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EFFECT OF INFLUENZA VIRUS ON THE INTERACTION OF CYTOSKELETON WITH CELL MEMBRANES

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Summary. — The effect of influenza virus on the organization of cytoskeleton (CS) associated with cell surface plasma membranes (PM) of chick embryo cells (CEC) was investigated. The CS isolated from virus-treated PM contained more proteins and more actin than the CS isolated from control membranes. Virus particles were found to be associated with this CS, although the purified virus alone, treated in the same manner, did not sediment at low speed in the residue insoluble in Triton X-100. Autophosphorylation of CS, especially of the polypeptides 24 K, 45 K, 65 K and 105—110 K, was increased in CS prepared from virus-treated membranes. Superprecipitation of the CS prepared from CEC was stimulated by addition of the virus. Electron microscopy of the Triton X-100 insoluble residue from virus-treated PM showed more structures similar to microfilament bundles than Triton X-100 insoluble residue from control membranes. Based on these results we suggest that already in early stages the virus infection leads to a reorganization of membrane-associated CS.

Key words: influenza virus; cytoskeleton; membranes; phosphorylation

Introduction

Actin is the main contractile protein of CS. It is present together with myosin in the majority of eukaryotic cells being of crucial importance in different cell functions. Based on coisolation of actin microfilaments (MF) with PM (Meyer and Burger, 1979; Condeelis, 1981; Tawata and Field, 1982; Carraway *et al.*, 1983; Davies, 1984; Painter *et al.*, 1985), on localization of MF at PM (Willingham *et al.*, 1981; Bennet and Condeelis, 1984; Goodloe-Holland and Luna, 1984), and on the isolation of CS structures from PM by Triton X-100 extraction, the conclusion was drawn that MF (i.e. the actin network) and PM were directly connected. There is broad circumstancial evidence indicating that microfilament-membrane interaction mediates some membrane functions. For example morphology of cells, clustering and global redistribution and endocytosis of ligand-receptor complexes, secretion (Friedman *et al.*, 1980; Condeelis, 1981; Tawata and Field, 1982;

Tsilibary and Williams, 1983; Bourguignon and Bourguignon, 1984; Woda and Woodin, 1984; Jesaitis *et al.*, 1985) as well as the transmission of extracellular signals to the cell nucleus. Various authors have shown that after specific ligand binding the cell surface receptors become associated with cytoskeletal structure (White *et al.*, 1983; Woda and Woodin, 1984; Stewart and Crawford, 1985). Influenza virus receptors are situated within the membranes of susceptible cells. The presented work followed the influence of influenza virus on the organization and on some properties of the CS associated with the CEC membranes.

Materials and Methods

Chick embryo cells (*CEC*) were prepared by trypsinization from 11-day-old embryos.

Influenza virus A (WSN) (HlNl) was adapted to CEC and purified by adsorption — elution on chick erythrocytes and by differentional centrifugation.

Membranes from CEC were prepared as described (Križanová *et al.*, 1985), or purified by centrifugation on a Ficoll gradient according to Wolosin *et al.* (1983), or purified by centrifugation on the Ficoll gradient of membranes after previous separation on 35 % sucrose.

Cytoskeleton (*CS*) was isolated from PM by the methods described: 1. by Wolosin *et al.*, 1983; 2. by Carraway *et al.*, 1983; 3. by Faucault *et al.*, 1984 or 4. according to method of Meisheri and Ruegg (1983) described for preparation of skinned fibres. The latter procedure yielded the most suitable material for superprecipitation of CS.

Influenza virus-treated PM were obtained by incubation of 1 mg PM with 4000—10 000 HAU (haemagglutination units) of influenza virus for 15 min at 0° C and then at 30° C for 15 min. Mock-treated membranes were allowed to react with physiological saline under the same conditions.

Autophosphorylation of cytoskeletal proteins, analysis in sodiumdodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometric scanning analysis as well as protein determinations were made as previously described (Križanová *et al.*, 1985).

Actin concentration after depolymerization in guanidin-HCl buffer was measured by DNase-inhibition test (Blickstad *et al.*, 1978) or by densitometric scanning analysis of the gels.

Superprecipitation of isolated CS was made under the conditions as described Meisheri and Ruegg (1983) by measurement of OD at 550 nm (Hidaka *et al.*, 1979). To avoid quick sedimentation of large aggregates during measuring, the suspension of CS isolated from CEC was passed through a low-gauge needle (Bonneau *et al.*, 1985).

For electron microscopy, the CS prepared from control membranes and from influenza A virus-treated membranes were negatively stained with 2 % phosphotungstic acid (PTA) pH 7.5 or with 1 % uranyl acetate in distilled water. The samples were examined in Philips EM 300 electron microscope at 80 kV.

Results

The CS prepared from virus-treated membranes revealed an increase in the amount of both, CS proteins and actin as compared with CS purified from mock-treated membranes. Repeated measurements according to Lowry showed that total protein was 1.59—1.6 fold higher than in controls, while the densitometric measurements showed values exceeding the controls 1.62 times. The actin content in the CS from virus-treated PM showed 1.85 to 3 fold increase as detected by DNase inhibition test; and 1.88—2.94 fold increase as come from densitometric scanning of the gels. These results were obtained from 5—6 determinations.

When CS was prepared from CEC with adsorbed influenza virus, significant changes were neither found in total protein nor in actin content. Electron microscopy (Fig. 1), HA activity (Table 1) and SDS-PAGE (Fig. 2) indicated that influenza virions were associated with the CS prepared from virus--treated membranes. Purified influenza virus, when treated according to the procedure used for CS isolation, yielded only a little or none Triton X-100 insoluble residue at low speed centrifugation. Haemaglutination inhibition titres of CS prepared from control membranes pointed the presence of a small proportion of virus receptors in the CS.

Incubation of CS with γ^{32}P-ATP resulted in ^{32}P incorporation into CS proteins which were probably phosphorylated by the endogenous protein-kinase (Sahyoun *et al.*, 1985; Landreth and Rieser, 1985). The proteinkinase activity was higher in the CS isolated from virus-treated membranes, ^{32}P in-

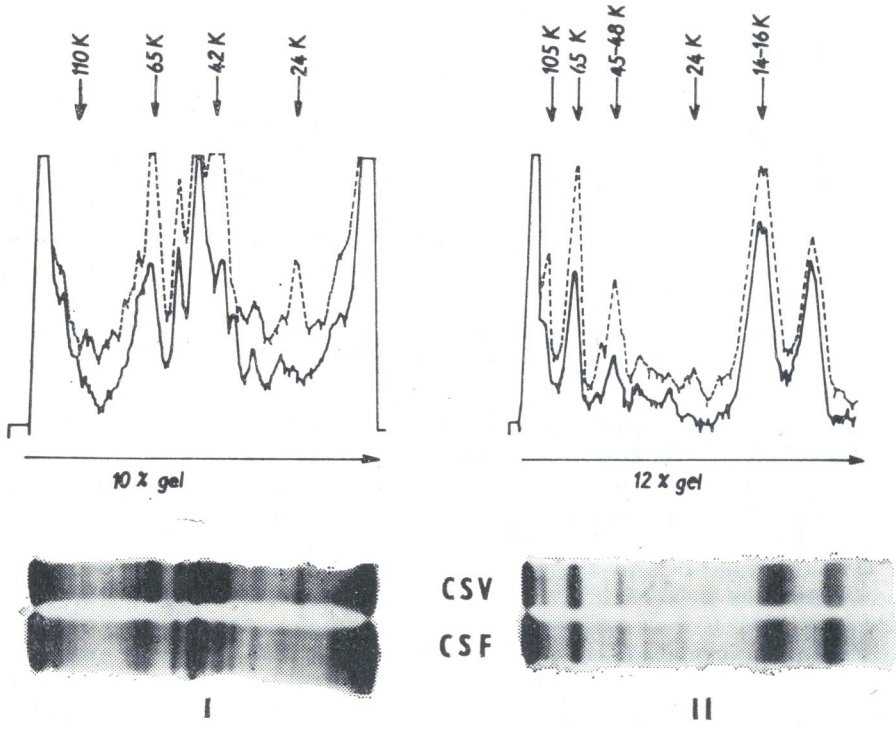

Fig. 3.

Densitometry of autoradiograms showing the effect of influenza virus on endogenous phosphorylation of CS proteins

CS-F from control membranes(——)
CS-V from virus-treated membranes (— — —)
Arrows indicate relative mol. mass; ^{32}P-ATP phosphorylation, electrophoresis, autoradiography and densitometry made as described by Križanová *et al.*, 1985. I — gel concentration 10 %; II — gel concentration 12 %.

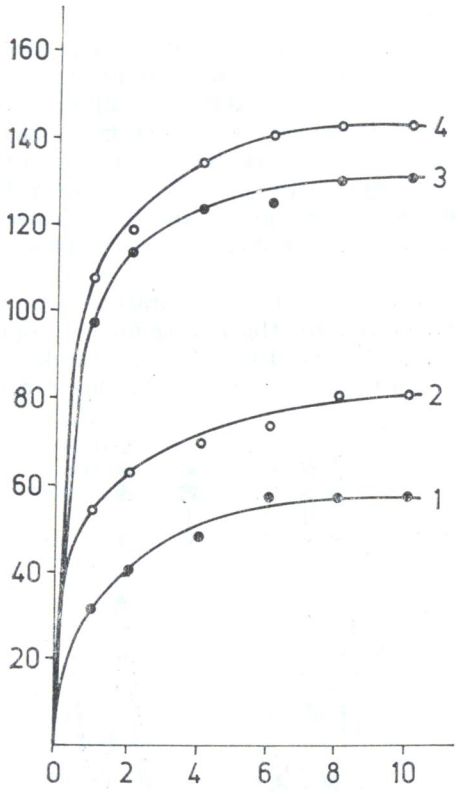

Fig. 4.
Effect of influenza virus on CS superprecipitation
1 — CS prepared from control CEC (Meisheri and Ruegg, 1983)
2 — CS prepared from virus-treated CEC
3 — CS isolated from noninfected CEC and then virus added
4 — CS isolated from virus-treated CEC and virus added
Abscissa: time in min; ordinate: Δ OD 550 nm (relative change).

corporation was enhanced into polypeptides of relative mol. wts of 22—24 K, 40—45 K, 60—70 K, 105—110 K and 140 K (Fig. 3, Table 2). Larger deviations in the label content of the polypeptide of mol. mass 60—70 K might be due to its easier dephosphorylation (Križanová *et al.*, 1985).

According to Hidaka *et al.* (1979) superprecipitation of CS corresponds to in vivo contraction and along with the ATP-ase activity it correlates with the contractile activity (Silver *et al.*, 1984). Superprecipitation of CS was increased if CEC were treated with the virus before CS isolation. As shown on Fig. 4, superprecipitation occurred faster and was of greater extent than of CS prepared from control CEC. Influenza virus added to the isolated CS also stimulated superprecipitation (Fig. 4, curves 3 and 4). CS prepared in a similar manner from PM (i.e. the material sedimented at 10 000 × g) showed analogous superprecipitation in a preliminary experiment, although the OD 550 values were lower and their differences smaller perhaps due to the lower CS concentration. Superprecipitation of the latter material required higher Ca^{2+} concentrations (results not shown).

These experiments showed that interaction of influenza virus with PM induced changes in the organization and function of CS at the cytoplasmic

Table 1. Comparison of virus receptors and the amount of virus particles in the cytoskeletons prepared according to different procedures

Preparation procedure according to	CS – F			CS – V		
	Prot. mg/ml	HIT per ml	HIT per mg prot.	Prot. mg/ml	HAU per ml	HAU per mgprot
Foulcault*	2.6	850–1060	320–430	5.4	17 400	3 180
Wolosin*	2.13	1280	605	4.86	10 240	2 120
Meisheri*	1.0	160	160	2.95	3 650	1 265

CS – F = cytoskeleton prepared from control (saline-treated) membranes
CS – V = cytoskeleton prepared from purified virus-treated membranes
HIT = haemagglutination inhibition titre
HAU = haemagglutination units
* see References
The values are average of 2–4 experiments.

surface of the membrane. In addition, electron microscopy of CS prepared from virus-treated PM revealed more structures similar to microfilament bundles than Triton X-100 insoluble residue from control membranes. Fig. 5.

Discussion

It is known that reorganization of CS in the cell may change its functions. Reorganization of microfilaments (i.e. of CS) have been described after infection of the cell with certain viruses or in transformed cells as well as was described the aim to specify the role of CS in virus infection (Celis, 1979, Graessmann *et al.*, 1980; Pautrat *et al.*, 1980; Meyer *et al.*, 1981; Bussereau

Table 2. Virus induced enhancement of ^{32}P incorporation into cytoskeletal proteins

Zone of relative mol. wt.	Gel concentration		
	12%	10%	6%
105–110 K	–	3.3–4.4	2.6
60–65 K	1.1	1.8	1.1
40–48 K	1.2	1.7	1.5
22–24 K	4.6	7.6	–

The values in Table represent the ratio CS – V/CS – F calculated from per cent of area shown by densitometric scanning. For details see Mat. and Methods and Fig. 3. For abbreviations see legend to Table 1.
– not separated

and Perrin, 1982; Lösse *et al.*, 1982; Howard *et al.*, 1983; Kasamatsu *et al.*, 1983; Keski-Oja *et al.*, 1983; Murti and Goorha, 1983; Notter and Balduzi, 1984; Stanislawsky *et al.*, 1984). Bedows *et al.* (1983) believe that the changes of the microfilament organization are different depending on the virus used. Certain viruses cause disassembly of microfilaments, another did not change the ratio of G and F actin in infected cells. All up to now published effects of viruses on the CS were followed in whole cells but not in isolated cell fractions.

The infection always begins with adsorption of virions to the cell membrane followed by penetration of the infective unit into the cell. Our results with isolated cell membranes have shown that already in this first step of infection virus may induce alterations in the organization of microfilaments (i.e. of the CS) associated with PM. Virus interaction with PM enhances the amount of total protein as well as actin content in the Triton X-100 insoluble residue, changes the extent of phosphorylation of certain CS components and stimulates superprecipitation of the CS derived from CEC. Electron microscopy revealed more and longer fibres in the CS isolated from virus -treated PM as compared to those seen in the CS prepared from control membranes. Similar results were reported by others autors, for example: 1. an increased actin amount in the CS of polymorphonuclear leukocytes after chemotactic stimulation (White *et al.*, 1983); 2. induction of contraction and redistribution of actin and myosin after activation of platelets (Painter and Ginsberg, 1984); 3. quick polymerization of actin and CS reorganization after thrombin-induced activation of platelets (Burn *et al.*, 1985).

Mechanism of actin reorganization induced by interaction of the virus with corresponding cell receptors and the role of this change in virus infection remains to be elucidated.

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Explanation of Figures (Plates XXV—XXVII):

Fig. 1. CS isolated according to Wolosin *et al.* (1983) from influenza virus-treated membranes. Arrow indicates the association of influenza virus with the actin microfilament. 2% PTA, magn. × 175 000.

Fig. 2. Analysis of the isolated CS in SDS-PAGE.
CS-F prepared from control membranes; CS-V prepared from virus-treated membranes.
A = actin; M = myosin; 55 and 25 are virus components; HMW = high molecular mass standard. Columns 2,3: equal volumes; columns 4,5: equal protein concentrations.

Fig. 5. CS isolated according to Wolosin *et al.* (1983) from virus-treated (I) and control (II) membranes. 2 % PTA, magn. × 66 000.