Physarum Vitronectin-like Protein has Extensive Homology to Dihydrolipoamide Acetyltransferase

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Key words: cell-adhesive protein/vitronectin/Physarum/dihydrolipoamide acetyltransferase/protein chemistry/immunocytochemistry

ABSTRACT. Physarum vitronectin-like protein with a molecular mass of 70 kDa cross-reacts with anti-bovine vitronectin and promotes cell-spreading (Miyazaki, K. et al. 1992. Exp. Cell Res., 199: 106–110.). The amino-terminal sequence of Physarum vitronectin-like protein is, however, distinct from those of animal vitronectins but shows significant sequence homology with dihydrolipoamide acetyltransferase, a component of pyruvate dehydrogenase complex. We have investigated the structural relationships between Physarum vitronectin-like protein and dihydrolipoamide acetyltransferase by using both antibody and protein-chemical methods. The vitronectin-like protein reacted with both anti-bovine vitronectin IgG and anti-rat pyruvate dehydrogenase complex IgG, indicating that it shares common antigenic determinant(s) with rat pyruvate dehydrogenase complex. Furthermore, sequencing studies of peptides obtained by lysylendopeptidase digestion indicated that internal sequences of Physarum vitronectin-like protein show significant homology with dihydrolipoamide acetyltransferase, but do not show any homology with the primary structures of authentic vitronectins. Immunocytochemistry revealed that the protein is widely localized in cytoplasm and nuclei of Physarum polycephalum, but is not present in the central area of vacuoles. Our results indicate that Physarum vitronectin-like protein is a molecule structurally and immunologically related to dihydrolipoamide acetyltransferase but functionally similar to animal vitronectin, although its localization is unique.

Vitronectin (for reviews, see 6, 23, 31) is a glycoprotein existing in animal blood plasma, extracellular matrix, and platelets. Vitronectin mediates attachment and spreading of animal cells through an Arg-Gly-Asp (RGD) sequence. It also binds thrombin-antithrombin III complex, plasminogen activator inhibitor-1, and terminal complement complex, whereby it may regulate blood coagulation, fibrinolysis, and cytolysis. Our knowledge of vitronectin is mainly based on in vitro experiments using human blood vitronectin. The primary structure deduced from the human cDNA sequence in 1985 (9, 28) provided the structural basis for the actions of vitronectin.

Since our establishment of a simple method to purify human blood vitronectin in 1988 (34), attempts to purify and characterize vitronectins from a variety of living organisms, especially primitive or easily manipulable experimental organisms, have started with the aim of identifying the essential structural and functional features of vitronectin. Vitronectins have been isolated from 14 mammalian and avian blood plasmas by using the simple purification method (11, 21), while cDNAs encoding rabbit (25) and mouse (27) vitronectins have also been cloned. These 14 vitronectins are similar in primary structures and in cell-spreading, heparin-binding, and collagen-binding activities. They serologically cross-react with each other. In addition, proteins reacting with anti-vitronectin antisera have been identified in Drosophila melanogaster (21), Caenorhabditis elegans, (21), Physarum polycephalum (21), and chick egg yolk (20). There are also proteins reacting with anti-vitronectin in flowering plants (24, 33) and brown algae, Fucus (32).

We have focused on a protein reacting with anti-bovine vitronectin in Physarum polycephalum. The protein, referred as Physarum vitronectin-like protein, has been purified and found to have an RGD-dependent cell-spreading activity (19). The amino-terminal sequence of the protein is, however, dissimilar to those of
animal vitronectins, and instead shows partial homology with yeast dihydrolipoamide acetyltransferase. This led us to examine the relationship between the two proteins more precisely.

Dihydrolipoamide acetyltransferase is one component of the pyruvate dehydrogenase complex, which is composed of three different enzymes: pyruvate decarboxylase, dihydrolipoamide acetyltransferase, and dihydrolipoamide dehydrogenase. In eukaryotic cells, the pyruvate dehydrogenase complex is located within the inner membrane of the mitochondrial matrix and functions in oxidative decarboxylation of pyruvate, to generate acetyl-CoA (1). All dihydrolipoamide acetyltransferases reported so far are associated with the pyruvate dehydrogenase complex. The cDNA encoding dihydrolipoamide acetyltransferase has already been isolated from human (29), rat (15), and yeast (Saccharomyces cerevisiae) (4, 22). The primary structures of dihydrolipoamide acetyltransferase have no homology with animal vitronectins.

In this manuscript, we present immunological and biochemical evidence that the Physarum vitronectin-like protein may be a chimeric molecule of vitronectin and dihydrolipoamide acetyltransferase. Moreover, we show that the protein is localized in cytoplasm and nuclei of Physarum plasmodia.

**MATERIALS AND METHODS**

**Purification of Physarum vitronectin-like protein.** Physarum vitronectin-like protein was purified from Physarum polycephalum plasmodia as described previously (19). Briefly, Physarum vitronectin-like protein was extracted from plasmodia homogenates with 0.8% Triton X-100, then separated by chromatography on columns of anti-bovine vitronectin IgG-Sepharose and heparin-Sepharose, and finally purified by electrophoresis on SDS-polyacrylamide gels. The electroeluted protein was dialyzed against 10 mM ammonium hydrogen carbonate and kept frozen at -80°C until use.

**SDS-polyacrylamide gel electrophoresis and immunoblotting.** SDS-polyacrylamide gel electrophoresis was carried out according to the procedure described previously (18). Briefly, purified Physarum vitronectin-like protein (116 μg) was precipitated with acetone, dissolved in 0.4 M ammonium hydrogen carbonate buffer (pH 7.9) containing 8 M urea, and reduced with 4 mM dithiothreitol at 50°C for 45 min under N2 gas. The reduced protein was S-carboxyamidomethylated with 10 mM iodoacetamide for 15 min at room temperature in the dark. After dilution with distilled water to make a urea concentration of 2.76 M, the S-carboxyamidomethylated protein was digested at 37°C for 8 h by lysylendopeptidase (14) from Acrobacter lyticus M497-1 (Wako Pure Chemical Industries Ltd.) at an enzyme-to-substrate ratio of 1:50. The digest was chromatographed on a reversed-phase C8 column (2.1 × 150 mm, V Bondapak S5, C8; Waters) using a Waters Model 625 LC system equipped with a Waters Model 991J photodiode array detector. High-performance liquid chromatographic (HPLC) separation of the digest was performed at room temperature with a linear gradient of 0-40% acetonitrile in 0.1% trifluoroacetic acid for 40 min and then 40-80% acetonitrile for 20 min at a flow rate of 0.2 ml/min.

**Amino acid sequence determination and homology search.** Amino acid sequence analysis of the peptides was carried out with an Applied Biosystems Model 473 sequencer. The partial sequences of the peptides derived from Physarum vitronectin-like protein were compared with all entries in the Protein Sequence Database of the Protein Identification Resource (release no. 33, June 1992), National Biomedical Research Foundation (USA). The database was accessed at National Institute of Genetics at Mishima on the basis of the algorithm of the computer program FLAT, version 1.3.

**Immunofluorescence microscopy.** Plasmodia of Physarum polycephalum cultured on the surface of 1.5% agar were obtained by reactivation from sclerotia, which were a kind gift from Dr. M. Ishigami (Shiga Univ.). The plasmodia were fixed with a periodate-lysine-paraformaldehyde fixative (2% paraformaldehyde, 10 mM sodium metaperiodate, 7 mM lysine, and 38 mM sodium phosphate buffer, pH 7.4) (17) for 2 h at room temperature. The specimens were permeabilized with 0.5% Triton X-100 in PBS for 10 min at room temperature, then rinsed thoroughly with PBS. The specimens were infiltrated with Holt solution (0.88 M sucrose, 1% gum arabic, and 0.1 mg/ml thymol) for 2-18 h at room temperature and

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Table I. Sequence homology between the N-terminal 20 residues of Physarum vitronectin-like protein and dihydrolipoamide acetyltransferase.

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Sequence Homology</th>
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<tbody>
<tr>
<td>Physarum vitronectin-like protein</td>
<td>1 A S Y P V 2 P Q K L G M P A L R P T S G Y 30 Q</td>
</tr>
<tr>
<td>Yeast dihydrolipoamide acetyltransferase</td>
<td>1 A S Y P E 2 H T I I G M P A L M Q I V L P A L S P T M T 20 Q</td>
</tr>
<tr>
<td>Rat dihydrolipoamide acetyltransferase</td>
<td>126 S S Y P V 145 S P T M T M</td>
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Note. Single letter amino acid abbreviations are used. In the 5th, 12th, 15th, and 17th cycles of Edman degradation, two or four amino acids were detected (19). Amino acid sequences of dihydrolipoamide acetyltransferases were deduced from nucleotide sequences (15, 22). The numbers above the sequences refer to the respective position in the corresponding protein. The boxed amino acid residues are identical.

Frozen in OCT mounting medium (Miles Laboratories) in liquid N2. Frozen sections (6 μm thick) of Physarum plasmodia were mounted on glass slides subbed with 2% aminopropyltriethoxysilane in dry acetone (3). Sections on the slides were immersed in PBS, blocked with 50% normal goat serum in PBS, then incubated with primary antibodies diluted at 1:200 in 50% normal goat serum in PBS for 2 h at room temperature. After being rinsed with PBS, the slides were incubated with fluorescein isothiocyanate-conjugated anti-rabbit IgG (MBL Co., Ltd.) at a dilution of 1:100 in 50% normal goat serum in PBS for 2 h at room temperature. Nuclei were stained with 4',6-diamidino-2-phenylindole dihydrochloride (Nacalai Tesque Inc.) at 2 μg/ml in PBS for 5 min at room temperature.

Immunoelectron microscopy. Physarum plasmodia fixed with the periodate-lysine-parafomaldehyde fixative as described above were infiltrated with a mixture of 10% polyethyl-

![Image of gel analysis](image)

**Fig. 1.** Immunological cross-reactivity of Physarum vitronectin-like protein with anti-bovine vitronectin IgG (B) and anti-rat pyruvate dehydrogenase complex IgG (C). Whole protein of Physarum plasmodia (lane 1), purified Physarum vitronectin-like protein (lane 2), and rat heart homogenate (lane 3) were stained with a silver staining kit for proteins (A). The proteins were electroblotted on nitrocellulose sheets and allowed to react with anti-bovine vitronectin (B) or anti-rat pyruvate dehydrogenase complex (C). Bound antibody was visualized by incubations with horseradish peroxidase-conjugated second antibody then with o-dianisidine and H2O2. Molecular size (in kDa) is indicated at the left. Arrows at the right indicate dihydrolipoamide acetyltransferase (E2, 68 kDa), protein X (X, 52 kDa), and α subunit of pyruvate decarboxylase (E1α, 43 kDa) of rat pyruvate dehydrogenase complex.
ene glycol and 2.3 M sucrose in PBS for 3 h at room temperature. The specimens were cut into pieces of about 2 x 2 x 2 mm³, placed on a specimen holder of a cryosectioning device (Reichert-Nissei FC-4E; Nissei Sangyo Co. Ltd.), and frozen in liquid N₂. Ultrathin frozen sections were made, and immunolabeling was performed according to the method described in our previous report (26), with some modifications. Briefly, the sections were treated with 0.05% Tween 20 and normal goat serum for 10 min to avoid non-specific staining. Primary antibodies at a final concentration of 1 : 200 in 0.05% Tween 20 and normal goat serum were then allowed to react for 2 h at room temperature. The sections were rinsed thoroughly with PBS and stained with colloidal gold-labeled goat anti-rabbit IgG (Amersham International Inc.) overnight at room temperature. The diameter of the colloidal gold particles was 10 nm. Specimens were rinsed with PBS, fixed with 2% glutaraldehyde in PBS containing 2% polyvinyl alcohol (30). They were observed in a transmission electron microscope (JEM 100C; JEOL).

RESULTS

Immunological cross-reactivity between Physarum vitronectin-like protein and dihydrolipoamide acetyltransferase. The amino-terminal sequence of Physarum vitronectin-like protein shows no homology with those of animal vitronectins (19). Further, a computer-assisted homology search indicated a significant sequence identity with the amino-terminal sequence of yeast (Saccharomyces cerevisiae) dihydrolipoamide acetyltransferase (22) (~65% for the 20 amino acids) and with an internal sequence of rat dihydrolipoamide acetyltransferase (15) (~50% for the 20 amino acids) (Table I), even

<table>
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<th>Table II. Homology of amino acid sequences of 10 lysylendopeptidase peptides from Physarum vitronectin-like protein with reported sequences of dihydrolipoamide acetyltransferase.</th>
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Note. Physarum vitronectin-like protein was digested with lysylendopeptidase and chromatographed on a reversed-phase column. Amino acid sequences of 10 major peaks (K-21, K-22, K-23, K-24, K-27, K-28, K-29, K-30, K-56, and K-63) are listed. The amino acid sequence of yeast dihydrolipoamide acetyltransferase (YE₂) and that of rat dihydrolipoamide acetyltransferase (RE₂) were deduced from their nucleotide sequences (15, 22). Single letter amino acid abbreviations are used. X indicates an unidentified residue. The boxed amino acid residues are identical. The numbers at the left of the sequences refer to the respective positions in the corresponding protein.
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Fig. 2. Immunofluorescence staining of Physarum polycephalum plasmodia with anti-bovine vitronectin IgG. Longitudinal (A) and cross (B, C) cryosections through a plasmodial strand cultured on the surface of 1.5% agar were allowed to react with a 1:200 dilution of rabbit anti-bovine vitronectin (A, B), then fluorescein isothiocyanate-conjugated goat anti-rabbit IgG. Control staining was performed in parallel with rabbit preimmune serum instead of rabbit anti-bovine vitronectin (C). Bar = 100 μm.

though a variety of animal vitronectins (21) including human (9, 28), rabbit (25), and mouse (27) have no such homology. Therefore, we examined the immunological cross-reactivity of the two proteins using anti-rat pyruvate dehydrogenase complex and anti-bovine vitronectin. Physarum plasmodia homogenate, Physarum vitronectin-like protein, and rat heart homogenate were electrophoresed on SDS-polyacrylamide gels and transferred to nitrocellulose sheets. Polyclonal anti-bovine vitronectin IgG recognized both a 70-kDa polypeptide in plasmodia homogenate (Fig. 1B, lane 1) and purified Physarum vitronectin-like protein (Fig. 1B, lane 2) on the nitrocellulose sheet. In contrast, no protein was recognized in rat heart homogenate (Fig. 1B, lane 3). On the other hand, polyclonal anti-rat pyruvate dehydrogenase complex IgG reacted strongly with the component enzymes of pyruvate dehydrogenase complex in rat heart homogenate (Fig. 1C, lane 3), i.e., dihydrolipoamide acetyltransferase (E2, 68 kDa), protein X (52 kDa), and α subunit of pyruvate decarboxylase (E1α, 43 kDa). The band of dihydrolipoamide dehydrogenase (56 kDa) in the immunoblot was hardly recognized, probably due to the low immunogenicity of the protein on the membrane, as pointed out by De Marcucci et al. (5). Furthermore, the antiseraum recognized 70 kDa Physarum vitronectin-like protein in both plasmodia homogenate (Fig. 1C, lane 1) and the purified preparation (Fig. 1C, lane 2). These results indicate that Physarum vitronectin-like protein and dihydrolipoamide acetyltransferase are immunologically cross-reactive and share common antigenic determinant(s). Physarum homogenate contained 4 other proteins that cross-reacted with anti-rat pyruvate dehydrogenase complex (Fig. 1C, lane 1). Their molecular masses were 56 kDa, 44 kDa, 41 kDa, and 40 kDa. These proteins may be the Physarum homologues of the components of rat pyruvate dehydrogenase complex.

Internal amino acid sequences of Physarum vitronectin-like protein. To probe the internal amino acid sequences of Physarum vitronectin-like protein, S-carboxymethylated Physarum vitronectin-like protein was digested with lysylendopeptidase and the resulting peptides were separated on a reversed-phase HPLC column. Partial amino acid sequences of 14 major peaks were determined by an automated amino acid sequencer. Five peaks showed multiple phenylthiohydantoin derivatives at each cycle of the Edman degradation. Nine peaks showed a single phenylthiohydantoin derivative. Five peaks showed multiple phenylthiohydantoin derivatives at each cycle of the Edman degradation. Nine peaks showed a single phenylthiohydantoin derivative at each cycle. The sequences of 10 peaks (K-21, K-22, K-23, K-24, K-27, K-28, K-29, K-30, K-56, and K-63) are listed together with the relevant sequences of yeast and rat dihydrolipoamide acetyltransferases in Table II. Nine of these ten peptides showed substantial sequence identity with the corresponding regions of dihydrolipoamide acetyltransferase. On the other
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hand, none of them showed homology with amino acid sequences of vitronectins from human, rabbit, and mouse. Interestingly, the sequence of peptide K-22 showed no homology with either vitronectin or dihydrolipoamide acetyltransferase; the highest homology found in a search using the protein sequence database was 58% with yeast GAC1 protein. These results suggest that K-22 is derived from a unique domain of Physarum vitronectin-like protein.

Immunohistological localization of Physarum vitronectin-like protein in Physarum plasmodia. Animal vitronectins are present in blood plasma and platelets as a soluble form and in extracellular matrix as an insoluble form. In eukaryotic cells, the dihydrolipoamide acetyltransferase is located within the inner membrane of mitochondrial matrix (1). Physarum vitronectin-like protein is fractionated in insoluble fraction of Physarum homogenate, suggesting association with or enclosure in membranous organella (19). To examine further the localization of Physarum vitronectin-like protein, Physarum plasmodia were fixed, sectioned, and incubated with antibodies against bovine vitronectin or rat pyruvate dehydrogenase complex followed by fluorescein isothiocyanate-labeled second antibodies. As shown in Fig. 2, anti-vitronectin antibody uniformly stained the plasmodia (except for vacuoles) in both longitudinal (Fig. 2A) and cross (Fig. 2B) sections through a plasmoidal strand. The protein was not restricted to the outer surface of plasma membrane or to mitochondria, in contrast to the expected localization for extracellular matrix or for pyruvate dehydrogenase complex. No gradient of labeling intensity was observed along the plasmoidal strand. Similar staining patterns were obtained with anti-rat pyruvate dehydrogenase complex (data not shown). Nuclei detected with 4',6-diamidino-2-phenylindole dihydrochloride were also stained with anti-vitronectin (data not shown), suggesting that Physarum vitronectin-like protein also exists in nuclei.

To examine the ultrastructural localization more precisely, immunogold electron microscopy of ultrathin cryosections was performed. The fine ultrastructure of Physarum plasmodia might not be well preserved due to the cryosectioning method employed, which was designed to minimize loss of the immunoreactivity of vitronectin-like protein. Anti-bovine vitronectin stained cytoplasm and nucleus of Physarum plasmodia (Fig. 3), confirming the immunofluorescence observations (Fig. 2). Gold particles were mainly localized in cytoplasm without significant enrichment in particular organella (Fig. 3A). A few particles were seen in mitochondria in some specimens. The particle density was less in the nucleus than in cytoplasm. Similar patterns of gold particles were observed using anti-pyruvate dehydrogenase complex instead of anti-vitronectin (data not shown). The background signal on specimens processed in parallel but with preimmune serum was negligible (Fig. 3B).

DISCUSSION

Physarum polycephalum contains the so-called Physarum vitronectin-like protein, which cross-reacts with anti-bovine vitronectin and has RGD-dependent cell-spread activity (19). The amino-terminal sequence of the protein, however, was shown to be distinct from those of animal vitronectins and partially homologous with dihydrolipoamide acetyltransferase. We found that Physarum vitronectin-like protein was recognized by antibodies against bovine vitronectin and against rat pyruvate dehydrogenase complex (Fig. 1). Further, not only the amino-terminal amino acid sequence (Table I), but also 9 internal partial sequences (Table II) of the protein showed significant identity with the corresponding regions of dihydrolipoamide acetyltransferase. For instance, peptide K-56 corresponds to the amino-terminal sequence of yeast dihydrolipoamide acetyltransferase and peptide K-63 corresponds to the sequence of residues 381 to 410 of the yeast enzyme. Since the apparent molecular weights of the two proteins are similar, the overall sequence of Physarum vitronectin-like protein might be very similar to that of dihydrolipoamide acetyltransferase.

On the other hand, anti-bovine vitronectin reacted with Physarum vitronectin-like protein but not with rat dihydrolipoamide acetyltransferase, while rat anti-pyruvate dehydrogenase complex reacted with both proteins (Fig. 1). This result indicates that rat dihydrolipoamide acetyltransferase does not contain a vitronectin-like region, whereas Physarum vitronectin-like protein does. This is not inconsistent with the results mentioned in the previous paragraph, since the total number of residues sequenced (144; Table II) accounts for only about 25% of the 70 kDa Physarum vitronectin-like protein. The vitronectin-like sequence which is responsible for RGD-dependent cell-spread activity with anti-vitronectin presumably lies in the remaining 75% of the molecule. Nevertheless, the vitronectin-like region in Physarum vitronectin-like protein may be smaller than the enzyme-like region. We could postulate that Physarum vitronectin-like protein might be a chimeric molecule of vitronectin and dihydrolipoamide acetyltransferase.

In Physarum homogenate, 4 proteins in addition to

Fig. 3. Localization of Physarum vitronectin-like protein by immunoelectron microscopy. Physarum plasmodia were fixed, sectioned, and labeled with rabbit anti-bovine vitronectin (A) or rabbit preimmune serum (B) followed by treatment with goat anti-rabbit IgG-collodial gold (10 nm diameter particles) as described under Materials and Methods. Bar = 1 μm. Abbreviations are nu, nucleus; cy, cytoplasm; mt, mitochondria.
the vitronectin-like protein cross-reacted with anti-pyruvate dehydrogenase complex (Fig. 1C, lane 1). These proteins might be Physarum homologues of the components of rat pyruvate dehydrogenase complex.

Immunofluorescence staining showed that the localization of Physarum vitronectin-like protein is homogeneous, with no variation of labeling intensity between the tip and end or between central and peripheral areas of a plasmodial strand (Fig. 2). Immunoelectron microscopy (Fig. 3) indicated that the vitronectin-like protein is not associated with particular cytoplasmic structures, but is broadly distributed in cytoplasm and nucleus. In contrast, vitronectin is a soluble blood protein or an insoluble extracellular matrix protein (the latter seems to be derived from the former) (7), while dihydrolipoamide acetyltransferase is generally located in the inner membrane of the mitochondrial matrix, although it was also recently found on the surface of biliary epithelial cells in primary biliary cirrhosis (10). Thus, the localization of Physarum vitronectin-like protein is completely different from those of animal vitronectins and dihydrolipoamide acetyltransferase.

Concerning the function of RGD-containing proteins in nuclei, Brake et al. (2) reported that human immunodeficiency virus type 1 (HIV-1) transactivation protein, tat, contains an RGD-sequence which promotes cell-spreadning. HIV-1 tat increases viral gene expression and replication. Kobayashi et al. (12) reported that primer protein, an enzyme component catalyzing protein-primed DNA replication of bacteriophages, contains an RGD-sequence, which is responsible for the DNA replication. Since Physarum vitronectin-like protein is present in nuclei (Fig. 3A), it might have some function in the regulation of gene expression or DNA replication.

It is clear that, despite the similarities of Physarum vitronectin-like protein to vitronectin and dihydrolipoamide acetyltransferase, it is entirely different from those in them localization, and it probably also differs from them in function. If we can understand the role of this protein in the slime mold Physarum, a primitive organism, it should lead to new insights into the evolutionary origin of vitronectin and dihydrolipoamide acetyltransferase. The next steps should be cDNA cloning and complete sequencing of the Physarum vitronectin-like protein.

Acknowledgements. This work was supported in part by the Hayashi Memorial Foundation for Female Natural Scientists, by a research grant from the Ministry of Education, Science and Culture of Japan and by the Special Coordination Funds of the Science and Technology Agency of the Japanese Government. We thank Ms. M. Abe for experimental help, Dr. M. Ishigami (Shiga Univ.) for providing sclerotia of Physarum polycephalum, Mr. W.R.S. Steele for linguistic advice, and Ms. K. Hayashi for secretarial assistance.

REFERENCES

Physarum vitronectin-like protein


(Received for publication, September 21, 1993 and in revised form, October 12, 1993)