72. Distribution of Cadmium in the Cross-Section of Cadmium Treated Abdominal Skin of a Bullfrog

Analysis of Cd with an Electron Probe X-ray Microanalyzer

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We have investigated the effects of Cd on the Na active transport by frog skin, and disclosed that epidermally applied Cd increased the Na active transport, whereas dermally applied Cd did not. We analyzed the distribution of Cd in the cross-section of frog skin by using an electron probe X-ray microanalyzer (XMA) in order to elucidate the mechanism of this directional difference of Cd effects on frog skin. It is hard to know the exact distribution of elements with an ordinary chemical quantum analysis. Even the histochemical method has a difficulty, because of its low resolution and specificity. XMA technique has overcome these difficulties, and we have succeeded for the first time in demonstrating Cd in the skin treated with Cd.

The abdominal skin of a bullfrog (Rana catesbeiana) were used in this experiment. The frogs were captured around the Saitama district, Japan. They were kept at room temperature (19–21°C) in a bath for a week or two without feeding. The frogs were anaesthetized with intrathecal injection of 0.5 ml urethane solution (0.25 g/ml). The measurement of potential difference (PD) of the skin in the open circuited condition and intermittent measurement of short circuit current (SCC) in the closed circuited condition were performed through the methods described in the previous paper. The excised abdominal frog skin was mounted on the tip of the glass cannula (1 cm in diameter), with epidermal side facing the inside of the cannula in case of the epidermal application of Cd, and with dermal side facing the inside of the cannula in case of the dermal application of Cd. The cannula was vertically held, and Ringer’s solution in both sides of the cannula was maintained at the same levels so that there could be no difference in hydrostatic pressure between epidermal and dermal sides of the skin. The composition of normal SO₄-Ringer’s solution was as follows; Na₂SO₄ 55 mM, K₂SO₄ 1 mM, Ca gluconate 1 mM, glucose 10 mM, sucrose 60 mM, tris-H₂SO₄ buffer
The Ringer's solution in both sides of the cannula was aerated in order to stir the solution and also to supply oxygen. When the PD became constant approximately 2 hr after the setup of skin, 2 mM Cd was administered to the skin for 20 min. Skin was washed with SO4-Ringer's solution for several seconds, and fixed with oxine-saturated 2% glutaraldehyde-1/14 M veronal buffer (pH 7.2) for 2 hr at 0–4°C. The fixed skin was freeze-dried with liquid freon-liquid nitrogen system, sectioned in 10 μm thickness by using the cryostat, and freeze-dried in the cryostat chamber. The section was mounted.
on the carbon plate (5 mm φ) coated with chloroprene containing adhesive. Carbon coating for an energy dispersive method and Pt-Pd coating for a wave length dispersive method were carried out over the surface of the sections.

Elements in the cross-section of abdominal skin were analyzed with an analytical electron microscope (JEOL JEM-100C/SEG/ASID/NDS with a multichannel analyzer, Ortec Model 6200) (EDX). We also used a scanning electron microscope JEOL JEM-U3S with a wave length dispersive analyzer (WDS) for line and spot analyses of Cd in the cross-section of abdominal skin.

Scanning electron micrograph of the cross-section of frog skin is shown in Fig. 1a. The epidermis is a stratified epithelium with 1 to 2 layers of cornified squamous cells in the stratum corneum (1), 1 to 3 layers of cuboidal cells in the s. granulosum (2) and s. spinosum, and a basal layer of columnar cells in the s. germinativum (3). Between epidermis and dermis, there is a layer called basement membrane (4). The dermis is divided into a loose layer, the spongiosum (5), and a deeper and more compact layer, the s. compactum. The outermost layer of the s. compactum is called the sieve layer (6). Tela subcutanea (7) is located at the inner surface of the skin.

Fig. 1b shows a typical example of X-ray energy spectrum at the sieve layer ((6) in Fig. 1a) of frog skin which was treated by 2 mM Cd from dermal side for 20 min. No change in SCC was observed in case of dermal application of Cd. Cadmium (La 3.13 keV) were clearly demonstrated in the “sieve layer”. Calcium (Kα 3.69 keV, Kβ 4.01 keV), P (Kα 2.01 keV), and Cl (Kα 2.62 keV) were also demonstrated in this spot. Counts ratio of Ca to P were approximately 2 : 1 and this ratio coincides with the value obtained in paraffin section of frog skin. It is difficult to say that Cl was derived from the biological specimen because Cl was also observed even in the supporting material. A small amount of Cd was found in the following layers: (2) – (5), and (7) of Fig. 1a. Small amounts of Ca and P were proved to exist in the layers (1) – (5) in Fig. 1a.

In case of epidermal application of Cd of 2 mM for 20 min, SCC was increased by 41.3%. Cadmium was not demonstrated in the sieve layer (Fig. 1c), though Ca and P were observed as reported previously.

A quantitative analysis of Cd by using WDS for 20 sec were carried out on the same preparations. In the skin treated with dermal application of Cd, counts of Cd specific X-ray in the sieve layer were 247±29 (mean ±1 S.E.) and those in the s. germinativum, were 41.2±10.2, while those in the s. germinativum of the control skin were
Fig. 2. Line analyses of Cd specific X-ray in the cross-sections of abdominal frog skin. White linear lines indicated by arrows in each ordinary scanning electron micrograph show the positions of the line analyses of Cd specific X-ray. a: The skin treated with epidermally applied Cd (2 mM, 20 min). b: The skin treated with dermally applied Cd (2 mM, 20 min). e, epidermis; p, s. spongiosum; s, sieve layer; c, s. compactum. c: The control skin.
2.0±1.0. Therefore, there is a remarkable significant difference in the counts of Cd specific X-ray between Cd treated and untreated skin (P<0.01). When Cd was applied to the skin from the epidermal side, counts of Cd specific X-ray in the sieve layer were 3.5±1.5 and those in the s. corneum were 20.0±8.0. We can not deny the null-hypothesis that there is no significant difference between counts of Cd specific X-ray in the sieve layer treated with epidermally applied Cd and those of control. However, there is a clear significant difference between counts of Cd specific X-ray in the s. corneum and those of control (P<0.01).

Fig. 2 illustrates the line analyses of Cd specific X-ray in the cross-sections of Cd treated skin and in that of control. When Cd was administered to the epidermal side of the skin, Cd was demonstrated to be distributed in the s. corneum and the s. granulosum, but not in the other layers (Fig. 2a). On the other hand, in case of dermal application, the existence of a large amount of Cd was demonstrated in the sieve layer and a small amount of it was also discovered in the s. spongiosum, basement membrane, s. germinativum and s. granulosum. No cadmium was demonstrated in the s. corneum in this case (Fig. 2b). In the control skin, Cd was not detected (Fig. 2c). These data confirm the previous results obtained by EDX. These findings of Cd distribution in frog skin was also observed in the histochemical technique — dithizone staining (unpublished observation). The fact that the epidermally applied Cd is distributed exclusively in the s. corneum and s. granulosum is compatible with the following assumption: Cd decreases the resistance of outer facing membrane, thus increases the Na active transport by the skin.2)

Epidermally applied colloidal lanthanum invades only into the occluding zonules located at the outer border of the s. corneum and at the outer layer of the s. granulosum of the frog skin.4) It is very likely that epidermally applied Cd also invades only into this special region of the skin.

The reason that dermally applied Cd does not affect the Na active transport may be ascribed to two seeming barriers, namely, the sieve layer and the basement membrane, which seem to disturb Cd to attain the Cd-sensitive site of epidermis. Sieve layer might absorb dermally applied Cd for exchange of Ca because of the existence of a large amount of Ca and P(6,7) and the high absorbability of Na and Rb in that layer. The basement membrane may be the second barrier against dermally applied Cd, because in the isolated epidermis which retained the basement membrane, dermally applied Cd still does not affect the Na active transport, as was the case with the whole skin (unpublished observation).
References

2) ——: Ibid., 28, 63–73 (1978).