Castanospermine: a potent inhibitor of sucrase from the human enterocyte-like cell line Caco-2

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The addition of castanospermine (5–50 μM) to a culture medium of Caco-2 cells results in a specific suppression of sucrase activity without modification of the biosynthesis of the enzyme. This effect is due to a direct inhibiting effect of castanospermine on Caco-2 sucrase activity. This inhibition is time-dependent (half-maximum efficiency at 10 min for 100 nM), enhanced by preincubation (suggesting a strong interaction with the enzyme), dose-dependent (ED_{50} at 4 nM after 1 h preincubation period) and of the fully non-competitive type. The calculated K_i (2.6 nM) suggests that castanospermine is the most potent inhibitor of sucrase so far reported.

Castanospermine *Sucrase* *Protein glycosylation* *Enzyme inhibition* *Cell differentiation* *(Human colon cancer cells)*

1. INTRODUCTION

Previous studies from this laboratory have shown that the modulation of the enterocytic differentiation, and more particularly of the expression of sucrase in human colon cancer cells in culture such as HT-29 [1,2] and Caco-2 cells [3], was dependent on glucose metabolism, most likely through glucose-mediated modifications of protein glycosylation [4]. To investigate this relationship further we chose to study how castanospermine would affect the expression of sucrase in these cells. Castanospermine (1,6,7,8-tetrahydroxyoctahydroindolizine) (fig.1), an alkaloid isolated from the seed of the Australian legume *Castanospermum australiae* [5] was chosen because it is known to interfere with the metabolism of glycogen [6] and to inhibit several glycosidases [7], including glucosidase I [8], one of the enzymes which control the processing of glycoproteins [9–11] (and presumably of brush border-associated hydrolases as they are known to be highly glycosylated glycoproteins [12]). Our studies were conducted in the cell line Caco-2, as it is the cell line which shows the highest level of sucrase activity [3,13]. In the course of this study we observed that in Caco-2 cells treated with castanospermine the activity of sucrase, but not of other hydrolases, was totally abolished without modifications of the biosynthesis of the enzyme. The results reported here show that this effect is due to a strong inhibiting effect of castanospermine on Caco-2 sucrase. The main characteristics of this inhibition are described.

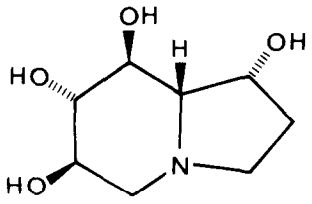

Fig.1. Structure of castanospermine (1,6,7,8-tetrahydroxyoctahydroindolizine) from [5].

2. MATERIALS AND METHODS

Caco-2 cells (obtained from J. Fogh, Memorial

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Table 1

Activities of brush border-associated hydrolases in control and castanospermine-treated Caco-2 cells

	Cell homogenate		P_2 fraction	
	Control cells	Castanospermine-treated cells	Control cells	Castanospermine-treated cells
Sucrase	83.6	0	1034	0
Aminopeptidase N	14.2	20.1	131.6	155.9
Dipeptidyl peptidase IV	61.3	72.5	830.3	969.2

Caco-2 cells were harvested on day 16 of the culture after a 48 h treatment with 25 μM castanospermine. Enzyme activities are expressed as mU/mg protein. Similar results were obtained in cells treated with castanospermine (5–50 μM) from day 1 to 16

Sloan Kettering Center, Rye, NY) were cultured in plastic flasks as described in [3] and used between passages 148 and 161. Enzyme assays were performed on day 16 of the culture, i.e. when the cells are morphologically fully differentiated [3,13], the enzyme activities increase exponentially [3,13] and their synthesis is maximum (unpublished). Cell homogenates and brush border-enriched fractions (P_2) were prepared as described in [3] according to [14]. Sucrase (EC 3.2.1.48) activity was determined according to [15], aminopeptidase N (EC 3.4.11.2) according to [16] using L-alanine-*p*-nitroanilide as substrate, and dipeptidyl peptidase IV (EC 3.4.14.5) according to [17] using 1.5 mM glycyl-L-proline-4-nitroanilide (at pH 8) as substrate. Enzyme activities are expressed as mU/mg protein, 1 unit representing the hydrolysis of 1 μmol substrate per min under the experimental conditions. Glycogen was measured as in [3]. For sucrase-isomaltase biosynthesis studies the same methods as in [18] were applied to both the cell homogenate and the P_2 fraction. Endo-β-acetylglucosaminidase H (Endo-H) [19] was obtained from Miles (France), castanospermine from Boehringer (Boehringer-Mannheim, FRG) and tunicamycin [20] from Calbiochem (Le Vésinet, France).

3. RESULTS

The addition of castanospermine to the culture medium of Caco-2 cells has no effect on glycogen content (not shown) and on the activities of aminopeptidase N and dipeptidyl peptidase IV, but

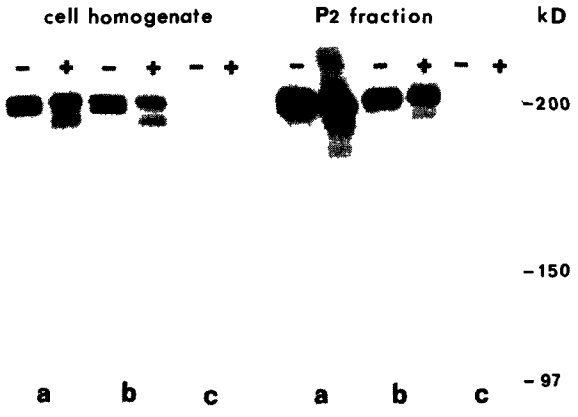

Fig.2. Autoradiographs of SDS-PAGE of immunoprecipitates of L-[^{35}S]methionine labeled sucrase-isomaltase, treated (+) or not (−) with Endo-H, from the cell homogenate and the brush border-enriched fraction (P_2) of Caco-2 cells. (a) Control cells, (b) cells treated for 48 h with 25 μM castanospermine, (c) cells treated for 48 h with 10 μM tunicamycin. Caco-2 cells were harvested on day 16 of the culture after a 3 h exposure to 500 μCi L-[^{35}S]methionine. Immunoprecipitates were obtained with the use of monoclonal antibody HBB2/614/88 [18] (obtained from H.P. Hauri, Biocenter of the University of Basel, Basel, Switzerland). As described in [18] the upper band corresponds to the complex form of the enzyme and the lower band, sensitive to Endo-H treatment, to the high-mannose form. Under our experimental conditions castanospermine has no effect on the biosynthesis of the enzyme, in contrast to tunicamycin, an inhibitor of the first step of the N-core glycosylation [20] which has a strong inhibiting effect on the biosynthesis of the enzyme.

results in a total absence of activity of sucrase (table 1). The concomitant absence of effect of castanospermine on the biosynthesis of the enzyme (fig.2) therefore suggested that the absence of sucrase activity was most likely the result of a direct inhibiting effect of the drug on Caco-2 sucrase.

This inhibition was investigated, using Caco-2 brush border-enriched fractions as a source for sucrase. As shown in fig.3 the inhibiting effect is time-dependent with, for 100 nM, a half-maximum effect at 10 min. This effect also depends on the time of preincubation (fig.4) suggesting that the drug has a strong interaction with sucrase. This strong interaction is further supported by the dose-response curves obtained with or without a 1 h preincubation (fig.5) which show that the ED_{50} decreases from 60 nM in non-preincubated samples to 4 nM in preincubated samples. It should also be noted that the slope of the curve obtained with preincubation is sharper

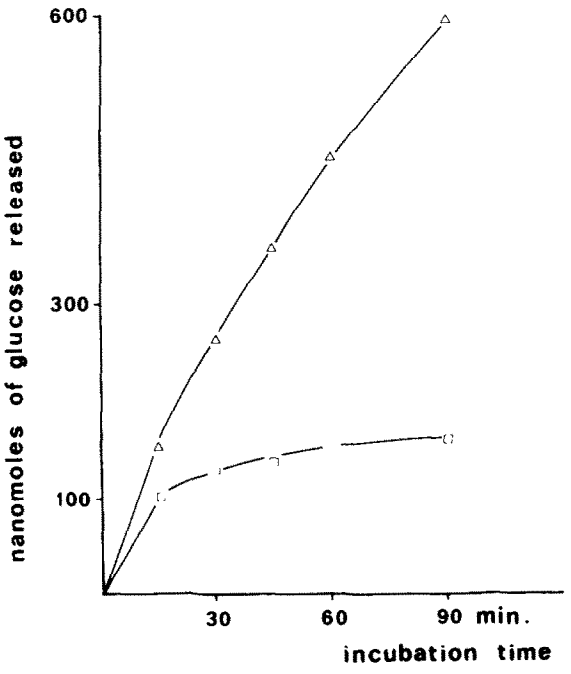

Fig.3. Time course of sucrase and castanospermine inhibition. P_2 fraction of control 16-day-old Caco-2 cells was used as a source for sucrase. Each value corresponds to 25 μg protein incubated in the absence (△) or presence (□) of 100 nM castanospermine.

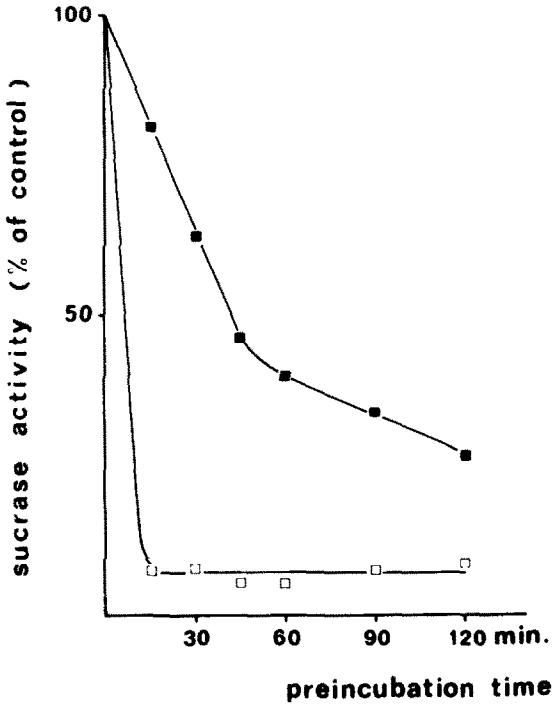

Fig.4. Effect of preincubation with castanospermine on the inhibition of Caco-2 sucrase activity. For each point 25 μg protein were used. Sucrose was added to the assay at the indicated time of preincubation with 10 nM (■) and 100 nM (□) castanospermine. The enzyme assay was performed within 30 min (steady-state velocity). For each point results are expressed as percent of the corresponding control sample (without castanospermine).

than in non-preincubated samples (fig.5), also indicating a strong interaction of castanospermine with sucrase. The Lineweaver-Burk plots derived from measurements of sucrase activities in extracts preincubated with increasing concentrations of castanospermine are reported in fig.6; this representation shows that the inhibition is of the fully non-competitive type and further indicates that the apparent K_m of Caco-2 sucrase for sucrose, which has not been reported before, is 9 mM. This fully non-competitive inhibition was confirmed by using the Henderson representation [21] (fig.7) which is more adapted to the very low concentrations of the inhibitor and further allows the calculation of the K_i value of the drug for sucrase (calculated K_i = 2.6 nM).

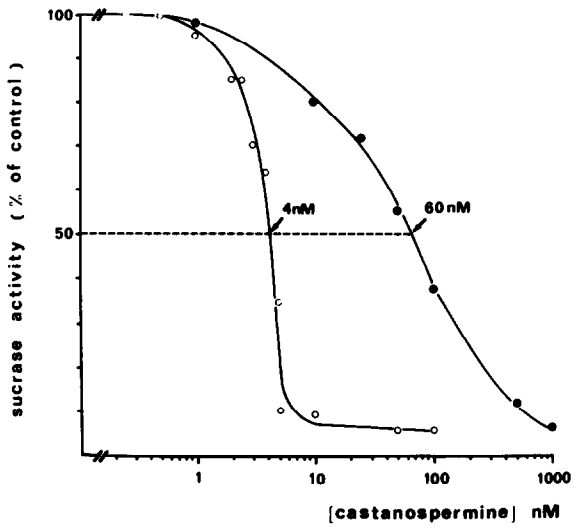

Fig.5. Dose-response curves of inhibition of sucrase activity by castanospermine without (●) or with (○) a 1 h preincubation. Experimental conditions as in fig.4.

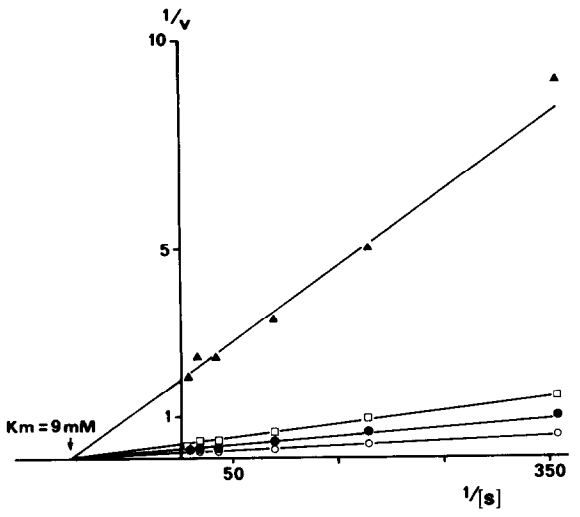

Fig.6. Effect of sucrose (*S*) concentration on the inhibition of sucrase activity (*V*) by castanospermine. Assays were performed in the absence (○) or after a 1 h preincubation with 5 nM (●), 10 nM (□) and 50 nM (▲) castanospermine. Results are plotted according to Lineweaver-Burk.

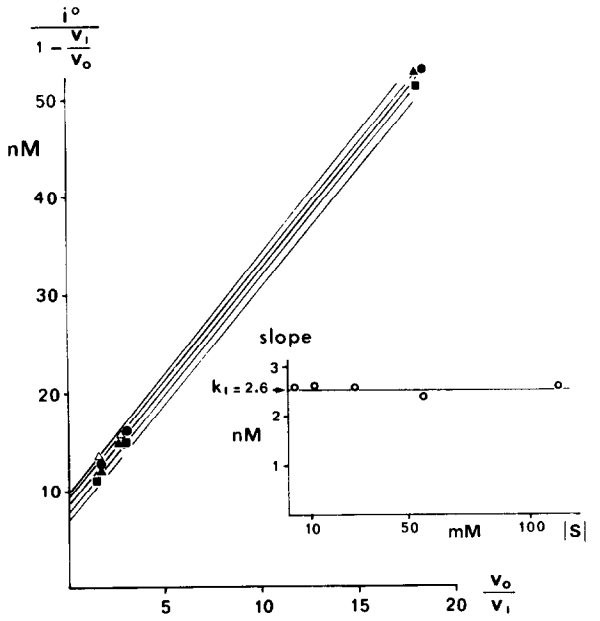

Fig.7. Henderson plot of the inhibition of Caco-2 sucrase by castanospermine: i^o, concentrations of castanospermine; v_i and v_o, the velocities of the reaction in the presence or absence of castanospermine, respectively. The concentrations of substrate (sucrose, in mM) were: 2.8 (●), 11.2 (△), 28 (▲), 56 (■) and 112 (□). Inset: the slopes of the primary plots were replotted against the concentration of sucrose (*S*); the K_i value is given by the intercept of this secondary plot with the slope axis.

4. DISCUSSION

The main conclusion to be drawn from this work is that castanospermine is a strong inhibitor of sucrase activity. As to the initial purpose of this work, which was to investigate whether castanospermine could modify the expression of brush border hydrolases in Caco-2 cells in culture via 2 of its known effects, i.e. modifications of glycogen metabolism [6] and inhibition of glucosidase I [8], it was observed that, under our experimental conditions, the drug had no effect on either glycogen or the biosynthesis of sucrase.

The inhibiting effect of castanospermine, which was suggested from the specific absence of sucrase activity in cells grown in the presence of the drug, was confirmed by direct inhibition studies. From these studies it can be concluded that castanosper-

mine acts through a fully non-competitive mechanism, by contrast with other drugs known to inhibit sucrase activity such as nojirimycin, deoxynojirimycin and acarbose [22]. This non-competitive characteristic of inhibition by castanospermine suggests that it could be a useful tool to study the relationship between the total splitting activity and the transglucosidation activity of sucrase [23,24]. Comparison of our results (K_i = 2.6 nM, fig.7) with those reported for other inhibitors [22,23] suggests that castanospermine is most likely the most potent inhibitor of sucrase so far described. Furthermore a strong interaction of the drug for sucrase is suggested by the preincubation-dependent increase of its inhibiting activity (figs 4,5) and by the differences in the slopes of the dose-response curves observed with or without preincubation. The fact that castanospermine inhibits sucrase activity is not totally surprising as it has been shown to inhibit the activity of some α-glucosidases [6,7].

These results imply that any study using castanospermine as an inhibitor of the process of glycosylation in intestinal cells should consider not only its strong inhibiting effect on sucrase activity, but also its strong interaction with the enzyme.

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