigel 601) was purchased from Bio-Rad, ADPR from P.L. Biochemicals, and [^{14}C]NAD from New England Nuclear.

2.2. *Measurement of poly-ADPR synthetase activity*

Muscle nuclei were prepared as described previously [12]. Poly-ADPR synthetase activity of muscle nuclei was measured as described [12].

2.3. *Measurement of poly-ADPR content in muscle nuclei*

Muscle nuclei were washed twice with 20% trichloroacetic acid (w/v), suspended in 4 ml of 0.3

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N KOH (10 times the volume of muscle nuclei), and incubated at 37°C for 16 h. After incubation, proteins, DNA and poly-ADPR were precipitated by the addition of 2 ml of 20% trichloroacetic acid, washed several times with 10% trichloroacetic acid and resuspended in 1 ml of 0.25 M CH_3COONa buffer (pH 8.8) containing 6 M guanidium chloride. Affinity chromatography was carried out by applying the sample to a 35 × 4.5 mm dihydroxyboryl-Sepharose column (0.4 ml packed gel) pre-equilibrated with 0.25 M CH_3COONa buffer (pH 8.8), containing 6 M guanidine hydrochloride. After the sample, an additional 10 ml of the buffer was passed through the column to remove all unbound materials. The unbound fraction was used for the measurement of DNA content [13]. Poly-ADPR were eluted from the gel with 0.25 M sodium acetate buffer (pH 4.1). After dialysis against redistilled water for 12 h using dialysis tubing (Spectra/por'6, cut-off 2000 Da, spectrum Med. Ind. Inc.), poly-ADPR was lyophilized and suspended in 200 μg snake venom phosphodiesterase. Digestion was carried out by incubating at 37°C for 3 h. The incubation mixture was lyophilized again and resuspended in 50 μl triethylamine buffer (pH 8.0). Nucleosides were separated by thin-layer chromatography (TLC) on microcrystalline cellulose (Avicel SF, Asahi Kasei Co.) using the same developing buffer as described by Shima et al. [14]. The gel spots of PR-AMP and 5'-AMP were scratched off the gel, nucleosides eluted with 10 mM HCl and measured by the fluorography method as described by Niedergang et al. [15]. ^{14}C-labelled poly-ADPR was obtained as described previously [12]. [^{14}C]PR-AMP and 5'-[^{14}C]AMP were counted directly by suspending the scratched gel into liquid scintillator.

Autoradiography was carried out by contacting the TLC gel with X-ray film (Kodak XAR film) at −80°C for 3 days.

3. RESULTS AND DISCUSSION

The role of poly-ADPR in cell differentiation has been suggested by several investigators [9–10,16,17]. Caplan et al. [9] have reported that the content of poly-ADPR in chicken muscle decreases slightly during later stages of muscle development. Terada et al. [10] have reported that during the differentiation of Friend erythroleukemia cells, the poly-ADPR synthetase activity decreases. Other investigators [16,17] have also reported that poly-ADPR synthetase activity decreases as cells differentiate.

Fig.1 shows the poly-ADPR synthetase activity of normal and dystrophic muscle nuclei during the development of muscle. Poly-ADPR synthetase activity of the normal muscle declined 1 week after hatching. Poly-ADPR synthetase activity of dystrophic muscle, however, was maintained at high levels for at least 4 weeks post-hatching, only decreasing in the 5th week (fig.1). Poly-ADPR synthetase activity of adult muscle was low in both normal and dystrophic muscle as compared with that of embryonic muscle (fig.1). These results confirmed our previous observations [12] and suggest that poly-ADPR levels decrease during later stages of muscle development, and that these events are delayed in dystrophic muscle.

The measurement of enzyme activity in the isolated nuclei is subject to many nonspecific influences. For example, if the poly-ADPR binding sites of nuclear proteins are occupied, apparent enzymatic activity may be low, in spite of the high content of poly-ADPR. Moreover, during the isolation of muscle nuclei, DNA damage may occur, which may affect the activity of poly-ADPR

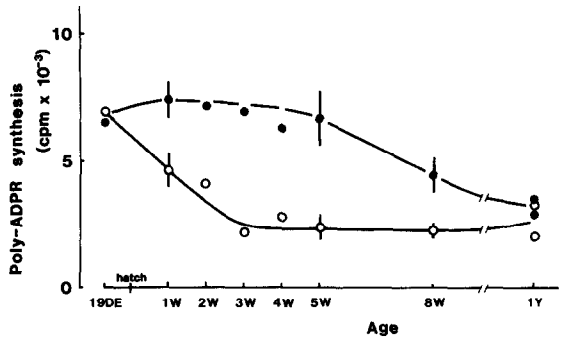

Fig.1 The activity of poly-ADPR synthetase of normal and dystrophic chicken breast muscle nuclei Poly-ADPR synthetase activity was assayed as described [12], and was expressed as 50 μg DNA/ml reaction mixture, since the activity expressed as DNA content increases linearly up to 50 μg DNA/ml [12] DNA content was determined by the method of Burton [13] The length of the vertical bar in the figure shows the standard errors of 4 (1w and 5w) or 3 (8w) samples ○, normal muscle; ●, dystrophic muscle.

Fig 2 The content of poly-ADPR of normal and dystrophic chicken breast muscle nuclei PR-AMP and 5'-AMP were obtained as described in section 2. Poly-ADPR content was expressed as the amount of PR-AMP, which was determined from the calibration curve established with various concentrations of ADPR The length of the vertical bar in the figure shows the standard errors of 4 (1w) samples. ○, normal muscle; ●, dystrophic muscle.

Table 1

Chain length of poly-ADPR

	Stage	Nor	Dys
Synthetic poly-ADPR	1w	8.2 ± 1.2	8.3 ± 0.7
	4w	8.4 ± 1.5	7.7 ± 1 3
Endogenous poly-ADPR	1w	6 5 ± 1 2	6.5 ± 1.0

Chain length of poly-ADPR was estimated by the method of Shima et al. [14] and is expressed as follows. Average chain length of poly-ADPR = (PR-AMP + 5'-AMP)/5'-AMP The length of poly-ADPR is estimated supposing the poly-ADPR to be straight. In case of the synthetic poly-ADPR, the values were the ratio of the counts (cpm) of PR-AMP to 5'-AMP The amount of PR-AMP (nmol) and 5'-AMP (nmol) of endogenous poly-ADPR were determined by the fluorescent method from the calibration curve of ADPR and 5'-AMP respectively Values are means ± SD from 4 different experiments

synthetase [11]. Poly-ADPR content of the nuclei of normal chick breast muscle during development was measured and shown to be decreased in 1-week-old chickens (1.8 nmol/mg DNA) and this increased slightly at adult stages (4 nmol/mg DNA) (fig.2). However, in dystrophic muscle, poly-ADPR levels remain high in younger stages and decline in the adult (fig.2).

The change of the activity of poly-ADPR synthetase and the content of poly-ADPR are parallel in younger stages. In older stages, however, in spite of the low activity of the poly-ADPR synthetase, poly-ADPR content is slightly increased (fig.2). The physiological role of poly-ADPR in old animals is rather obscure. Shimoyama et al. [18] reported that the stimulation of DNA synthesis by ADP-ribosylation of nuclear proteins was observed in chick embryonic liver nuclei, but the depression of DNA synthesis was observed in hen liver nuclei.

Stone et al. [19] reported that after hydrolysis of RNA in 0.25 N KOH, 15–50% of the poly-ADPR remained associated with the insoluble residue. In our case, however, 70–80% of the [^{14}C]poly-ADPR remained in the trichloroacetic acid insoluble residue. This discrepancy may be due to the different methods of adjusting pH after hydrolysis of RNA in 0.25 N KOH.

Poly-ADPR formed in vitro has varying chain length (n = 1–40), free or covalently bound to nuclear proteins [20]. Poly-ADPR is hydrolyzed at the pyrophosphate bond by snake venom phosphodiesterase [14], yielding PR-AMP and a small amount of 5'-AMP. From the molar ratio of PR-AMP to 5'-AMP, the chain length can be estimated by the method of Shima et al. [14]. Increased levels of poly-ADPR synthetase could be associated with the production of increased length of the same number of poly-ADPR chains (more elongation) or an increased number of chains of the same length (more initiation). As shown in table 1, poly-ADPR length is similar in both normal and dystrophic muscle. Thus, changes in poly-ADPR synthetase activity in isolated nuclei seem to be associated principally with changes in the number rather than with the length of the chains. However, by our estimation, the poly-ADPR length may be shorter than the true length, because we assumed the poly-ADPR to be straight, but it has been reported that poly-ADPR has many branches [20]. The ratio of PR-AMP to 5'-AMP may decrease as the number of branches increase.

The method described here determines only endogenous poly-ADPR levels, because mono-ADPR residues are rapidly destroyed at high pH

values when liberated from proteins by alkali [19]. The content of mono-ADPR and its physiological role in both normal and dystrophic muscle remains to be elucidated.

The high levels of poly-ADPR synthetase activity in dystrophic muscle nuclei could be the result of delay of inactivation or degradation of enzyme molecules during differentiation. We cannot exclude the possibility of the activation or synthesis of enzyme molecules in dystrophic muscle nuclei, or the production of more available sites on chromosomal proteins for initiation of polymer synthesis. We must also consider the possibility that dystrophic muscle may suffer from DNA damage, for the poly-ADPR synthetase activity is accompanied by the DNA repair [11]. So we must further investigate these points.

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