EVIDENCE FOR THE PRESENCE OF A PROTEIN WITH ATP-ASE AND ANTIGENIC SPECIFICITY IN *PARAMECIUM AURELIA*, VARIETY 4, STOCK 51*

by

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In lower organisms, the movement of flagella, the stroking of cilia, and ameboid movement are ascribed to contractile protein particles. WEIBULL has described the isolation of a protein fraction from flagella of *Proteus vulgaris*[1] and from the flagella of *Bacillus subtilis*[2]. This protein fraction has the characteristic X-ray pattern of a protein of the keratin-myosin class[3]. ENGELHARDT has isolated from a homogenate of sperm cells a protein fraction ("spermosin") which possesses enzymatic activity towards adenosinetriphosphate[4]. GOLDACRE AND LORCH have postulated that a protein, having the property of splitting adenosine-triphosphate is responsible for ameboid movement and cytoplasmic streaming[5]. KESZTYUS *et al.* reported on the antigenic properties of myosin[6].

We have succeeded in isolating from *Paramecium aurelia*, variety 4, stock 51 antigen type A(7), a protein preparation which has adenosinetriphosphatase activity and which gives a precipitin reaction with 51 A antiserum.

The isolation procedure for myosin as given by SZENT-GYÖRGYI[8] was followed.

100 l of a culture of *P. aurelia* (containing appr. 1500 *P. aurelia* per ml) was concentrated with the Sharples centrifuge. The bowl content (7.75 g) was suspended in 3 volumes of an ice-cold mixture of equal volumes of 0.3 *M* KCl and 0.15 *M* KH_2PO_4 (p_H 6.5). After 10 minutes 4 volumes of water were added and the mixture was gently stirred for 2 hours at room temperature. The suspension was centrifuged and the sediment was discarded. 1.5 volumes of ice-cold water were added slowly under vigorous stirring. A sheen-like precipitate was formed. The solution was allowed to stand in the cold for 12 hours and was centrifuged in the cold. The precipitate was washed repeatedly with 0.04 *M* KCl solution. The precipitate was then dissolved in 10 ml of 0.5 *M* KCl solution and tested for ATP-ase activity in the following manner. To 2 ml of the above solution (containing 16 γ N per ml) were added 1 ml of glycine buffer (p_H 8.3) and 0.5 ml of a 1% $CaCl_2$ solution. 1 ml of 10% trichloroacetic acid (TCA) was added to the control tubes. 1 ml of adenosinetriphosphate solution ($P_7 = 1.00 \cdot 10^{-4}$ *M*; p_H 7.5) was added to the control tubes and the experimental tubes. The experimental tubes were incubated

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for 30 minutes at 27°. At the end of the incubation period 1 ml of 10% TCA was added to the experimental tubes. The color was developed according to the procedure of FISKE AND SUBBAROW[9]. The contents of the tubes was brought to constant volume and the tubes were centrifuged in order to sediment the precipitated protein. The color was read in a Beckman B spectrophotometer. In a duplicate determination 4 ml of the *P. aurelia* extract was used. It can be seen from Table I that in both cases only one half of the available labile P was split from adenosinetriphosphate. Since doubling the concentration of the *P. aurelia* extract did not appreciably alter the amount of P_7 split, it can be concluded that the action of the enzyme present in the extract was specific for the hydrolysis of only the terminal phosphate group from adenosinetriphosphate. The preparation contained therefore an adenosinetriphosphatase.

TABLE I

SPLITTING OF ADENOSINETRIPHOSPHATE BY A *P. aurelia* EXTRACT

P. aurelia extract added ml	Total P_7 $\cdot 10^{-4}$ *M*	P_7 split $\cdot 10^{-4}$ *M*
0	1.00	0
1	1.00	0.54
2	1.00	0.56

A precipitate was formed when 0.2 ml of the extract was carefully layered on a serum prepared against *P. aurelia* of serotype 51A. No precipitate was formed with control serum.

SUMMARY

A protein with adenosinetriphosphatase activity has been isolated from *P. aurelia*, var. 4, stock 51. This protein gives a precipitate with an antiserum prepared against serotype 51A.

RÉSUMÉ

Nous avons isolé de *P. aurelia*, var. 4, stock 51, une protéine à activité adénosinetriphosphatasique. Cette protéine est capable de précipitation spécifique avec l'immun-sérum 51A.

ZUSAMMENFASSUNG

Wir haben aus *P. aurelia*, var. 4, stock 51 ein Eiweisspräparat mit Adenosinetriphosphataseaktivität isoliert. Dieses Eiweiss wird durch Antiserum gegen Typ 51A präzipitiert.

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