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EFFECT OF CONCANAVALIN A AND SUCCINYL-CONCANAVALIN A ON NUCLEOSIDE AND SUGAR UPTAKE IN MOUSE EMBRYO FIBROBLASTS

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SUMMARY: We have examined the effect of the tetrameric and dimeric form of Con A at a dose of 50μg ml^{-1} on nucleoside and glucose uptake using synchronized mouse embryo fibroblasts undergoing S phase. We have found that thymidine and uridine uptake were depressed by Con A but not significantly by succinyl-Con A. The inhibition was gradual, as it required a suitable time of incubation to become fully manifest and it was of non-competitive type. By contrast the uptake of 2-deoxy glucose was inhibited promptly and to a similar extent by Con A regardless of molecular structure. Kinetic analysis of the modalities of the sugar uptake process indicated an inhibition of competitive type.

INTRODUCTION

The effects of the lectin Concanavalin A (Con A) on lymphocytic cells have been extensively investigated and characterised (1). The lectin binds to glucosyl or mannosyl receptor moieties carried on cell surface macromolecules (2) and due to its tetravalent nature is able to crosslink these molecules (3). Such an interaction can thus result in the redistribution of macromolecular components of the cell surface and in the formation of clusters and caps (4-6). At the low concentration required to induce capping Con A can be mitogenic and stimulate lymphocytes to polyclonal proliferation and subsequent maturation (7-9). However, at higher concentrations Con A causes an inhibition of mitogenesis and an inhibition of growth in lymphoid and myeloid cell lines (10-14). Since similar results are elicited by insoluble forms of the lectin, the effects of Con A on lymphocyte proliferation have been ascribed to a direct action of the lectin at the cell surface (7, 15).

In cells of fibroblastic derivation concentrations of Con A higher than those inducing blast transformation can inhibit growth by maintaining cells at the G_1-G_0 stage of the cycle (12, 16, 17) or by exerting a G_2 block (17). A recent study of S phase lymphocytes has shown that Con A prevents replicon

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iniation but that it does not affect DNA elongation (18) thus allowing synthesis that has started to continue. Recently we have observed that when Con A is added to cells in S phase, a dissociation occurs between uptake of thymidine, which is inhibited, and rate of increase of cellular DNA content, which remains unaffected (17). In the present report we have examined the effect of the tetrameric and dimeric form of Con A on nucleoside and sugar uptake in synchronized S phase fibroblasts. We have found that uptake of both thymidine and uridine were markedly decreased by Con A, but only moderately by succinyl-Con A. The establishment of the effect required a suitable period of incubation to become manifest and the inhibition occured in a non-competitive manner. By contrast the uptake of the sugar 2-deoxy-glucose was inhibited promptly and to a similar extent by both Con a and succinyl-Con A. This inhibition was of competitive type.

MATERIALS AND METHODS

Cell Cultures Synchronous cultures of tertiary embryonic fibroblasts from C57 Bl mice were obtained without the use of metabolic inhibitors by a method exploiting the growth characteristics of the cells during passages in culture from the time of initial isolation. The tertiary fibroblasts were seeded in scintillation vials at a density (1.4×10^{-5} cells cm^{-2}) corresponding to half that expected at confluence, in Eagle's BHK medium containing 10% tryptose phosphate broth and 5% foetal calf serum in an atmosphere of 5% CO_2 in air, and incubated at 37°C in a water bath. Such cell populations have been shown to progress through the cell cycle with a good degreee of synchrony. Details of this method have been described elsewhere (19). To investigate the effects of lectins on nucleoside and sugar uptake the synchronised tertiary fibroblasts were used at 17 hr after seeding when cells were approaching the time of maximum thymidine uptake during the S phase.

Lectins Concanavalin A (Con A), 3 x crystallised, was obtained from Miles Laboratories. Dimeric succinylated concanavalin A (succinyl-Con A) was prepared by derivitization of the native tetrameric molecule with succinic anhydride by the method of Gunther et al (20). The lectins were dissolved at the required concentration in phosphate-buffered saline (PBS) immediately prior to use. An equal aliquot of PBS alone was added to control cultures. Lectins were added to the cells which were incubated for various periods up to 2 hr before the uptake measurements were performed.

Nucleoside Uptake To assess the nucleoside uptake the cultures were incubated in the presence of either 1.3×10^{-7} M methyl-^{3}H thymidine (18-25 Ci $mmol^{-1}$) or 0.6×10^7 5-^{3}H-uridine (25-30 Ci $mmol^{-1}$) for various times. The labelling was stopped by removing the medium by aspiration and washing the cultures twice with ice-cold PBS. The acid-soluble nucleoside pool was extracted by treating the cultures with 5% trichoroacetic acid (TCA) at melting ice temperature. The fixed cells were re-extracted with TCA, washed twice with

distilled water and dried. Incorporation of radioactivity into acid-soluble cellular material was assayed directly by adding 10 ml of toluene: butyl PBD scintillation cocktail (5g of 2(4'-t-butylphenyl-5(4"biphenyl)-1, 3, 4 oxadiazole per litre of toluene) to each dried vial and counting in a Beckman scintillation counter. The acid-soluble nucleoside pools were assayed by adding an aliquot of the TCA extract to 10 ml of PPO; POPOP; Triton; toluene scintillation cocktail (5g of 2, 5-diphenyloxazole; 0.1g of 1, 4-bis (2-(5-phenyloxazole)) benzene; 300 ml Triton X-100 and 700 ml toluene).

Uptake of 2-deoxy-D-glucose This sugar was selected to investigate monosaccharide uptake since it is transported and phosphorylated by the cells in the same way as glucose, but is not further metabolised. Immediately prior to the determination of the sugar uptake, the culture medium was replaced with glucose-free Eagle's BHK medium supplemented with 5% dialysed foetal calf serum in order that the glucose present in normal medium would not interfere with the uptake of the analogue. The cultures were incubated for various times in the presence of 0.12×10^{-7} M 2-deoxy-D-1 ^{3}H-glucose (15-25 Ci mmol^{-1}). The uptake of 2-deoxyglucose into intracellular pools was assayed as described above for nucleosides.

Lineweaver-Burk Analysis Qualitative estimations of the uptake process were carried out by incubating cells for a 5 minute period (while initial uptake was linear) in the presence of ^{3}H-thymidine, ^{3}H-uridine and ^{3}H-2-deoxyglucose at concentrations ranging from 0.05 to 0.5 M and assaying total uptake of each substrate as described above.

RESULTS

Overall Effect on uptake processes. The aim of this first set of observations was to examine the effect of different doses of the tetrameric and dimeric form of Con A on the uptake process as an overall phenomenon. In the experiments reported in Table 1 thymidine and uridine uptake were assessed at a time when pools had reached saturation. The data show that 1) uptake of both nucleosides was decreased in the presence of native Con A, 2) the effect was dose dependent and 3) that it became more marked with time of incubation. The effect of succinyl-Con A, on the other hand, was less pronounced and it was influenced to a minor degree, if at all, by the incubation time. Table 1 also shows that, in contrast to what observed in the case of the two nucleosides, glucose uptake was inhibited by Con A and by succinyl-Con A to a comparable extent and that the effect was prompt. Since the results obtained with 50 μg ml^{-1} of Con A were in all cased marked, this concentration was used for all further experiments.

Uptake Kinetics The overall phenomena reported in Table I were analysed further in terms of uptake two hours after the addition of lectin. Thymidine and

TABLE I Effect of various doses of Con A and succinyl-Con A on total uptake of thymidine, uridine and 2-deoxyglucose

Treatment	lectin (g ml-1)	Total uptake ^{3}H-thymidine CPM	% change	^{3}H-uridine CPM	% change	^{3}H-deoxyglucose CPM	% change	Time after lectin
Control	-	3687	-	33557	-	12888	-	
	5	3627	-2	33243	-4	12336	-4	
	50	3046	-17	27968	-17	9172	-29	
	100	3038	-18	30243	-10	8680	-33	
								30 min
succinyl-Con A	5	3634	-1	33041	-2	12148	-6	
	50	3591	-3	31-21	-8	9382	-27	
	100	3253	-12	30512	-9	9764	-24	
Control	-	4656	-	26329	-	12732	-	
	5	4350	-7	26536	+1	12588	-1	
Con A	50	2723	-42	11372	-57	8756	-31	
	100	2510	-46	11279	-57	7064	-45	
								2 hrs
	5	4176	-10	29934	+14	13898	+9	
succinyl-Con A	50	4098	-12	26374	0	9520	-25	
	100	3748	-20	25941	-1	7742	-39	

Lectins were added to cultures of tertiary embryonic fibroblasts 17 hr after seeding and total uptake measured 30 min and 2 hr later, using a 30 min pulse.

uridine in these experiments were used at concentrations which would favour entry into the cells by facilitated transport rather than free diffusion (21, 22). Figure 1A and 1C show that the rate of uptake of both nucleosides was decreased by Con A but only moderately affected by succinyl-Con A. Since the initial rates of uptake remained linear over a period of 5-10 minutes, measurements made within the first five minutes, a time when a steady state situation had not been reached and little lable was incorporated in the acid precipitable fraction, were used for a Lineweaver-Burk examination of the uptake kinetics. The results (fig. 1B and 1D) show that the V_{max} values were reduced in the presence of Con A by about 30 per cent in both cases while the K_m values remained unchanged, thus indicating an inhibition of non-competitive type. Analysis of the data obtained in the presence of succinyl-Con A showed no significant divergence from controls.

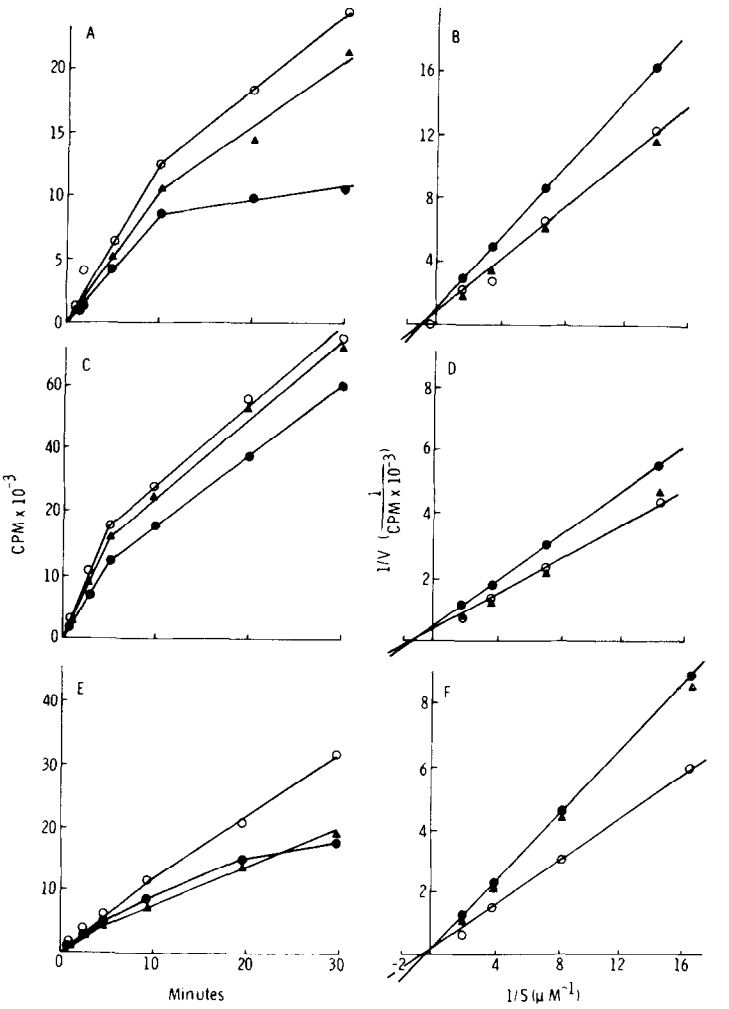

Fig. 1 Effect of Con A and succinyl-Con A on time course of total uptake and Lineweaver-Burk plots. A and B, thymidine; C and D, uridine; E and F, 2-deoxy-glucose. Lectin (50 $\mu g\ ml^{-1}$) was added at 17 hours after seeding and measurements carried out two hours later.

Sugar uptake was also examined using a concentration within the optimal range of the active transport system. The effect of the lectin on this process is shown in figure 1E. Contrary to what was observed for the two nucleosides the curve of uptake of 2-deoxyglucose was depressed to a similar extent by both dimeric and tetrameric Con A. Lineweaver-Burk analysis (fig. 1E) during the initial linear uptake (5 minutes) also showed that in contrast to the previous findings, the inhibition of the sugar uptake was of a competitive type as, while the V_{max} values did not change, the K_m values were doubled.

DISCUSSION

The present results demonstrate that Con A markedly inhibited thymidine and uridine uptake and that in both cases similar patterns of inhibition were elicited. A similarity of behaviour between the two nucelosides is in accord with the view that they both use the same transport system (23), but our results do not allow to distinguish the relative participation of the transport process as such and that of the specific kinases in the exertion of the inhibitory effect. The underlying condition(s) for the establishment of this inhibition is not known but it can be suggested that a rearrangement of macromolecular components of the plasma membrane is required. This is indicated by the fact that a period of incubation was necessary for the effect to become fully manifest and that little effect was exerted by succinyl-Con A which binds to the same surface determinant as Con A but has little cross-linking ability (24).

In contrast to the nucleoside findings the uptake of the glucose analogue 2-deoxyglucose was promptly and similarly inhibited by both Con A and succinyl Con A. The absence of a time-lag requirement for the effect to become manifest and the similarity of response with either form of lectin indicate that, in this case, the glucose uptake system was directly and immediately affected, possibly by the binding of the lectin to some structural component of the system. This is further supported by the kinetic analysis which demonstrates a loss of affinity for the substrate, as shown by the reduced K_m of the uptake process.

It is widely believed that the inhibitory effect of lectins on cell growth and division relates primarily to their cross-linking ability leading to rearrangement of surface macromolecules. Such an interaction would alter the operational state of the cell surface and a number of events related to growth. Our present results with the use of nucleosides are in accord with this view but our findings on sugar uptake cannot be explained by cross-linking. Instead they illustrate one example where a cell surface process is affected

in a fashion consistent with direct lectin binding and not through rearrangement ofmacromolecular components. Thus, more than one mechanism involving the cell surface may be responsible for the complexity of events initiated by lectins of which arrest of growth is the terminal phenomenon.

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