Membrane crystals of Ca^{2+}-ATPase in sarcoplasmic reticulum of developing muscle

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The vanadate-induced crystallization of Ca^{2+}-ATPase was analyzed on sarcoplasmic reticulum vesicles isolated between 10 and 28 days of development from pectoralis muscles of chicken. After exposure to Na_3VO_4 in a Ca^{2+}-free medium, Ca^{2+}-ATPase crystals begin to appear on portions of the surface of a few vesicles, isolated at 18 days of development. Thereafter, the number of vesicles containing Ca^{2+}-ATPase crystals rapidly increases and after 1 week of postnatal development (28 days), it reaches the adult level of about 30% of the vesicle population. These observations are discussed with reference to the mechanism of Ca^{2+}-ATPase crystallization and the regulation of sarcoplasmic reticulum biosynthesis.

Sarcoplasmic reticulum development *Skeletal muscle* *Ca^{2+}-transport ATPase*

1. INTRODUCTION

During the last week of development in ovo the rate of contraction and relaxation of chicken skeletal muscle increases several fold [1], accompanied by a steep increase in the Ca^{2+} transport activities of SR that continues into the first 2 weeks of postnatal development [2–4]. The increased Ca^{2+} transport activity is due to an increase in the concentration of Ca^{2+} transport ATPase in the SR membrane from about 200–400 ATPase molecules/μm^2 surface area in 12–14 day old embryos, to about 20000 ATPase molecules per μm^2 surface area in fully developed adult muscle [2–4]. Similar changes in Ca^{2+} transport activity and ATPase concentration were observed in developing rabbit muscles [5].

In SR vesicles isolated from adult rabbit or chicken muscle 2-dimensional crystalline arrays of Ca^{2+}-ATPase are induced by vanadate in a Ca^{2+}-free medium [6,7]. In SR isolated from adult fast-twitch muscles of rabbit, 70–80% of the vesicles contain ATPase crystals [6,8]; in slow-twitch skeletal and cardiac muscles, under identical conditions, the crystalline vesicles constitute less than 10% of the vesicle population [8]. The relative amount of crystalline vesicles is proportional to the Ca^{2+}-ATPase content, and ATPase or Ca^{2+} transport activities, suggesting that only the vesicles that contain Ca^{2+}-ATPase crystals are derived from the SR. Therefore, crystallization of Ca^{2+}-ATPase provides a specific method for the identification of SR elements containing Ca^{2+}-ATPase in heterogenous vesicle populations.

The aim of this work was to characterize SR preparations obtained at different stages of embryonic development, by electron microscope analysis of the distribution of Ca^{2+}-ATPase crystals induced by Na_3VO_4.

Abbreviation: SR, sarcoplasmic reticulum

2. MATERIALS AND METHODS

The experiments were carried out on New Hampshire chicken embryos and chickens. The fertilized eggs were incubated in a Ragus Austria-type incubator at 37°C and 75–80% relative humidity for 10–21 days. 2–7-day-old and adult chickens were also investigated. Microsome frac-

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tions were prepared from the cleaned breast muscles of 50–70 embryos in the earlier periods and fewer in the later stages, according to Nakamura et al. [9].

For crystallization of the Ca^{2+}-ATPase, microsome samples (1 mg protein/ml) in a medium of 0.1 M KCl, 10 mM imidazole (pH 7.4), 5 mM vanadate, 0.5 mM EGTA, and 5 mM $MgCl_2$ were incubated at 2°C for 24–48 h [6]. The surface structure of the vesicles was analyzed after negative staining with 1% uranyl acetate on a carbon coated formvar film, using a JEOL 100 B electron microscope at 80 kV accelerating voltage.

The Ca^{2+}-dependent ATPase activity of the samples was tested as described in [9]. For the analysis of the protein composition 6–18% gradient polyacrylamide gel electrophoresis was used [10].

3. RESULTS

The surface of the vesicles isolated from the superficial pectoralis muscles of chicken embryos between 10 and 16 days of development in ovo appeared to be smooth and featureless by negative staining with uranyl acetate (fig.1). The first morphological sign for the accumulation of the Ca^{2+}-ATPase in the isolated vesicles was the appearance of 4 nm diameter surface particles at 18 days of development. These particles have been previously associated with the Ca^{2+} transport ATPase [11]. In the presence of 5 mM Na_3VO_4 and 0.5 mM EGTA these particles associate into crystalline arrays (fig.2) that usually appear only on a small portion of the total surface area of the vesicles. At this stage of development the majority of the vesicles are still free from ATPase crystals, although they may contain randomly dispersed 4 nm surface particles. In microsomes isolated at 20 days of development, vesicles that are completely covered with Ca^{2+}-ATPase crystals are already visible, but the crystalline vesicles represent less than 10% of the total vesicle population (fig.3). At the end of the first postnatal week the frequency of

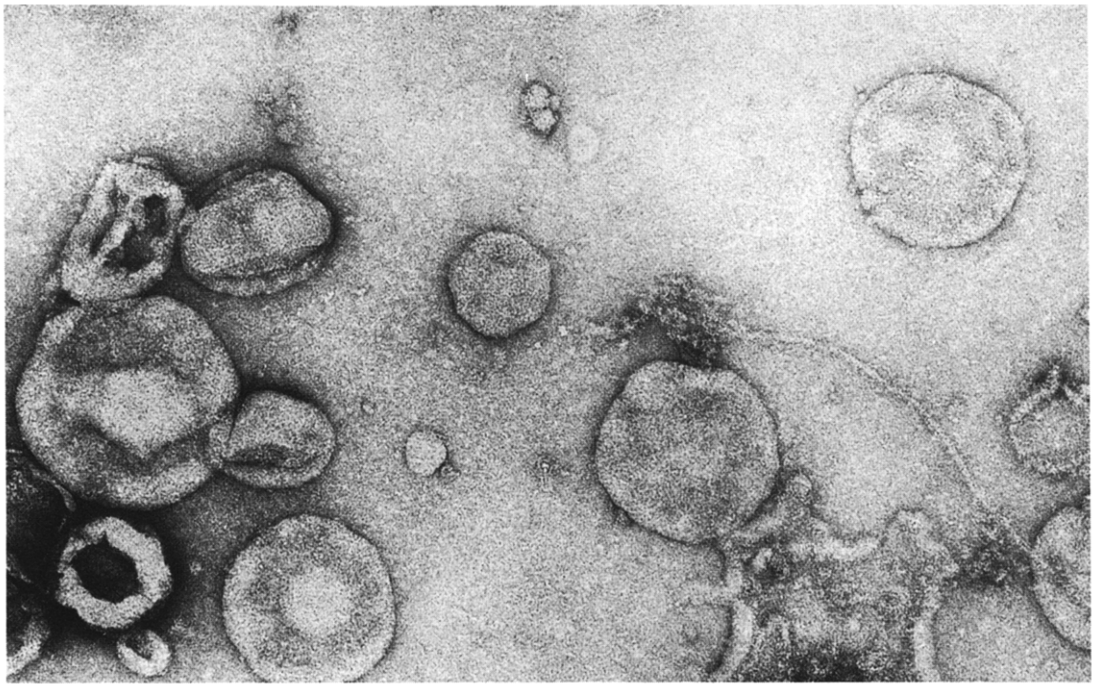

Fig.1. Microsome vesicles of 16-day-old embryonic chicken breast muscle. The sample was incubated for 48 h in a medium containing 0.1 M KCl, 10 mM imidazole (pH 7.4), 5 mM vanadate, 5 mM $MgCl_2$, 0.5 mM EGTA. Negative staining with 1% uranyl acetate. Magnification: × 124000.

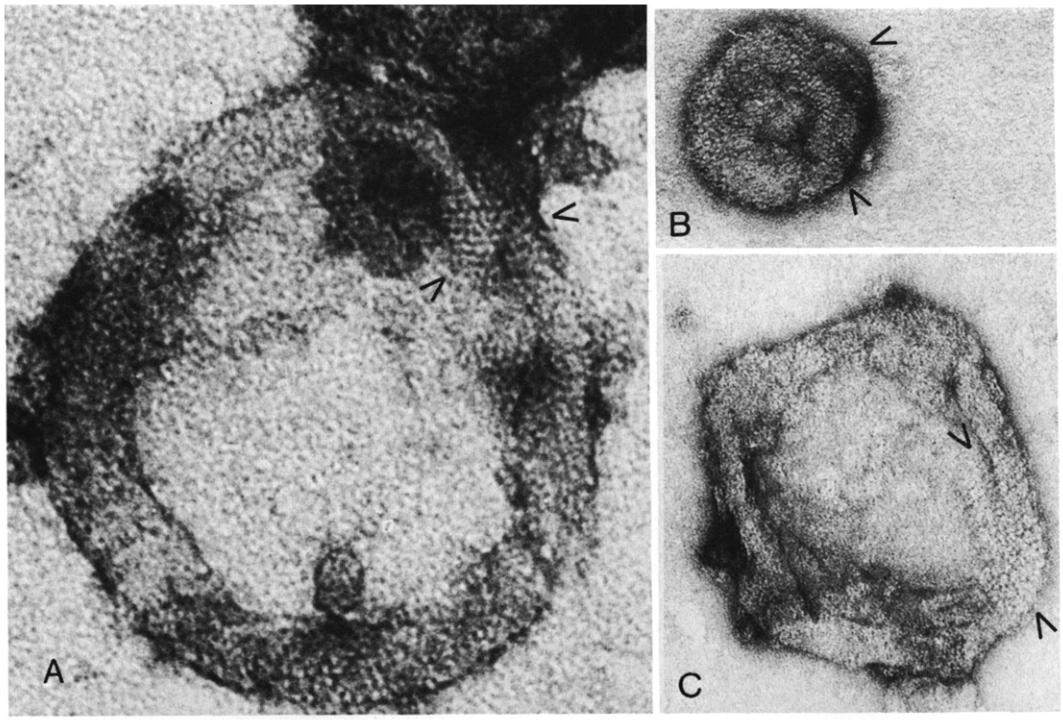

Fig.2. Crystalline areas on the surface of microsomal vesicles from 18-day-old chicken embryonic breast muscle. The sample was treated as described in fig.1. Negative staining with 1% uranyl acetate. Magnification: A, × 324000; B,C, × 196000.

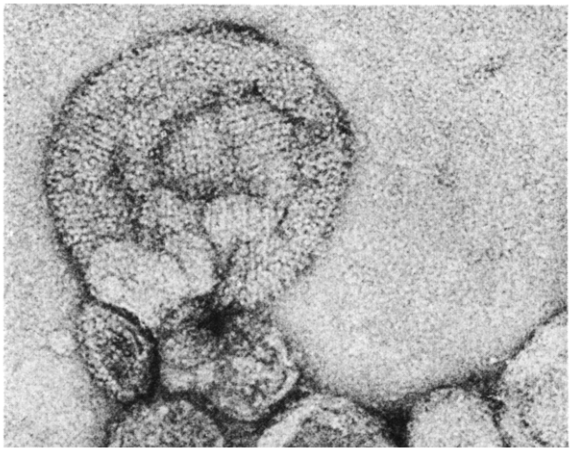

Fig.3. Microsome vesicles from 20-day-old chicken embryonic breast muscle. The sample was treated as described in fig.1. Ca^{2+}-ATPase crystalline arrays cover the surface of the whole vesicle. Negative staining with 1% uranyl acetate. Magnification: × 183600

the crystalline vesicles reaches 25–30% (fig.4); the same level is observed in microsomes isolated from adult chicken muscle. In microsome preparations isolated from chicken muscle the crystalline vesicles are of round or elliptical shape, and the cylindrical structures described in several mammalian species (fig.4, inset; [6–8]) are usually absent.

The Ca^{2+} transport ATPase (105-kDa protein) is a trace component of microsomes isolated from pectoralis muscle of 10-day-old chicken embryos (fig.5, lane A). The amount of 105-kDa protein steeply increases during development and it constitutes 25–30% of the protein content of microsomes isolated from chicks just before hatching (fig.5, lane B). The Ca^{2+}-ATPase content of chicken microsomes is generally lower and their protein composition is more heterogeneous compared with preparations isolated from rabbit muscle.

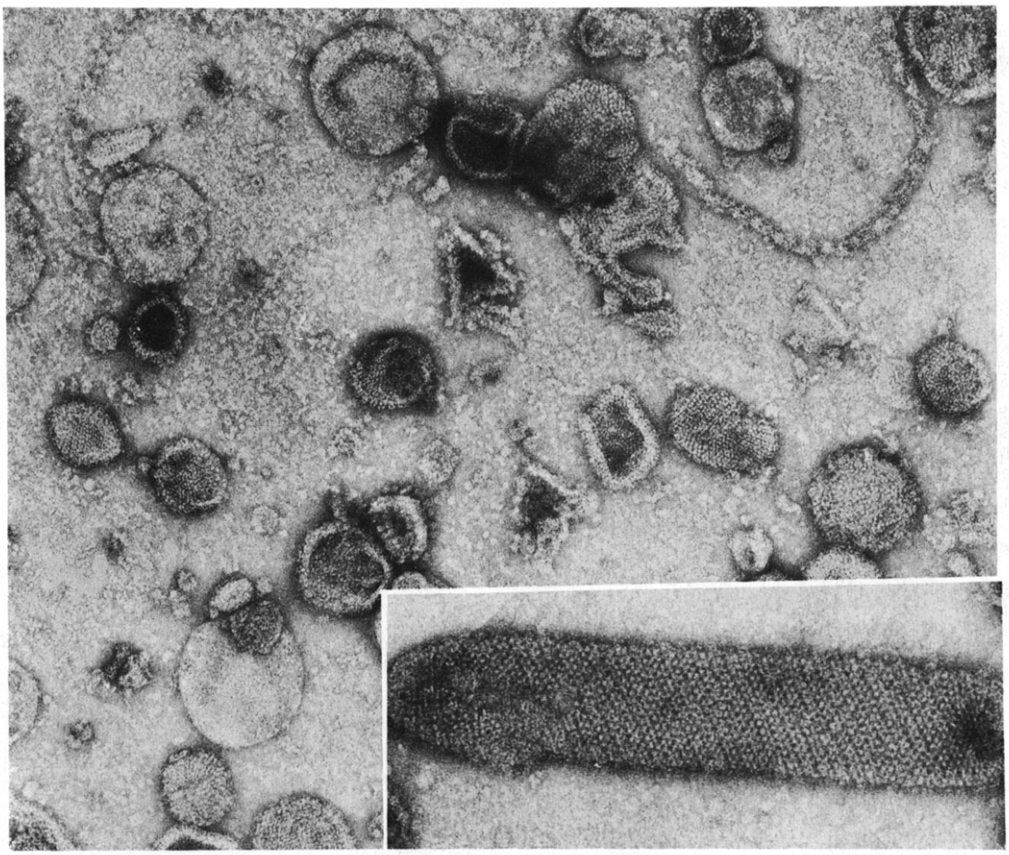

Fig.4. Microsome fraction from breast muscle of 7-day-old chicken, treated as described in fig.1. Negative staining with 1% uranyl acetate. Inset: membrane tubule covered with 2-dimensional Ca^{2+}-ATPase crystals from rabbit fast muscle, treated as described in fig.1. Negative staining with 1% uranyl acetate. Magnification: × 113000; inset: × 184000.

4. DISCUSSION

Two mechanisms are available for the regulation of Ca^{2+}-ATPase content of the muscle cell.

(i) Regulation of the amount of sarcoplasmic reticulum with a Ca^{2+}-ATPase content that is close to physical saturation. This mechanism operates in the adjustment of the Ca^{2+}-ATPase content to physiological requirements in slow and fast muscles of adult animals [4,12].

Therefore, in microsome preparations isolated from these muscles the vanadate-induced crystalline arrays cover the whole surface of the vesicles that contain Ca^{2+}-ATPase, and the frequency of crystalline vesicles is proportional to the Ca^{2+}-ATPase content of the preparation [8].

(ii) Regulation of the concentration of Ca^{2+}-ATPase within the evolving SR membrane [4,11,12]. This mechanism is operative in developing muscles, where the Ca^{2+}-ATPase content of the membrane progressively increases from the phospholipid rich endoplasmic reticulum in 10-day-old embryos to the fully developed SR of adult animals [4,11,12]. The synthesis of SR involves cotranslational insertion of Ca^{2+}-ATPase molecules synthesized on membrane-bound polysomes [11–13] in a process that may be regulated by cytoplasmic Ca^{2+} concentration [11,12,14].

The observations presented in this report conform with the second mechanism.

The vanadate-induced crystallization of Ca^{2+}-ATPase can be described as a form of fibrous condensation [15]. In processes of this kind

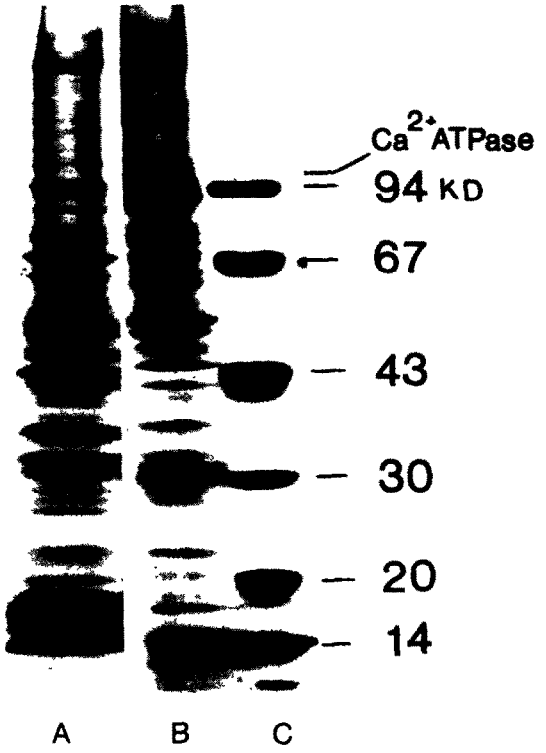

Fig.5. Protein composition of microsome samples from 10- and 21-day-old chicken embryo breast muscles. 6–18% gradient polyacrylamide gel. Lane A: 10-day-old embryo; lane B: 21-day-old embryo; lane C: molecular mass markers.

formation of protein polymers occurs only if the concentration of protein exceeds a certain minimal so-called 'critical' concentration; above this concentration the protein molecules associate into well-defined fibrous polymers. It seems that in developing SR the concentration of Ca^{2+}-ATPase first reaches this critical concentration at 18 days of development, resulting in the formation of small crystalline patches of Ca^{2+}-ATPase. These crystalline patches expand rapidly with continued insertion of ATPase molecules until the membrane becomes physically saturated. Based on earlier data [2,3] the critical Ca^{2+}-ATPase concentration for the formation of Ca^{2+}-ATPase crystals is between 1000–2000 ATPase molecules per μm^2 membrane surface area. Saturation of the membrane by Ca^{2+}-ATPase requires about 20000 ATPase molecules per μm^2 surface area [12].

The observations presented here, together with earlier findings [7,8] indicate that even in fully developed chicken muscle about 70% of the vesicles do not develop identifiable Ca^{2+}-ATPase crystals and may originate from structures other than SR. The identification of the origins of these membranes is an important task for the future.

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