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An Analysis of Amoeboid Movement
II. Mechanical Properties of Surface Structure of *Amoeba*

With 3 Text-figures

Fumikazu KANNO

Laboratory of Biology, Hosei University, Tokyo
(*Communicated by* H. KINOSITA)

In order to solve problems on the mechanism of amoeboid movement, many investigations have been made on mechanical properties of amoeba protoplasm (Harvey and Marsland, 1932; Heilbrunn and Daugherty, 1932; Landau, Zimmerman and Marsland, 1954, and many others). However, most of those studies dealt chiefly with amoeba protoplasm as a whole. Recently, a few approaches have been made with respect to local difference in mechanical property of a moving amoeba (Allen and Roslansky, 1959; Allen, 1960; Yagi, 1961; Goldacre, 1961). But the results of such experiments did not always agree, and there remained problems to be investigated in detail concerning the local mechanical properties of surface structure.

In the present work, mechanical properties, such as elasticity of surface structure in different regions of moving amoeba, were investigated by the use of the sucking method which was principally the same as the one reported by Mitchison and Swann (1954) and the results were compared with those of previous workers.

MATERIAL AND METHOD

A clone of *Amoeba* (*proteus* type) was used in this study and the culture methods were stated elsewhere (Kanno 1964). Only monopodal amoeba was selected as material, because the simple shape of the organism made it possible to conveniently divide the cell body into three general different regions, e.g., the anterior, middle and posterior regions. The lateral flat side of the surface structure, or the surface layer including plasmalemma and ectoplasmic gel, of moving amoeba was sucked horizontally into a glass capillary (inner diameter, 15–20 micra for the length of about 500 micra) by negative hydrostatic pressure generated by means of a mercury column manometer connected with the capillary (Fig. 1). The sucking force was easily calculated from the value of applied hydrostatic pressure and of capillary cross section. The tip of the glass capillary was always kept horizontal and perpendicular to the body surface. The amoeba was always immersed in inorganic culture solution contained in a trough (Kanno, 1964). All the experiments were carried out at room temperature (19°–23°C).

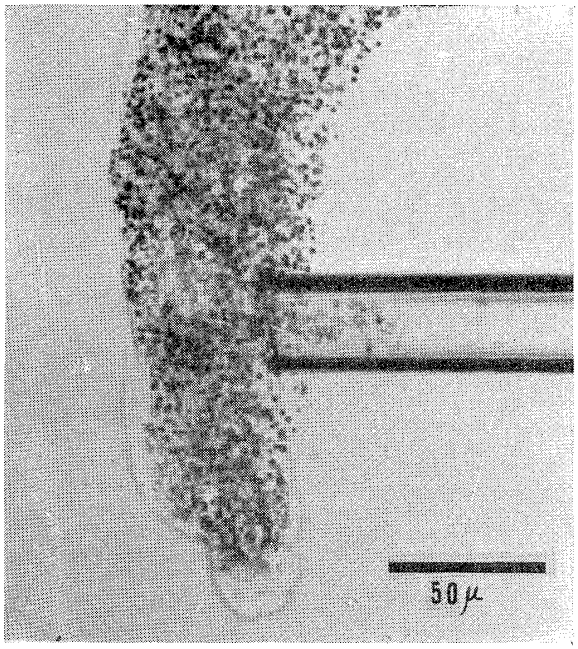

Fig. 1. Photomicrograph of a moving amoeba. A part of the surface structure is sucked into a glass capillary.

Results

If the sucking force was less than 0.25×10^{-2} dynes, the part pulled in the capillary (inner diameter; 15–20 micra) was less than 10 micra in length and usually consisted of hyaline layer. On the other hand, suction stronger than 6.0×10^{-2} dynes always caused the tearing away of the surface structure from the cell body. Therefore, sucking force applied to the cell surface was kept within a range from 0.25×10^{-2} dynes to 6.0×10^{-2} dynes except in the tearing experiment (Section 2).

When negative pressure was applied locally to the surface of amoeba, the surface structure was suddenly extended within the capillary and resumed a certain length for several seconds. After measurement of the length of the extended surface structure was completed, the extended part was pushed out of the capillary by application of slight positive pressure. The suction experiment was repeated succesively, when the amoeba retracted its extended pseudopod and regained the monopodal shape. Suction at the anterior region was made near the rear end of the hyaline cap, because the anterior tip of moving amoeba was highly sensitive to mechanical stimulation, and readily responded by a rapid cessation of streaming.

The amoeba thus captured by a suction pipett continued normal creeping and showed sliding movement of plasmalemma toward the anterior end as was reported by the former workers (Mast, 1926; Abé, 1961). It is interesting to point out that the plasmalemma covering the extended surface structure within the capillary has never shown any sign of sliding movement, while the plasmalemma covering the open surface very close to the capillary tip exhibited normal forward sliding.

1. Relation between the sucking force applied and the length of extended surface structure.

The surface structure of the three regions of moving amoeba was extended by sucking forces of various strengths. Results obtained on 50 amoebae are summarized in Figure 2. It is shown in this figure that the length of extended surface structure of amoebae was nearly proportional to the sucking force, and that the force-length line was the steepest at the anterior region (a). This suggests that the elasticity of the surface structure is the lowest at the anterior region and is highest at the posterior end.

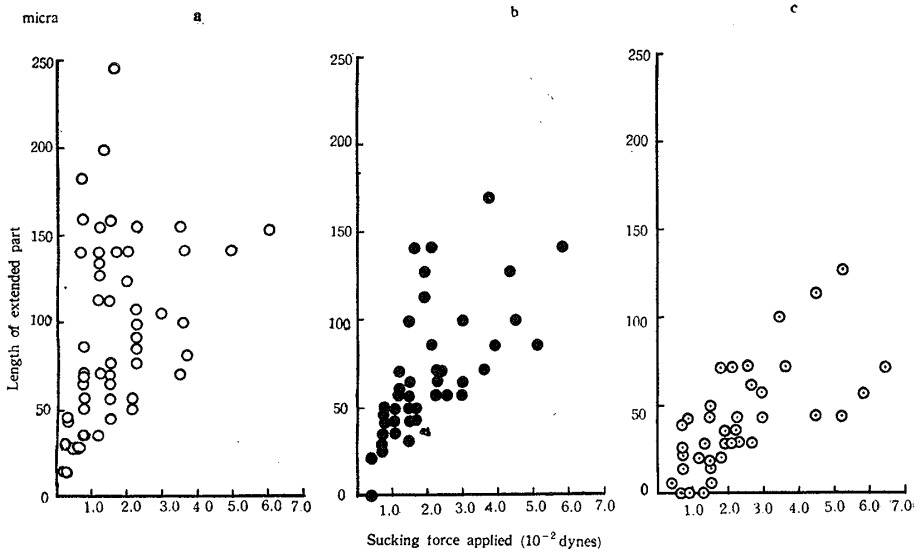

Fig. 2. Relation between the sucking force applied to the surface of moving amoebae and the length of extended part of surface structure. (a), the anterior region (hollow circles); (b), the middle region (solid circles); (c), the posterior region (double circles).

It should be noticed that the surface structure of the posterior region (c) could hardly be stretched by a sucking force weaker than a certain limit. It is also clear from the figure that the data in (a) are scattered more remarkably than those in (b) and still more than those in (c). Result of continued observation on the time change of elasticity of surface structure of the three regions of a single moving amoeba, as is shown in Figure 3, seems to explain the above-mentioned variation of the data in terms of the remarkable time change of elasticity value even in the same region.

An attempt was made to suck only plasmalemma and hyaline layer of moving amoeba into the capillary. Sucking force of 0.15×10^{-2} dynes was enough to pull them into the capillary, when it was applied locally at either the anterior or middle region. In this case the length of the part extended from the anterior region did not differ much from that pulled out from the middle region. The suction into the capillary of the plasmalemma and hyaline layer at the posterior region was impractical, because in this region the surface of the plasmalemma wrinkled and the hyaline layer was very thin. Thus it was impossible to demonstrate the topographical difference in elasticity of plasma-

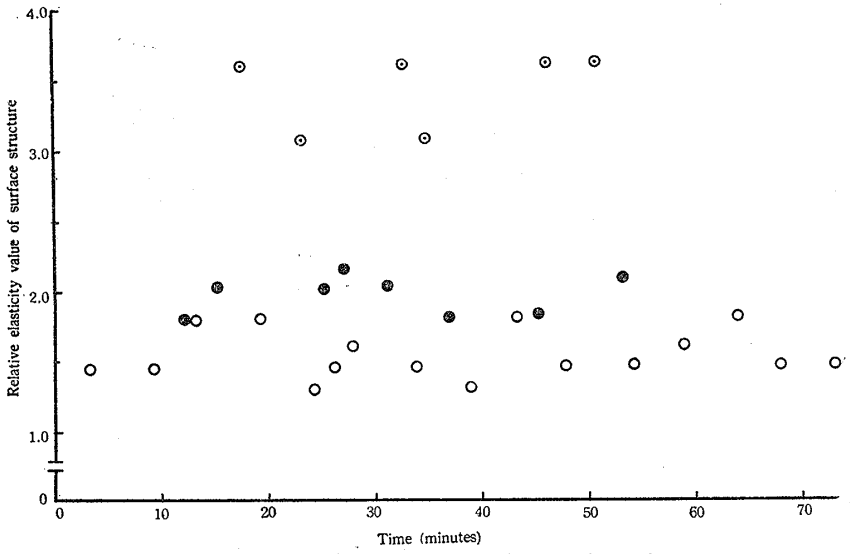

Fig. 3. Typical time change in elasticity of surface structure at three regions of an individual moving amoeba. Elasticity is expressed in terms of the reciprocal of the length of the surface structure extended within the capillary by the sucking force of 2.0×10^{-2} dynes. Hollow circles, the anterior region; solid circles, the middle region; double circles, the posterior region.

lemma by the method employed in the present experiment.

2. Sucking force sufficient to tear off the extended surface structure.

An attempt was made to tear a part of the surface structure from the rest of the cell body within the capillary by application of stronger sucking force. Three different types of tearing were distinguished, namely, (1) the extended part was torn off at the opening of the capillary, (2) the plasma-

Table 1

Sucking force required to tear off the extended surface structure

The surface structure was torn off (+) or failed to be torn off (−) by various sucking forces. (Capillary diameter: 30 micra)

No. of specimens	Region of the cell	Sucking force in dynes $\times10^{-2}$								
		0.9	1.8	2.7	3.6	4.5	5.4	6.3	7.2	8.1
1	Anterior	−	−	±	±	+				
	Middle	−	−	−	−	−	−			
	Posterior	−	−	−	−	−	−			
2	Anterior	−	±	+						
	Middle	−	−	−	−	−	±	+		
	Posterior	−	−	−	−	−	−	+		
3	Anterior	−	−	−	−	+				
	Middle	−	−	−	−	−	−	+		
	Posterior	−	−	−	−	−	−	−	−	+

lemma was broken at the tip of the extended part, with the consequent rapid flowing out of cell contents, (3) the extended part was broken at the middle portion within the glass capillary. In the present experiment, the capillary containing the extended surface structure was pulled away from the rest of the cell body when the length of the extended part reached a certain limit (25 micra). Thus the extended part within the capillary was exclusively made to be torn off at the opening of the capillary (above-mentioned case 1). The critical values of sucking force required to tear off the extended part are shown in Table 1. It is shown in this table that the critical values are the lowest at the anterior region; that is, the surface structure at the anterior region is mechanically weaker when compared with that of the other region.

It should be noted that whenever the extended part was being torn off from the cell, a fine protoplasmic thread connecting the extended mass and the cell body appeared and the length of this fine thread was always longer at the anterior part than that at the posterior region.

3. Magnitude of elastic return of extended surface structure.

When the sucking force within the capillary was suddenly removed, the length of the extended part within the capillary quickly decreased to a certain level. Such a decrease in length, when expressed in percentage of the initial length before the negative pressure was released, was shown to be dependent on the region of the cell from which the surface structure was sucked into the capillary as is shown in Table 2. It is clear from the table that the part extended into the capillary from the anterior region demonstrated very little tendency for elastic return. The tendency of elastic return was clearly shown

Table 2

Magnitudes of elastic return of extended surface structure of Amoeba

The sucking force of 4.2×10^{-2} to 16.8×10^{-2} dynes which pulled the surface structure into the capillary (diameter: 20 micra) was suddenly removed. The measured length of elastic return of the surface structure was expressed in percentage of the initial length (100 micra) of surface structure within the capillary just before the negative pressure was cut off.

No. of specimens	Moving amoeba			Rounded amoeba
	Anterior region	Middle region	Posterior region	
1	3	13	20	30
2	0	15	25	
3	0	10	15	
4	0	10	20	
5	2	8	25	26
6	2	13	18	
7	4	10	18	33
8	0	15	17	33
9	0	14	20	
Mean	1	12	20	31

in the middle region and still more clearly in the posterior part, suggesting the existence of significant difference in colloidal nature between the anterior part and the rest of the cell body. It may be also pointed out from the table that the elasticity was the highest in the case of the rounded motionless cell, in which no clear distinction was found between the surface structure and the endoplasm.

Discussion

Through the use of low speed centrifuge microscope methods, Allen (1960) pointed out that the posterior ectoplasm of moving amoeba (*Amoeba proteus*, *A. dubia* and *Chaos chaos*) was less rigid than the ectoplasm of the anterior part. On the other hand, Yagi (1961), measuring the elasticity values of ectoplasmic gel of *Amoeba* (*proteus* type) by his electromagnetic method, reported that there exists an antero-posterior gradient of ectoplasmic elasticity; the anterior part being the lowest and the posterior end the largest.

In the present study, the elasticity of the surface structure was represented by the reciprocal of the length of the surface structure pulled into the capillary by the sucking force of a definite magnitude. Slight suction methods, such as adopted by Mitchison and Swann (1954) in their elasticity measurement on sea urchin egg, were found not to be applicable in the present case, because only the hyaline layer was pulled out by the slight suction. On the other hand, absence of difference in length of extended plasmalemma and hyaline layer between the anterior and the middle region seems to indicate that the remarkable antero-posterior gradient of elasticity of surface structure shown in sections 1 and 3 is due to the similar gradient of elasticity of ectoplasmic gel which acts as a main mechanical resistance against the sucking force applied to the surface structure. Additional experimental evidence to show that the plasmalemma did not take any important part in elasticity of surface structure is that the plasmalemma of the surface structure within the capillary has never shown any sign of sliding movement, whereas the plasmalemma covering the open surface very close to the capillary tip exhibited normal sliding movement, suggesting the semi-fluid nature of the plasmalemma. Mast (1926) and Pappas (1956) maintained that the plasmalemma has a certain solid structure. The discrepancy between their results and mine will be left unsolved at least for the present state of investigation. It was found in the present experiment that the elasticity of surface structure (Fig. 2), as well as the threshold value of sucking force to tear off the surface structure (Table 1), was different at various regions of amoeba, the values being the lowest at the anterior region and the highest at the posterior region, in spite of the gradual change in position of the ectoplasmic inclusions relative to the moving amoeba (Fig. 3).

Yagi (1961) measured the elasticity of ectoplasmic gel from the displacement of nickel particles inserted into ectoplasm of moving amoeba placed in magnetic field, and demonstrated the antero-posterior gradient of ectoplasmic elasticity quite similar to the present result. A large elasticity value at the anterior part, suggestive of the pulling force, generated at the "fountain zone" and strong enough to cause forward streaming of endoplasm as was mentioned

by Allen (1961), could not be found in the present experiment.

Very little, if any, tendency of elastic return observed on the surface structure extended from the anterior region shown in Table 2 may be due to the adhesive nature of the anterior surface. In this connection it is interesting to point out that the length of the fine thread appearing between the cell body and the extended mass torn off from the cell was always longer at the anterior region than that at other parts of the cell.

As is shown in Table 2, the magnitude of elastic return of the extended surface structure was the highest in the rounded cells. This corresponds with the result obtained by Yagi (1961), who stated that the interior of the cell body of rounded amoeba was highly ectoplasmic.

Goldacre (1961) reported that the least suction force required to rupture the amoeba was of the order of 30×10^{-2} dynes. The force sufficient to tear off the surface structure was shown in the present experiment to be about 10×10^{-2} dynes. This value coincided fairly well with that of the former even though the detailed procedure was different.

SUMMARY

1. The surface structure of moving *Amoeba* (*proteus* type) was locally sucked into a glass capillary by negative force produced by Hg manometer. The length of surface structure pulled into the capillary was taken to indicate the relative elasticity of the surface structure of amoeba.

2. It was found that the elasticity of the surface structure differed at different regions of moving organism. At the posterior region, the surface structure was shown to be most rigid.

3. Force sufficient to tear the extended surface structure from the rest of the cell body was measured at different regions of moving amoeba. The threshold value of the force was found to be the lowest at the anterior region and the highest at the posterior region.

4. Elastic return of the extended surface structure in the capillary occurred when the sucking force was cut off. The magnitude of the elastic return was larger at the posterior region than that at the middle region. Very little tendency toward elastic return of surface structure was found at the anterior region.

5. The present method of measuring elasticity of surface structure, as well as the mechanical properties of plasmalemma and that of the ectoplasmic gel of amoeba, was discussed in relation to those of previous workers.

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