J. Cell Sci. 5, 313-319 (1969)
Printed in Great Britain

THE EFFECT OF ETHYLENEDIAMINETETRA-ACETATE WASHING ON THE STRUCTURE OF SPINACH THYLAKOIDS

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SUMMARY

The particles removed from spinach thylakoids by washing with ethylenediaminetetra-acetate were found to correspond to particles on the outside surface of the thylakoid, and not to the quantasome cores which remain within the membrane.

INTRODUCTION

Negatively stained preparations of buffer-washed spinach thylakoids show that particles 8·0–10·0 nm in diameter are attached to the thylakoid. Howell & Moudrianakis (1967*a*, *b*), Moudrianakis, Howell & Karu (1968) and Murakami (1968) found that these particles were largely removed from the thylakoids by washing the thylakoids in 1 mM ethylenediaminetetra-acetate (EDTA). The particles so removed were identified as the Ca^{2+}-dependent ATPase. Howell & Moudrianakis (1967*a*) further asserted that the particles removed by washing with EDTA were, in fact, the quantasomes, described by Park & Biggins (1964) and Branton & Park (1967).

We repeated the experiments of Howell and Moudrianakis and found that thylakoids which were 'particle free' by the criterion of negative staining still possessed quantasome cores (Park & Pfeifhofer, 1969) when viewed by fracturing and deep-etching techniques (Park & Pfeifhofer, 1968). This finding is consistent with our interpretation that the quantasome core lies largely within the thylakoid membrane and is exposed during the fracturing process.

In the previous paper (Park & Pfeifhofer, 1969) we described the character of the external, deep-etched surfaces of thylakoids and their relationship to the internal fracture faces. In this paper we show that the 8·0–10·0 nm thylakoid-bound particles seen by negative staining can also be visualized by deep etching. We show further that the 8·0–10·0 nm particles are situated on the A' surface of the thylakoid and are distinct from the quantasome core which lies largely embedded within the membrane.

MATERIALS AND METHODS

Thylakoids were prepared by the methods of Howell & Moudrianakis (1967*a*), including density-gradient purification of the EDTA-washed material. Material for deep etching was frozen in water and treated as described earlier (Park & Shumway, 1968). Glutaraldehyde fixation of some thylakoid preparations was carried out at room temperature in 10 % glutaraldehyde for 1 h. Negative staining was carried out with 2 % ammonium molybdate solution which contained 0·1 % sucrose to facilitate even spreading of the stain. Freeze etching was performed following modifications (Branton & Park, 1967; Park & Shumway, 1968) of the method initially described by Moor, Mühlethaler, Waldner & Frey-Wyssling (1961).

OBSERVATIONS AND DISCUSSION

A negatively stained preparation of spinach thylakoids before EDTA washing is shown in Fig. 1. This negatively stained thylakoid preparation is similar in appearance to many reported in the literature (Howell & Moudrianakis, 1967*a*; Murakami, 1968). The thylakoids are covered with a number of particles about 10·0 nm in diameter. Thylakoids washed in 1 mM EDTA, purified on a density gradient and then negatively stained, are shown in Fig. 2. These thylakoids appear smooth and were thought by Howell and Moudrianakis to be quantasome-free. Figure 3 is a preparation of EDTA-washed thylakoids which were purified on a density gradient and then freeze-fractured with no etching. Though these thylakoids were smooth when observed by negative staining, they showed typical *B*-fracture faces. That the quantasome cores upon the *B* face of the EDTA washed, density-gradient purified thylakoids can exist in paracrystalline quantasome arrays is shown in Fig. 3.

Experiments such as those reported earlier (Park & Pfeifhofer, 1968) and confirmed in Fig. 3 support the idea that it is not the *B*-face particles which are removed by EDTA washing, but they do not reveal which particles are removed by this treatment.

An experiment designed to show which particles are removed from the thylakoids by EDTA washing is shown in Figs. 4 and 5. Figure 4 presents a typical fractured and deep-etched control thylakoid in which the *A′* surface and *B* face is exposed. Particles are seen on both the *A′* surface and on the *B* face. A similar observation on a thylakoid preparation after EDTA washing and density-gradient purification is shown in Fig. 5. It is evident that the *B*-face particles remain and that it is the large particles on the *A′* surface which have been removed by EDTA washing. Deep etching has caused some destruction of the *B* face in Fig. 5 (see Park & Pfeifhofer, 1969). Observation of the interior surface of the thylakoid (*D* surface) and its corresponding *C*-fracture face (Figs. 6, 7) show that here little or no change is produced by EDTA washing and density-gradient purification.

Howell & Moudrianakis (1967*b*) and Murakami (1968) have found that the particles on the *A′* surface possess Ca^{2+}-dependent ATPase activity. We have observed further that glutaraldehyde fixation tightly links this particle to the thylakoid so that it is no longer removed by EDTA washing. This suggests that the *A′*-surface particle may be bound to the thylakoids by a protein–protein association in addition to divalent ions as suggested by the EDTA effect.

We conclude from these experiments that the 10·0-nm particles removed from spinach thylakoids by EDTA washing are located on the *A′* surface of the thylakoids. The quantasome cores on the other hand are largely buried within the thylakoid and are not removed by the EDTA treatment.

This work was supported by the National Institute of General Medical Sciences Grant GM-13943-03.

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(*Received* 11 *November* 1968)

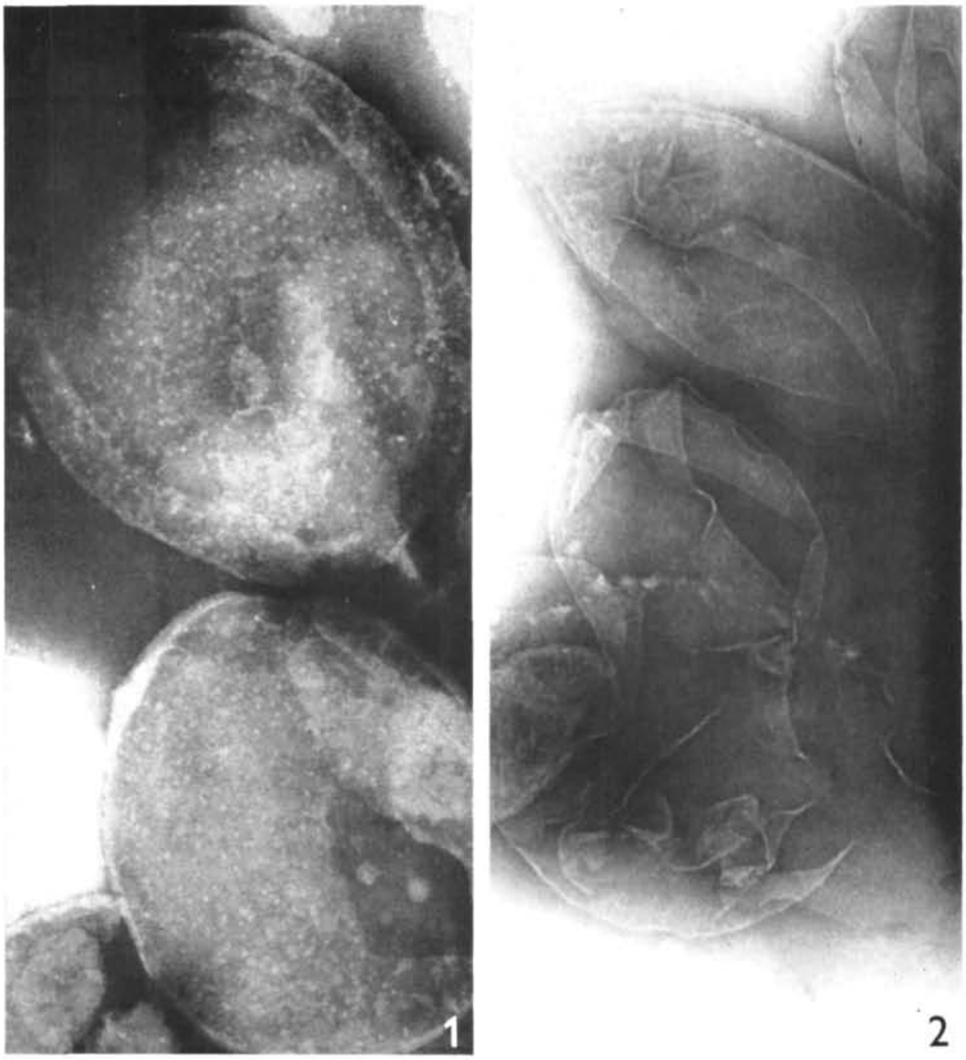


Fig. 1. Spinach thylakoids negatively stained with ammonium molybdate before washing with 1 mM EDTA. ×80000.

Fig. 2. Spinach thylakoids negatively stained with ammonium molybdate after washing with 1 mM EDTA and purifying on a density gradient. ×75000.

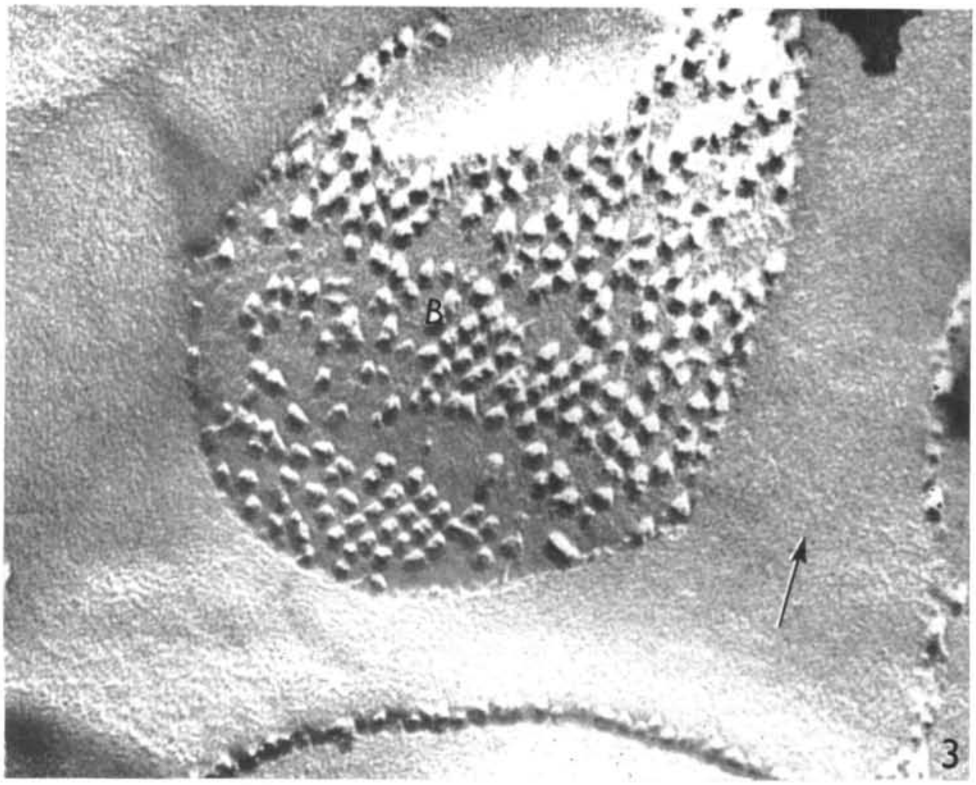


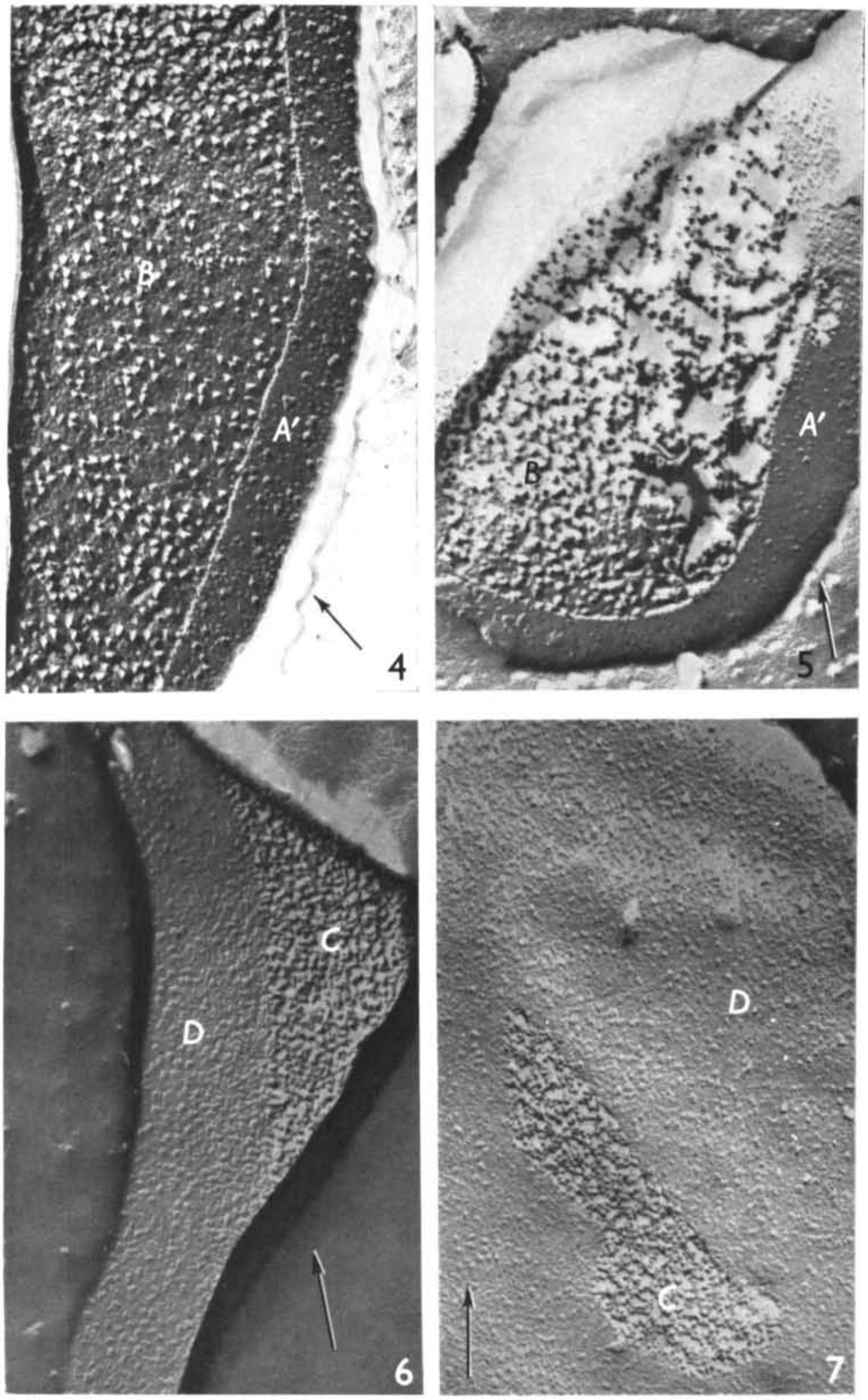
Fig. 3. Spinach thylakoids freeze-fractured with minimum etching after washing with 1 mM EDTA and purifying on a density gradient. Arrays of quantasome cores are evident. Arrow indicates shadow direction. $\times 160000$.

Fig. 4. Spinach thylakoid fractured and deep etched so as to show the *A'* surface and *B* face before washing with 1 mM EDTA. × 80000.

Fig. 5. Spinach thylakoid fractured and deep etched so as to show the *A'* surface and a somewhat damaged *B* face after washing with 1 mM EDTA and purifying on a density gradient. × 80000.

Fig. 6. Spinach thylakoid fractured and deep etched so as to show the *D* surface and *C* face before washing with 1 mM EDTA. × 80000.

Fig. 7. Spinach thylakoid fractured and deep etched so as to show the *D* surface and *C* face after washing with 1 mM EDTA and purifying on a density gradient. × 70000.


B
A′
4
B
A′
5
C
D
6
D
C
7