Effect of Concanavalin A on the Kinetics of Ecto-5′-adenosine Monophosphatase (5′-Adenosine Monophosphate Phosphohydrolase) in the Outer Surface of Intact Neural Cells in Culture†

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ABSTRACT: Oncogenic cultured rat C6 astroblastoma cells display strikingly high ecto-5′-adenosine monophosphatase (ecto-5′-AMPase) activity, 4.23 ± 20 μmol of P_i liberated by intact cells from 3 mM extracellular 5′-AMP (mg of protein)$^{-1}$ h^{-1}, as compared with 0.15 ± 0.01 for nononcogenic cultured hamster astroblasts. A further rise in C6 cell ecto-5′-AMPase activity occurs with increase in cell density during growth. Less than 2 pg of the lectin, concanavalin A (Con A), bound per cell reversibly inhibits most of the cellular ecto-5′-AMPase activity. Inhibition by Con A binding is independent of cellular temperature. Con A binding suppresses phosphohydrolase activity of a pK = 7.4 functional group on the cell surface. A direct proportionality is observed between quantity of Con A bound to the cell surface and simultaneous relative decreases both in Michaelis constant and maximum velocity of ecto-5′-AMPase in the intact cell. The findings suggest that a major consequence of the specific high affinity binding of Con A to the C6 cell surface is the inactivation of the enzyme–substrate complex of ecto-5′-AMPase.

It is a relatively unexplored possibility that the mitogenic and other biological effects (Burger & Noonan, 1970) of glycose-specific lectins operate in part through their influence upon the activities of cell-surface glycoprotein enzymes. We have reported earlier in a preliminary communication (Stefanovic et al., 1975) that ecto-5′-AMPase of C6 cells but not nonspecific phosphatase or the Ca^{2+}-dependent ATPase is highly sensitive to the binding of concanavalin A (Con A) to the cell surface. The effect of Con A binding on the activity of the ecto-5′-AMPase of this neural cell in culture is presented in detail in the present study. Interest in the characteristics of lectin suppression stems from the hypothesis that this ectoenzyme may function in cellular interregulatory mechanisms. There is mounting evidence that extracellularly released 5′-AMP may be enzymatically hydrolyzed by ecto-5′-AMPase at the mammalian cell surface to adenosine (Stefanovic et al., 1976; DePierre & Karnovsky, 1974). This latter compound may function as a potent exogenous activator of adenylate cyclase for neural cells and for lung, fat, and bone cells, platelets, and lymphocytes (Blume & Foster, 1975; Sturgill et al., 1975; Clark & Seney, 1976; Mills & Smith, 1971).

Materials and Methods

Culture of Cells. Normal, NN, hamster astroblasts were obtained from North American Biologicals. Transformed, C6, rat astrocytoma cells were obtained from the American Type Culture Collection. Approximately 10^5 cells were seeded in 5 mL of Dulbecco's modified Eagle's medium supplemented, per milliliter, with 100 μL of Gibco fetal calf serum, 50 units of sodium penicillin G, and 25 μg of streptomycin sulfate. The cells grew under a humidified atmosphere of 95% air–5% carbon dioxide at 37 °C on a 25-cm^2 growth surface in Falcon plastic petri dishes. For developmental studies, cell growth continued for 8 days, with medium changes on the third, fifth, and seventh days; lectin-binding and enzymatic experiments took place on the day following each medium change.

Lectin Treatment of Cells. We removed the culture medium. We then washed the cells twice with a buffered salt solution consisting of 130 mmol of sodium chloride and 1 mmol of magnesium chloride per liter of 40 mM tris(hydroxymethyl)aminomethane hydrochloride at pH 7.4. The washed cells interacted over timed intervals and at several temperatures with Con A (Sigma), succinyl-Con A (a gift from Dr. M. Burger), *Ricinus sanguinis* agglutinin, RSA (Aunis et al., 1975) (a gift from Dr. J. P. Zanetta), *Dolichos biflorus* agglutinin, DBA, or wheat germ agglutinin, WGA (Vector). The cells were exposed to varying concentrations of these lectins, up to 100 μg/mL, in the buffered salt solution. Details regarding concentrations of lectins, temperature, time periods of exposure to lectin, pH, and ionic strength are specified in the appropriate sections under Results, below. Radioactive measurements of tritium indicated the amounts of Con A bound to cells in the form of [^{3}H]Con A (New England Nuclear). For tritium counting, we utilized a toluene–ethanol based scintillation mixture (Buhler, 1962) containing omnifluor as scintillator to which we added a concentrated formic acid solution of the whole cells scraped from the dish, after first having doubly rinsed each sample with buffered salt solution. To correct for nonspecific lectin binding, we added lectin in the presence of an excess of a competing saccharide ligand (100 mM methyl α-D-glucoside). In experiments in which we varied ionic strength, pentaerythritol (Barton & Rosenberg, 1973) substituted for sodium chloride to maintain osmolarity in the extracellular medium.

Ecto-5′-AMPase Assay. We removed the nutrient medium or the lectin treatment medium and washed the fully attached cells gently three times with about 10 mL of the pH 7.4 buffered salt solution described above. We then added the buffered salt solution plus adenosine 5′-monophosphate (5′-AMP) to the cell cultures, generally 1.5 mL of solution containing 3 mM 5′-AMP. Hydrolysis of 5′-AMP to adenosine and inorganic phosphate by the cellular ecto-5′-AMPase proceeded for 10 min at 37 °C. Longer periods were employed for time-course studies. At the end of each incubation, we poured off the extracellular fluid and measured inorganic phosphate in aliquots of the latter by the colorimetric procedure of Gomori (1942), retaining the cells for protein analysis by the method of Lowry et al. (1951) employing bovine serum albumin as standard. The contribution of nonspecific ectophosphohydrolase activity to the hydrolysis of

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Table I: Comparison of 5′-AMPase of Intact and Disrupted Nervous System Cells in Culture

cell line	5′-AMPase activity[a]	
	intact cells (ecto)	cell homogenate[b]
C6 rat glioma	4.23 ± 0.20	6.80 ± 0.33
NN hamster astroblasts[c]	0.15 ± 0.01	0.43 ± 0.09
N18 mouse neuroblasts	0.12 ± 0.02	0.21 ± 0.07

[a] Micromoles of inorganic phosphate liberated (microgram of cell protein)$^{-1}$ hour^{-1}. Approximately 10^6 cells were exposed to 3 mM 5′-AMP in 1.5 mL of buffered isotonic sodium chloride, pH 7.4, at 37 °C for 10 min. Inorganic phosphate was measured colorimetrically. [b] Cells were scraped into 1.5 mL of buffered isotonic saline, transferred to an all glass Potter-Elvejhem homogenizer, and homogenized by six strokes at 0 °C. [c] NN astroblast cells are a continuous line of cultured nononcogenic cells. A primary culture of hamster astroblasts grown out directly from embryonic brain tissue showed higher activity: 2.93 ± 0.07 μmol of inorganic phosphate (mg of cell protein)$^{-1}$ h^{-1}, total activity; 0.99 ± 0.01 ecto activity.

Table II: Rise in Specific Activity of Ecto-5′-AMPase of Neural Cells in Culture

cell line	ecto-5′-nucleotidase activity[a]			
	days in subculture[b]			
	2	4	6	8
N18	0.11	0.12	0.13	0.13
NN	0.12	0.15	0.18	0.18
C6	1.20	2.30	2.80	2.60

[a] Micromoles of P_i liberated from mM 5′-AMP hour^{-1} (milligram of cell protein)$^{-1}$. Nonsaturating 5′-AMP concentration was employed to avoid cytotoxicity. Mean values (n = 6); SD ± 6%. [b] Approximately 10^5 cells (100 μg of cell protein) were seeded per initial culture. Ecto-5′-AMPase activity was measured every second day. Increase in cell protein by the eighth day was 20-fold, 28-fold, and 30-fold for N18, NN, and C6 cells, respectively.

5′-AMP, as measured by the inclusion of *p*-nitrophenyl phosphate (Stefanovic et al., 1975) in the incubation medium, was estimated and disregarded, being in no case more than 2%.

Results

Ecto-5′-AMPase of Intact Cells. In Table I we list (a) the ecto-5′-AMPase activity of several neural cell lines in culture and (b) the corresponding activity in whole cell homogenates produced from these cells in buffered isotonic sodium chloride. Ecto-5′-AMPase activity of the glial oncogenic C6 astrocytoma cell line is high compared with other cell types. A neuronal, N18, cell line, included for comparison, displays lower activity, as does also the normal glial NN astroblast line. In all of these cell lines, ecto-5′-AMPase activity rose significantly with increasing cell density, which we estimated in terms of increasing cell protein per dish. These results are shown in Table II. Over an 8-day period of culture, by the end of which the increase in cell protein per dish had either ceased entirely or slowed considerably, the initially high ecto-5′-AMPase activity of the C6 cell line per dish had risen more than 500%, while that of the nononcogenic NN cell line had approximately doubled. In contrast with these neural astroblast cells (Benda et al., 1968), the N18 neuronal cell line displayed little increase in specific activity, but this was a rapid increase per dish, reflecting rapid cell growth. For subsequent studies employing lectins, we selected a 3-day time period in cell culture at which time the specific ecto-5′-AMPase activity, i.e., activity per milligram of cell protein, had reached approximately the half-way mark in its cell-density dependent rise in activity.

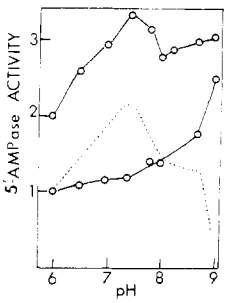

FIGURE 1: Dependence of Con A inhibition of ecto-5′-AMPase of rat C6 astrocytoma cells upon pH. Cells were incubated with Con A at a concentration of 30 μg/mL in pH 7.4 buffered isotonic salt solution for 5 min at 37 °C. 5′-AMP was then provided at a concentration of 3 mM at the desired pH, and incubation was continued for an additional 10 min. At the end of this period, inorganic phosphate in the extracellular medium was measured colorimetrically. (O–O) Upper, without the addition of Con A; lower, Con A inhibited activity; (···) differences curve between control and Con A inhibited activity.

Effect of Extracellular Con A Concentration on Binding of Con A to the Cell Surface. Measurement of the nonspecific binding of [^{3}H]Con A to the surface of intact C6 cells in monolayer culture in the presence of competing ligand indicated a low degree of such binding, which occurred as a temperature-dependent, quasi-linear function of the concentration of Con A in the treatment medium. Exposure of the cells for 10 min in a pH 7.4 buffered salt solution containing 40 mM tris(hydroxymethyl)aminomethane, 1 mM magnesium chloride, 100 mM methyl α-D-glucoside, and 100 μg of Con A per milliliter gave values for nonspecific binding of Con A per milligram of cell protein as follows: 0.86 μg at 37 °C, 0.59 at 18 °C, and 0.27 at 4 °C. Lower concentrations of Con A gave lower nonspecific binding, in direct proportion to concentration of Con A in the extracellular medium.

Binding was rapid at pH 7.4. Specific binding, i.e., total binding in the absence of competitive saccharide ligand minus nonspecific binding of [^{3}H]Con A to the cell surface, proceeded at the initial reaction velocity over a period of 10 min at 4 and 18 °C; at 37 °C, the initial rate of binding of Con A ended after approximately 8 min.

Effect of Con A Binding on pH Dependence of Ecto-5′-AMPase of C6 Cells. Figure 1 presents the effect of Con A binding on activity of ecto-5′-AMPase of C6 cells as a function of pH. The rate of attachment of Con A to the cells was observed to be a function of pH, being 145% at pH 6, 110% at pH 7, and 80% at pH 8 as compared with attachment at pH 7.4 taken as 100%. Activity at each pH was measured with 4.5 μg of Con A attached per milligram of cell protein, an amount of Con A which inhibits ecto-5′-AMPase approximately 66% at the pH optimum of 7.4. Analysis of the pH curves by subtraction of the Con A inhibited from the Con A free function suggests that Con A depresses the activity of a functional group with a pK value near 7.4. Residual phosphohydrolase activity and potentiation of enzymatic hydrolysis of 5′-AMP by hydroxyl ion are clearly discernible in the hyperbolic rise in activity with increasing pH for the Con A inhibited activity curve.

Quantitative Relationship between Con A Bound and Inhibition of Ecto-5′-AMPase. Figure 2 shows the effect of the quantity of Con A bound specifically to the intact cell on the activity of ecto-5′-AMPase. The inhibitory effect of specific Con A binding on ecto-5′-AMPase increases linearly with the amount of Con A bound and appears to be biphasic, with a break point around 2 μg of Con A/10^6 cells. This quantitative effect is independent of the temperature at which the Con A is bound, although temperature exerts an influence on the rate

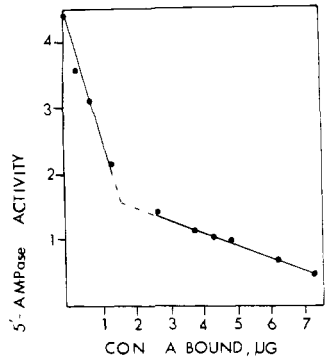

FIGURE 2: Quantitative effect of specific Con A attachment to the surface of C6 cells on the activity of ecto-5′-AMPase as measured in terms of initial velocity of the enzymatic reaction with an extracellular substrate concentration of 3 mM. Conditions for the attachment of Con A at pH 7.4 and the measurement of ecto-5′-AMPase at this optimum pH for enzymatic activity are described under Methods in the text. Con A was attached to cells at three different temperatures: 4, 18, and 37 °C. The amount of Con A attached was measured radioactively with [^{3}H]Con A, and the enzymatic reaction in each case was run at 37 °C.

of binding and inhibition is directly proportional to the amount of bound lectin. Nonspecific binding of Con A, i.e., in the presence of 100 mM competing saccharide, had no significant influence upon ecto-5′-AMPase activity below 300 ng of Con A bound nonspecifically per 10^6 cells. Inhibition of 5′-AMPase was measurable upon greater binding, e.g., 12% inhibition upon nonspecific binding of 400 ng and 15% at 800 ng of Con A bound. Specific binding of this latter quantity of Con A produced roughly three times as great an inhibition of ecto-5′-AMPase, as shown in Figure 2. When pentaerythritol substituted for sodium chloride in the incubation medium to give a medium of lowered ionic strength but undiminished osmolarity, a small but measurable decrease in inhibition took place, e.g., a 9% change upon complete replacement of sodium chloride with 260 mosm pentaerythritol.

Effect of Con A Binding on the Kinetics of Ecto-5′-AMPase Activity. The ecto-5′-AMPase activity of the intact C6 cells in monolayer culture obeyed Michaelis–Menten kinetics with or without Con A bound to the cell surface. The pH activity curve suggests (Figure 1) that Con A attachment represses the activity of a pK 7.4 functional group. We measured the effect of substrate concentration on the initial velocity of enzymatic hydrolysis of 5′-AMP by ecto-5′-AMPase at this pH and obtained evidence that, in a formal sense, the attachment of Con A to the cell surface operates to stabilize the enzyme–substrate complex. The reciprocal initial velocity–substrate concentration plots for various amounts of Con A attached to the cell surface gave a series of lines of apparently equal slope. V_{max} decreased with increasing Con A binding; K_m decreased proportionately. These findings are shown graphically in Figure 3. When shifted from pH 7.4 to 9, cells with 3.9 μg of attached Con A per 10^6 cells showed the same proportional reduction in ecto-5′-AMPase activity due to Con A binding as at pH 7.4 taking into account that the control V_{max} value is lower at pH 9. However, there was no significant effect of Con A on K_m values at this considerably higher pH, in contrast with the pronounced effect on K_m near the pH optimum of 7.4.

Effect of Substrate Binding on Con A Inhibition of Ecto-5′-AMPase. We exposed the cells to 5 mM 5′-AMP in order nearly to saturate the active site with substrate. Cells were then treated with Con A at various concentrations for 10 min by addition of the lectin to the incubation medium. At the end of this time, less than 3% of the 5′-AMP had been

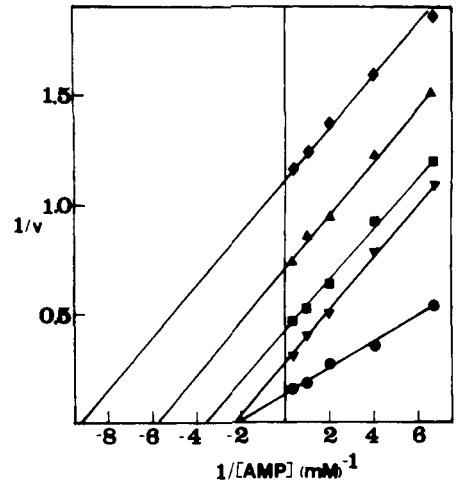

FIGURE 3: Effect of Con A attachment to C6 cells on kinetic parameters of ecto-5′-AMPase activity. Conditions for the attachment of Con A and the measurement of ecto-5′-AMPase activity at pH 7.4 are described under Methods in the text. (●–●) Con A free cells; (♦–♦) 7.3 μg of Con A attached per 10^6 cells; (▲–▲) 6.0 μg of Con A; (■–■) 3.7 μg of Con A; (▼–▼) 2.2 μg of Con A.

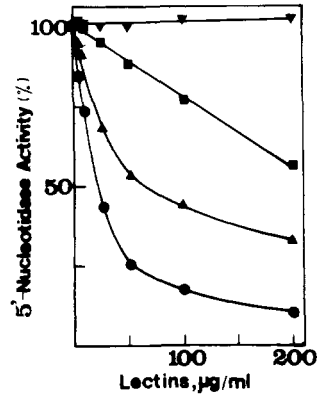

FIGURE 4: Inhibitory effect of various lectins on ecto-5′-AMPase activity of C6 cells. Cells were incubated in buffered isotonic salt solution at pH 7.4 with each lectin in the absence of competing ligand at the concentrations shown for 10 min at 37 °C. The lectin solution was then poured off, the cells were washed twice with buffered salt solution, and ecto-5′-AMPase measurement was made as described under Methods in the text. (▼–▼) *Dolichus biflorus* lectin (DBA); (■–■) *Ricinus sanguinis* lectin (RSA); (▲–▲) *Triticum vulgaris* lectin (WGA); (●–●) *Canavalia ensiformis* lectin (Con A).

hydrolyzed by ectoenzymatic activity. We poured off the solution of Con A plus 5′-AMP and measured ecto-5′-AMPase activity of the cells. We could not discern any measurable difference between the inhibitory effect of Con A attachment to the substrate-saturated and the substrate-vacant ecto-5′-AMPase of the intact C6 cells.

Comparison of the Effects of Lectins Other than Con A on Ecto-5′-AMPase of C6 Cells. DBA had no inhibitory effect on ecto-5′-AMPase. RSA binding inhibited ecto-5′-AMPase, but far less effectively than Con A. Exposure to RSA for 10 min at concentrations up to 200 μg/mL of treatment medium at 37 °C nonspecifically inhibited ecto-5′-AMPase in direct proportionality with the concentration of RSA in the medium. WGA was more strongly inhibitory than RSA under initial velocity conditions for lectin binding and, although not quite as effective as Con A, showed similar saturation characteristics as evidenced by the hyperbolic function produced upon plotting activity against lectin concentration. These findings are depicted graphically in Figure 4.

Effect of Ionic Strength on Release of Con A Inhibition by Methyl α-D-Mannoside. Treatment of the Con A inhibited

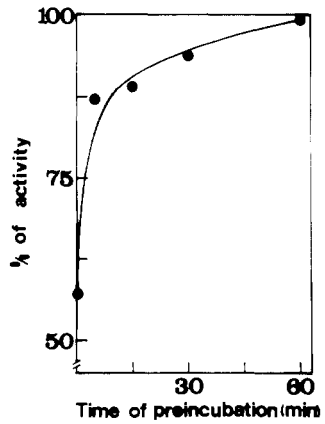

FIGURE 5: Rate of deinhibition of ecto-5′-AMPase of C6 cells upon exposure at pH 7.4 and 37 °C to methyl α-D-mannoside. The intact C6 cells were preincubated with 25 μg at Con A/mL of buffered salt solution for 5 min. The solution was poured off, and the cells were rinsed twice with the isotonic salt solution. The salt solution was replaced and the addition of 260 mM methyl α-D-mannoside made for the time periods indicated. This solution was then poured off, cells were rinsed twice, and ecto-5′-AMPase activity was measured as described under Methods in the text.

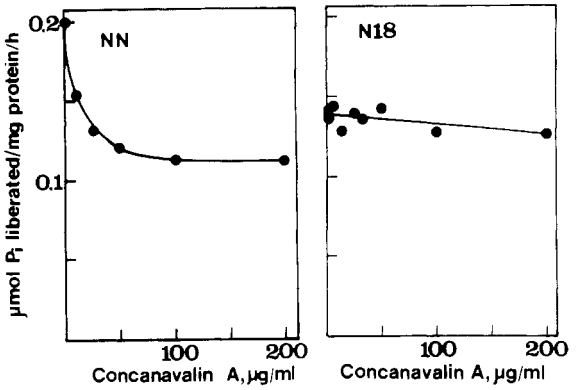

FIGURE 6: Inhibition of ecto-5′-AMPase by Con A attached to NN and N18 cells. Conditions are the same as described for Figure 2.

cells with methyl α-D-mannoside either in buffered isotonic salt solution or in isoosmolar, low ionic strength pentaerythritol solution restored ecto-5′-AMPase activity in full. Like inhibitory attachment, deinhibitory release displayed a quasihyperbolic time course as shown in Figure 5.

Comparison of the Effect of Con A on Ecto-5′-AMPase of C6, NN, and N18 Cells. Con A had no significant inhibitory effect on the 5′-AMPase of the N18 neuroblastoma cells. A very small degree of inhibition, observed upon exposure to high concentrations of lectin, was attributable to nonspecific binding. Inhibition of ecto-5′-AMPase of the normal glial NN astroblast cells, like the oncogenic C6 glial cells, showed saturability. A maximum of 38% of the total ectoactivity was inhibited upon Con A treatment; approximately 91% of C6 ecto-5′-AMPase is inhibited. In Figure 6, a comparison is made of the effect on ecto-5′-AMPase of exposure of these two, former, cell lines to increasing concentrations of Con A.

Effect of Partial Desialylation of C6 Cells on Lectin Inhibition of Ecto-5′-AMPase. No significant change in the effect of any of the lectins tested occurred as a result of partial removal of sialic acid from the C6 cells by treatment with *V. cholerae* sialidase (neuraminidase).

Discussion

Con A may cross-link mobile glycoprotein receptors on the cell surface and cause an alteration in enzymes associated with the plasma membrane of mammalian cells (Edelman et al., 1972). Detailed information is scarce which can lead to an elucidation of the mechanisms by which this and other lectins may alter the properties of cell-surface enzymes. 5′-AMPase is an ectoenzyme, functionally facing outward, on the surface of a large number of hyperplastic or tumorigenic mammalian cells (Bingham & Burke, 1972; LeLievre 1973; Gurd & Evans, 1974; Woo & Manery, 1975; Newby et al., 1975). The oncogenic and extremely rapidly growing C6 rat astrocytoma cell displays an elevated level of ecto-5′-AMPase activity which, unlike some other phosphohydrolases in the cell surface, is extremely sensitive to interaction of the intact cell with Con A (Stefanovic et al., 1975). This lectin, which possesses one saccharide binding site per protomer, reportedly is dimeric in solution below pH 6 and tetrameric above neutrality (Kalb & Lustig, 1968). Upon exposure of intact living cells in culture to increasing concentrations of lectin above pH 6 for brief periods, rat C6 glioma cells bind as much as 8 μg of Con A/10^6 cells, an extraordinarily high quantity. Essentially all of the bound lectin is released upon extensive incubation of these cells with methyl α-D-mannoside.

The C6 astrocytoma cells display a level of ecto-5′-AMPase activity some 30-fold that of a nontumorigenic NN line of astroblasts. A neuronal, N18 line of cells displays an even lower ecto-AMPase activity than the NN cells; unlike the glial cells tested, it is not inhibitable by exposure to Con A. The relatively small differences in rate of binding of inhibitory Con A to the cell surface at different temperatures suggest that the activation energy of formation of Con A–cell receptor complexes to produce the Con A inhibited state of ecto-5′-AMPase is rather low. We have observed a linear relationship between Con A inhibition of ecto-5′-AMPase and Con A binding to the cell surface over the range of "high affinity" binding of Con A, i.e., below 1.5 pg bound per cell. This latter produces some 60% of the maximum ecto-5′-AMPase inhibition obtainable by Con A binding. The lack of influence of temperature, which affects membrane fluidity, suggests that the effect of Con A is related to direct binding to the enzyme molecule in the cell surface. The saccharide binding sites for Con A are completely released by exposure of the lectin-bound cells to exogenous α-mannoside. The failure of large changes in ionic strength to alter significantly the Con A inhibition of ecto-5′-AMPase suggests that the inhibitory binding of lectin to the ectoenzyme represents, essentially, a nonionic interaction.

With sufficient cell binding of Con A to produce at the pH optimum of 7.4 a 66% inhibition of total activity, acid–base titration of the ecto-5′-AMPase activity of the intact cells in isotonic sodium chloride solution reveals that Con A binding represses the activity of a major pK = 7.4 functional group. We suspect that this may represent the pK of the active enzyme–substrate complex. A simple tentative explanation for our finding of quasiparallel lines in the reciprocal initial velocity–reciprocal substrate concentration plots for ecto-5′-AMPase upon attachment of various quantities of Con A may be proposed: a simultaneous blockage of dissociation of both substrate and product from the active site of the enzyme. The blocked product may be either inorganic phosphate or adenosine or both. Con A binding inhibits both the substrate-free and substrate-saturated ectoenzyme. Upon shifting from a bulk medium pH of 7.4 to a high pH, e.g., 9, the effect of Con A binding on K_m is relieved, but reduction in V_{max} persists. These findings may indicate that the Con A effect is not simply steric inhibition. The order of binding, i.e., exposure of the ectoenzyme to 5′-AMP before the attachment

of Con A, or the reverse, has no demonstrable effect, and there is no compelling evidence that allosteric modifications lie at the basis of this inhibition. This is not to say that conformational changes may not occur, especially in the attached Con A molecule which has been shown to be susceptible to pH-dependent rearrangement upon ligand binding (Pflumm et al., 1971). When exposed alternatively to succinyl Con A and Con A, the C6 cells displayed a maximum "saturation" inhibition of ecto-5′-AMPase by succinyl-Con A which is less than half that produced by Con A. At the pH tested, 7.4, succinyl-Con A reportedly is a dimeric molecule, while Con A is tetrameric (Gunther et al., 1973). The associative properties of the protomers of Con A have been suggested to be determinants in the capping of Con A bound to the cell surface. The altered nature of succinyl-Con A may account for the absence of capping when succinyl-Con A is bound (Gunther et al., 1973). These considerations suggest that ecto-5′-AMPase may be a relatively mobile enzyme in the cell surface. Neither Ca^{2+},Mg^{2+}-dependent ecto-ATPase nor ectoacetylcholinesterase is inhibitable by Con A.

DBA, having a carbohydrate specificity for *N*-acetylgalactosaminyl groups, did not inhibit ecto-5′-AMPase in the C6 cell. RSA exerted a lower and linearly increasing initial rate of inhibition with respect to increasing lectin concentration and no saturability of inhibition of activity over the wide range of lectin concentration tested, suggesting that the influence of RSA binding may not result from direct interaction with ecto-5′-AMPase. In contrast, the *N*-acetyl-β-glucosaminide-binding lectin, WGA, exerted an inhibitory effect on ecto-5′-AMPase whose order of magnitude and saturation characteristics greatly resembled that of the α-D-mannoside (glucoside)-binding lectin, Con A. Release from WGA inhibition by *N*-acetylglucosamine displayed kinetic features similar to those for Con A and α-mannoside. In view of the reported ability of WGA to bind sialosyl groups (Greenway & LeVine, 1973), we partially desialylated the cell surface with *V. cholerae* sialidase (neuraminidase). This treatment had no effect either on the control activity or on the influence of any of the lectins tested. In contrast, ectoacetylcholinesterase is markedly activated by partial desialylation of the cell surface (Stefanovic et al., 1975) but is unaffected by the attachment of Con A. From this, we infer that enzymatically susceptible bound sialic acid on the cell surface neither blocks access of the lectins to ecto-5′-AMPase nor functions as an important enzymatic component. These findings support the notion, derived from the ineffectiveness of DBA and RSA as inhibitory agents, that neither galactosyl nor *N*-acetylgalactosaminyl groups are important determinants of ecto-5′-AMPase activity in the cell surface. These glycosyl groups expectedly would be uncovered by the removal of sialic acid from cell surface sialoglycoproteins and become available to DBA and RSA. Conversely, the potent saturable inhibitory activity of both Con A and WGA point to a mannosyl- and *N*-acetylglucosaminyl-containing glycoprotein structure for ecto-5′-AMPase. Its cell density dependent increases in activity to levels where a relatively high extracellular concentration of 5′-AMP is entirely degraded to inorganic phosphate and free adenosine within minutes at 37 °C, and the adenylyl cyclase activating effect of this latter compound when supplied extracellularly (Clark & Seney, 1976), suggest a role for ecto-5′-AMPase in the rapid growth of these mammalian cells. It is conceivable that cell-elaborated lectins may influence the activity of this cell surface ectoenzyme in a manner analogous to that demonstrated for certain plant lectins in this study.

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