Na^+-DEPENDENT, ELECTRONEUTRAL L-ASCORBATE TRANSPORT ACROSS BRUSH BORDER MEMBRANE VESICLES FROM HUMAN SMALL INTESTINE

Inhibition by D-erythorbate

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1. Introduction

In the small intestine of rats, rabbits or hamsters L-ascorbate is taken up by a low-capacity, Na^+-independent system showing 'diffusion' kinetics ([1–4] and Caspary, unpublished quoted [5]). In at least two of the mammalian species in which this substance is a vitamin, viz., man and the guinea pig, L-ascorbate is taken up by a saturable system of larger capacity which is activated by extracellular Na^+ [5–7].

In the guinea pig, we have recently shown that L-ascorbate transport into brush border membrane vesicles is not due to the system(s) transporting D-glucose. It is carrier-mediated and electrically neutral with an L-ascorbate:Na^+ flux ratio of 1. The epimer of L-ascorbate in C_5, erythorbate (D-isoascorbate) was found to be transported by the same system as L-ascorbate, thus acting as a (weak) fully-competitive inhibitor ($K_i \simeq 18$ mM, as compared with the K_m for L-ascorbate, 0.3 mM) [4].

In man, to the best of our knowledge, few kinetic studies in vitro have been performed. In particular, no investigation up to now has utilized brush border membrane vesicles, which are the only preparation capable of providing unequivocal information as to the type of transport system involved. Yet in man it is particularly important to closely scrutinize the interaction of erythorbate with the transport system for L-ascorbate since:

(i) Population groups may derive their daily intake of L-ascorbate mainly from the ascorbate present as an additive to foodstuffs [8];
(ii) Erythorbate, which is indeed absorbed in human intestine [9], is being used in some countries as an additive to foodstuffs for technological reasons;
(iii) Erythorbate and L-ascorbate compete for the same membrane transport systems in peripheral tissues (e.g. [10]).

The major difficulty in working with man is, of course, that of obtaining sufficient amounts of suitable material. In fact, for uptake studies of this kind, neither peroral biopsies (too small) nor post-mortem material (quickly degraded) are adequate.

Our studies in man, therefore, were perforce less extended than those in the guinea pig [4]. The results below do show, however, that the L-ascorbate transport systems in the small intestinal brush border membranes of the two species are similar. In man the system also:

(i) Shows saturation by the substrate;
(ii) Is activated by external Na^+;
(iii) Is electroneutral (and can thus be expected to have a L-ascorbate:Na^+ flux ratio of 1);
(iv) Is not identical with the system(s) transporting D-glucose;
(v) Is inhibited fully-competitively by D-erythorbate (probably a substrate for the system).

Judging from the estimated K_m- and K_i-values, ery-

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Table 1
Dependence of L-ascorbate uptake on the time of incubation – Inhibition by D-isoascorbate (D-erythorbate)

Incubation time (min)	L-Ascorbate concentrations (mM) yielding half-maximum uptake ($\bar{x}$ ± SE)	L-Ascorbate uptake at infinitely large L-ascorbate concentration (pmol . mg protein^{-1}) ($\bar{x}$ ± SE)	Apparent K_i-value for D-isoascorbate (mM)
1	0.20 ± 0.127	254 ± 45	11
1.5	0.432 ± 0.22	366 ± 83	13
2	0.489 ± 0.07	521 ± 38	38

Brush border membrane vesicles from human jejunum were incubated at 20°C with various concentrations of L-[^{14}C]ascorbate (0.3, 0.5, 0.7 or 1.0 mM) in 300 mM D-mannitol, 10 mM Hepes/Tris (pH 7.5) with an initial Na_2SO_4 gradient (50 mM out, 0 in). The app. K_i-values were estimated from Dixon plots similar to those of fig.1

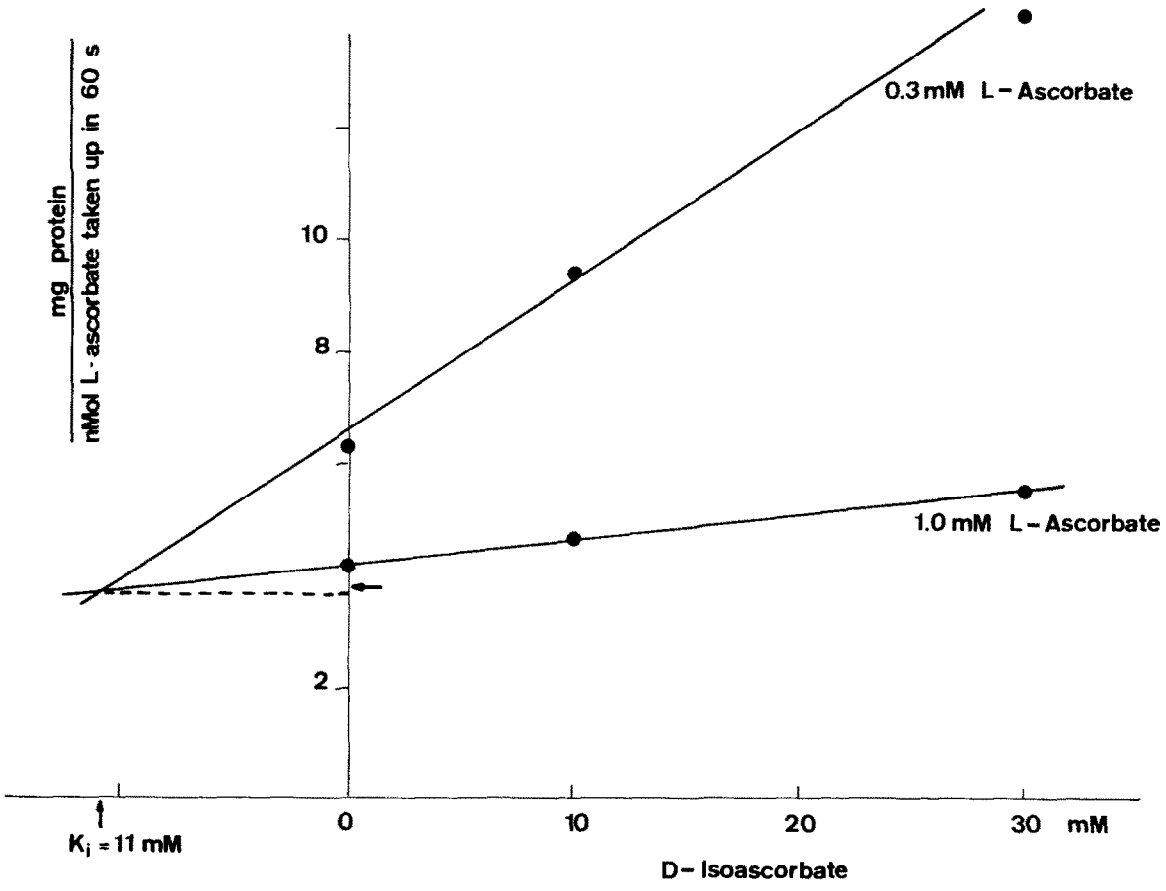

Fig.1. Dixon plot of the inhibition of L-ascorbate uptake into brush border membrane vesicles by D-isoascorbate (D-erythorbate). Sixty-second L-ascorbate uptake was measured at 20°C in the presence of an initial Na_2SO_4 gradient (50 mM out, 0 in) and the indicated concentrations of L-[^{14}C]ascorbate and D-isoascorbate. The buffer was in all cases 10 mM Hepes/Tris (pH 7.5) plus 300 mM D-mannitol. The arrow indicates the reciprocal of the L-ascorbate concentration (mM) giving maximum uptake (60 s), as calculated from double-reciprocal plots in parallel experiments.

thorbate must inhibit L-ascorbate uptake in human small intestine at least as much as it does in the guinea pig.

2. Materials and methods

Macroscopically normal pieces of human jejunum or ileum were obtained during operations on the small bowel and were kept at −20°C for no longer than a couple of weeks. After thawing, brush border membrane vesicles were prepared via Ca^{2+} precipitation ([11] as modified in [12]). The uptake of L-[^{14}C]ascorbate into these vesicles was measured at 20°C in 10 mM Hepes/Tris (pH 7.5), 100 mM D-mannitol, plus the salts and at the ascorbate concentration(s) indicated in the legends to the figures. The incubations were stopped by addition of ice-cold 250 mM NaCl, the suspension filtered through a Sartorius filter (0.6 μm pore size) under suction, and washed once with the same ice-cold solution. The radioactivity in the filter was measured in a scintillation counter. For details, refer to [4].

3. Results and discussion

In man, as in the guinea pig [4], the capacity of L-ascorbate transport is too low (and not compensated for by a particularly high specific radioactivity of the substrate available) to allow determination of K_m- and K_i-values from the short initial linear uptake (discussed in [13,14]). We thus resorted to estimating them from incubations as short as possible (table 1, and fig.1). Clearly, the transporter for L-ascorbate shows saturation and fully-competitive inhibition by D-erythorbate. The latter phenomenon is most likely due to this substance being transported by the same system: in guinea pig small intestinal vesicles erythorbate elicits counterflow of L-ascorbate [4]. L-Ascorbate transport is activated by extravesicular Na^+ (compare the 60 s uptake values in Na_2SO_4 and $NaNO_3$ with those in K_2SO_4 and KNO_3, respectively, in the lower panel of fig.2).

Contrary to the transport of D-glucose in the same preparation (upper panel of fig.2) (see also [12,15]), that of L-ascorbate is insensitive to differences in the electric potential, as induced by highly permeant

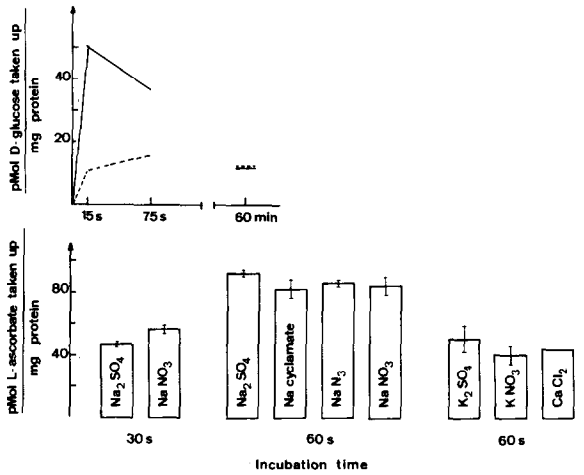

Fig.2. Lower panel: effects of cations and anions on L-[^{14}C]ascorbate uptake. The initial concentrations in the incubation media were: L-[^{14}C]ascorbate, 0.5 mM; buffer and D-mannitol, as in fig.1; salts: Na_2SO_4, K_2SO_4 or $CaCl_2$, 50 mM; $NaNO_3$, Na cyclamate, NaN_3 or KNO_3, 100 mM. Upper panel: In the same vesicles under similar conditions D-glucose uptake was electrogenic. D-[^{3}H]Glucose (5 μM) uptake was measured in the presence of either a NaSCN gradient (100 mM out, 0 in) (——), or a Na_2SO_4 gradient (50 mM out, 0 in) (– – –). The uptake in the presence of a $NaNO_3$ gradient was the same as in the presence of a NaSCN gradient (data not shown). Other conditions, as in the experiment of the lower panel.

anions (e.g., NO_3^- or N_3^-) compared with relatively impermeant ones (e.g., SO_4^{2-} or cyclamate) (lower panel in fig.2). Thus, on the basis of the criteria discussed elsewhere (e.g. in table 2 of [4]), the electroneutral Na^+-dependent L-ascorbate transport in human small intestine also is due to a L-ascorbate, Na^+-cotransporting carrier with (under the experimental conditions used here) a flux ratio of 1.

The system for L-ascorbate transport is not identical with that mediating D-glucose transport because:

(i) Phlorizin, a strong fully-competitive inhibitor of intestinal D-glucose transport (e.g. [13,16]) does not detectably inhibit (−3% ± 9%) L-ascorbate uptake at concentrations (0.25 mM) which strongly inhibit D-glucose uptake;

(ii) L-Ascorbate does not inhibit D-glucose uptake (data not shown);

(iii) D-Glucose only marginally inhibits L-ascorbate uptake (data not shown). (Presumably an example of heterologous inhibition [4]).

Summing up, L-ascorbate transport across small intestinal brush border membranes is very similar in man and in guinea pig, and is quite different from that in rat, hamster or rabbit. In the last 3 species it is Na^+-insensitive and has the kinetic characteristics of a 'diffusion' [1–5]. It is well known, of course, that man and guinea pig belong to the few mammalian species which are unable to carry out the complete synthesis of L-ascorbate and for which this substance is thus a vitamin. The possible nutritional relevance of the inhibition of L-ascorbate uptake by D-erythorbate is difficult to assess from in vitro experiments. The maximum accepted daily intake of this inhibitor (as high as 5 mg/Kg body wt in some countries), the amount of L-ascorbate occurring in the diet and the K_i of the fully-competitive inhibition by D-erythorbate (~11 mM) are the main factors which can be expected to play a role.

Acknowledgements

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