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Proline transport across the intestinal microvillus membrane may be regulated by membrane physical properties

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There is now abundant evidence that integral membrane protein function may be modulated by the physical properties of membrane lipids. The intestinal brush border membrane represents a membrane system highly specialized for nutrient absorption and, thus, provides an opportunity to study the interaction between integral membrane transport proteins and their lipid environment. We have previously demonstrated that alterations in this environment may modulate the function of the sodium-dependent glucose transporter in terms of its affinity for glucose. In this communication we report that membrane lipid–protein interactions are distinctly different for the proline transport proteins. Maximal transport rates for L-proline by either the neutral brush border or imino transport systems are reduced 10-fold when the surrounding membrane environment is made more fluid over the physiological range that exists along the crypt-villus axis. Furthermore, in microvillus membrane vesicles prepared from enterocytes isolated from along the crypt-villus axis a similar gradient exists in the functional activity of these transport systems. This would imply that either the functional activity of these transporters are regulated by membrane physical properties or that the synthesis and insertion of these proteins is coordinated in concert with membrane physical properties as the enterocyte migrates up the crypt-villus axis.

Introduction

Each cell in the body has a wide variety of proteins designed for a diverse range of functions. In order for each to function properly their immediate microenvironment is carefully controlled to provide optimal conditions for protein function. While this principle is evident for enzymes located in the aqueous environment of the cell it has been more difficult to demonstrate for membrane bound transport proteins. The microvillus membrane of the enterocyte provides a useful model to study these phenomena since it represents an environment highly specialized for the transport of a wide variety of nutrients. Furthermore, since this cell has one of the most rapid turnover rates in the body it is a relatively simple task to examine the maturation of this membrane, both in terms of protein function as well as the maturation of membrane lipids with the resultant physical environment they provide for integral membrane proteins. Previous work with this model has examined the interaction between the sodium-dependent glucose transporter and the physical properties of the microvillus membrane. Several groups have reported that manipulations producing a more fluid microvillus membrane are associated with decreased rates of glucose transport [2,4,6]. Recent work from our group has demonstrated that the functional activity of sodium-dependent glucose transport increases in the microvillus membrane as the enterocyte matures along the crypt-villus axis of the rabbit. Coincident with increased rates of glucose transport the microvillus membrane becomes progressively less fluid [10]. However, the two phenomena do not appear to be causally related since fluidization of villus-tip microvillus membrane, to simulate a crypt-like microenvironment, produced only a slight decline in maximal rates of glucose transport. These studies did reveal, however, that the conformational change in the glucose transporter initiated by sodium binding could only occur in membranes of a certain minimal fluidity. Thus, the affinity of this transport system for glucose in the presence of sodium appears to be dependent upon membrane physical properties. These observations have recently been confirmed in studies with diabetic rats performed by Dudeja et al. [5]. Increases in rates of

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glucose transport seen with this model appear to be secondary to recruitment of enterocytes further down the villus rather than the alterations in membrane physical properties also observed. Thus, the interaction between membrane physical properties and the sodium-dependent glucose transporter appear to be restricted to the conformational changes associated with sodium binding, at least over the physiological range of membrane physical properties studied.

The conformational alterations that occur with transporter activation are unlikely to be identical for different transport systems, therefore, the influence of membrane physical properties would also be expected to differ between carriers. In order to test this hypothesis we have examined the influence of membrane physical properties on the carrier-mediated transport of L-proline across the microvillus membrane. Transport of L-proline is more complicated than glucose in that this molecule has at least three separate pathways across the microvillus membrane. These include two active Na^+-dependent cotransport systems; the neutral brush border (NBB) system, which transports other neutral amino acids such as L-alanine, and the imino system which is specific for L-proline and imino acids such as L-pipicolate [18]. in this paper we present data demonstrating that the interaction of these transport proteins with membrane physical properties are distinctly different from those observed for the glucose transporter.

Materials and methods

Cell and microvillus membrane isolation

New Zealand White rabbits, weighing between 0.75 and 1.0 kg, were killed with an overdose of intravenous pentobarbitol. The jejunum was rapidly removed and flushed with ice-cold buffer containing PBS and 1 mM DTT. Cells from along the crypt-villus axis were isolated using a modification of the method described by Rowling and Sepulveda [12]. Following isolation, each fraction was pooled and microvillus membrane vesicles were prepared using the calcium precipitation method described by Kessler [7]. The final membrane pellet was suspended in a buffer containing 100 mM KCl, 10 mM Hepes, 10 mM Tris and mannitol in a concentration producing iso-osmotic conditions with the reaction buffer. The resultant suspensions were stored in liquid nitrogen [17].

Membrane marker studies

Sucrase activity of membrane isolates and their homogenates was determined by the method of Dahlqvist [3]. To identify the proportion of crypt enterocytes in each population, rapidly dividing cells were labelled with bromodeoxyuridine (BrdU). This was accomplished by injecting BrdU (100 mg/kg iv) 3 h prior to killing. Cytospins were prepared from each isolated cell fraction and stained for the presence of BrdU according to the method of Thomas and Williams utilizing a fluorescein conjugated anti-BrdU antibody [24]. The number of fluorescent nuclei were expressed as a percentage of total enterocytes in each population.

Proline transport

Vesicle uptake of ^{3}H-labeled L-proline was measured using a rapid filtration technique. Proline uptake rates were measured at final concentrations ranging from 50 μM to 8 mM. A 5-s incubation time was used as preliminary studies, over the concentration range to be used, demonstrated that uptake rates were linear for up to 10–20 s depending upon concentration. All experiments were conducted at 21°C. A reaction buffer was prepared containing 150 mM NaCl, 100 mM KCl, 10 mM Hepes, 10 mM Tris and various concentrations of L-[^{3}H]proline (pH = 7.5). Studies were performed under voltage-clamped conditions using 40 μM valinomycin. Transport was initiated by rapidly mixing 10 μl of the membrane preparation with 20 μl of the reaction solution. The reaction was terminated with the rapid addition of 2 ml of an ice-cold solution consisting of 100 mM KCl, 300 mM mannitol, 10 μM Hepes and 10 mM Tris (pH = 7.5). The resultant mixture was rapidly filtered through a 0.45 μM filter (Millipore/Continental Water Systems, Bedford, MA) and subsequently dissolved in scintillation cocktail (Beckman). L-[^{3}H]Proline trapped in the vesicles was quantitated in a liquid scintillation counter (Beckman Instruments Inc., Palo Alto, CA). Values for nonspecific binding of the isotope to the vesicles and the filters were determined by measuring uptake at time zero.

To determine rates of sodium-independent uptake, 10 μl of the membrane preparation was rapidly mixed with 20 μl of a reaction solution containing 100 mM KCl, 300 mM mannitol, 10 mM Hepes, 10 mM Tris. The reaction was quenched and uptake measured as previously described. Rates of sodium-independent proline uptake over the concentration range of 50 μM to 8 mM were consistently less than 5% of total uptake when determined in vesicles isolated from mucosal scrapings. However, in vesicles obtained from isolated enterocytes a greater diffusional component was evident. Since we wished to examine the functional activity of the membrane bound protein transporters this component was extracted from the raw data as described below.

In separate studies the NBB carrier was inhibited by the addition of a large excess of L-alanine (100 mM) under iso-osmotic conditions. Uptake experiments were performed using a proline concentration of 150 μM.

In additional studies the influence of altered membrane physical properties were evaluated using microvillus membrane vesicles isolated following a light

jejunal scrape. These represent largely villus tip and mid-villus cells. Vesicles were suspended in a buffer containing 100 mM KCl, 300 mM mannitol, 10 mM Hepes, 10 mM Tris and 15 mM of either methanol, ethanol, propanol, butanol, pentanol or hexanol. The reaction solution contained 150 mM NaCl, 100 mM KCl, 10 mM Hepes, 10 mM Tris, and 15 mM of one of the alcohols mentioned above.

Fluidity determinations

The term membrane fluidity in this paper is used to refer to the relative motional freedom of molecules within the lipid bilayer. These properties were assessed with steady state fluorescence polarization techniques and a variety of probes that allowed both the static and dynamic component of membrane fluidity to be estimated [13,19]. The former was evaluated with the probe 1,6-diphenyl-1,3,5-hexatriene (DPH) while the dynamic component was evaluated at different depths in the bilayer using several isomers of *n*-(9-anthroyloxy)stearic or palmitic fatty-acid probes ($n = 2$, 6, 9, 12, or 16). Data are reported as the steady-state anisotropy parameter and the total fluorescence for a constant amount of membrane and probe. The latter measurement is important since it provides an estimate of fluorescent lifetime, total fluorescence being directly proportional to fluorescent lifetime. Finally, for the data obtained with DPH both the limiting hindered anisotropy (r_∞) and the membrane order parameter (S_{DPH}) were calculated using a previously published empirical relationship [20].

Chemicals

All chemicals were of the highest available grade and were purchased from either Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). L-[^{3}H]Proline was purchased from New England Nuclear (Boston, MA) and the fluorescent probes were purchased from Molecular Probes (Junction City, OR).

Statistical analysis

Statistical analysis was performed using the commercial program Systat (Evanston, IL). Comparisons between groups were performed using ANOVA and a Tukey test for post-hoc comparisons. Within group homogeneity of variances was first established with a Bartlett test. For comparison of kinetic curves and estimation of kinetic parameters a recently described method was employed [8]. This method allows for parameter estimation by direct nonlinear regression with data weighted in proportion to within sample variances. From recent work by Stevens et al. [16] it is apparent that L-proline can utilize two distinct brush border carriers. Thus, rates of L-proline uptake determined in the presence of 100 mM *cis* sodium were initially fit to Eqn. 1.

$$J = \left(\frac{J_1^{max} \cdot C}{K_m^1 + C}\right) + \left(\frac{J_2^{max} \cdot C}{K_m^2 + C}\right) + P \cdot C \qquad (1)$$

This model assumes two carrier mediated processes and a linear 'diffusional' component with a slope of P. Rates of carrier-dependent L-proline uptake can be obtained by removing the contribution of $P \cdot C$ from the raw data. Since nonlinear regression algorithms are most precise when the minimum number of parameters are solved for, situations where no clear distinction between the two carriers could be made were fit to Eqn. 2 which describes a system composed of a single carrier and a linear diffusional component.

$$J = \left(\frac{J_1^{max} \cdot C}{K_m^1 + C}\right) + P \cdot C \qquad (2)$$

This situation arose for several reasons. First, in some instances the regression based upon Eqn. 1 converged with the two carriers having identical K_m's, suggesting that only a single carrier could be dissected mathematically from the data. Secondly, in other cases two distinct carriers appeared to fit the data but the total contribution of the second carrier was insignificant. Since we know that two significant routes exist this solution does not provide useful information on a second carrier. Finally, in all cases the 'goodness of fit' of the data to the model, as assessed by the methods previously outlined [8], was no better with the two carrier model. Thus, data is presented as carrier-dependent rates of L-proline uptake and a single J^{max} and K_m are given. This does not imply that only a single carrier exists, only that the two carriers are so similar in terms of handling L-proline that they cannot be kinetically distinguished when using L-proline alone as a substrate. The K_m represents the average value for two carriers and the J^{max} represents the sum of each individual value. Comparisons between parameter estimates were performed as described [8].

Results

Sensitivity of proline transport to alterations in membrane physical properties

The first question addressed was whether rates of L-proline uptake varied when membrane physical properties were modified in a graded fashion. Such experiments must be performed under conditions that do not alter the sodium permeability of the vesicles as this will artificially reduce rates of sodium-dependent nutrient transport. Unfortunately, fluidizing agents such as benzyl alcohol have been shown to dramatically increase

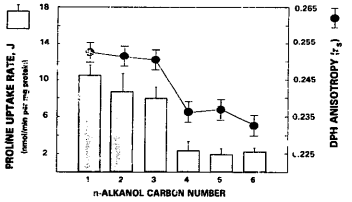

Fig. 1. Proline transport as a function of fluidity. Rates of L-proline uptake, mean ± S.E. from five membrane preparations, were determined at a fixed *cis* concentration of 1 mM in the presence of several alcohols (15 mM). The alcohols used were a homologous series of *n*-alkanols ranging from methanol to hexanol. Shown in the filled circles are the steady-state anisotropy parameters for the probe DPH in each membrane preparation. With increasing alcohol hydrophobicity an abrupt increase in membrane fluidity was observed with the addition of butanol that coincided with a significant decline in rates of L-proline uptake.

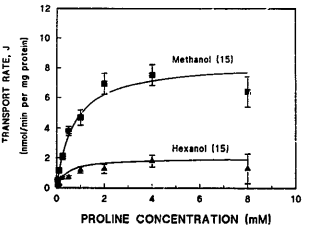

Fig. 2. Kinetics of proline uptake in fluidized vesicles. Experiments illustrated represent the kinetic curves for L-proline uptake in five membrane preparations (mean ± S.E.) as a function of concentration in vesicles incubated with either methanol or hexanol (15 mM). Parameter estimates derived from these experiments are presented in Table I.

the sodium permeability of such vesicles and, thus, are not useful under these circumstances. However, we have previously demonstrated that a series of linear *n*-alkanols provide efficient fluidization of microvillus membrane vesicles and do not alter either sodium permeability or initial rates of sodium-dependent glucose transport [10]. Fig. 1 illustrates the effect of incubating microvillus membrane vesicles in a fixed concentration of alcohol (15 mM) of progressively increasing chain length. Rates of L-proline uptake were determined under conditions of a *cis* sodium concentration of 100 mM and 1 mM L-proline. For this homologous series of alcohols an abrupt increase in membrane fluidity was seen with the addition of butanol that persisted with the addition of either pentanol or hexanol. The data shown utilize the fluorescent probe DPH but similar results were observed with the (anthroyloxy)stearic acid derivatives (data not shown). Coincident with the dramatic increase in membrane fluidity was a significant reduction in rates of L-proline uptake. Since only a single L-proline concentration gradient was used, these studies cannot be used to address the question of whether alterations in membrane physical properties altered maximal rates of L-proline uptake or the affinity of the proline transport systems for substrate.

To determine which kinetic parameter(s) were specifically affected rates of L-proline uptake were determined over a substrate concentration range of 50 μM to 8 mM in either fluidized or non-fluidized vesicles. In the non-fluidized vesicles methanol was used as an osmotic control for hexanol. These data are depicted in Fig. 2 with the calculated kinetic parameters in Table I. As illustrated, fluidization of these vesicles produced a 10-fold reduction in the J^{max} for L-proline uptake with no significant alteration in the K_m of this transport system. Furthermore, vesicle size was not significantly different between groups. These data suggested that increasing motional freedom within the microvillus membrane did not alter the affinity of the proline transport system for its substrate, but rather, had a profound effect upon either functional transporter number or turnover time.

A final question concerning these data was whether increasing membrane fluidity had a preferential effect upon one or other of the L-proline transport pathways. To address this concern we evaluated rates of proline uptake into fluidized and non-fluidized vesicles in either the presence or absence of large excesses of L-alanine to inhibit proline transport via the NBB pathway. These data are shown in Fig. 3 and represent rates of transport at a single 150 μM proline concentration. Once again it was evident that the addition of hexanol dramatically reduces total rates of L-proline

TABLE I

Kinetic parameters for proline transport

Each value represents best fit parameter estimate from the experiments depicted in Fig. 2. In brackets are the 95% confidence intervals for each determination. Also included are the steady-state anisotropy parameter values for the membranes in which L-proline transport rates were determined.

Alcohol added (mM)	J^{max} (nmol/min per mg)	K_m (μM)	DPH anisotropy (r_s)
Methanol (15)	8.33 (7.03, 9.63)	692 (513, 872)	0.251 ± 0.002
Hexanol (15)	1.99 (≈ 1.42, 2.57) *	431 (178, 685)	0.219 + 0.002 *

* $P < 0.05$ vs. methanol-treated membranes.

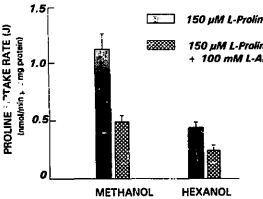

Fig. 3. Contribution of the imino carrier in fluidized and non-fluidized vesicles. Rates of L-proline uptake were determined at a fixed *cis* concentration of 150 μM. In order to determine the contribution of the imino system the neutral brush border transport system was saturated with 100 mM L-alanine. Total rates of L-proline uptake were significantly reduced with the addition of hexanol (15 mM). The addition of L-alanine also significantly reduced rates of L-proline uptake, however, the relative proportion that the imino transport system contributed to total L-proline uptake was constant in either experiment. Each set of data represents the mean ± S.E. from at least five membrane preparations in each group.

uptake, however, the contribution of the imino transport system (measured in the presence of L-alanine) was constant in both fluidized and non-fluidized states (55.5 ± 11.1 and 41.0 ± 5.7%, respectively). Thus, the effect of fluidization upon L-proline transport systems appeared to be shared equally between the two major transport systems.

L-Proline transport along the crypt-villus axis

Since the foregoing data suggested that L-proline transport was exquisitely sensitive to membrane physical properties we elected to examine rates of L-proline uptake under conditions where these are known to vary physiologically; enterocyte maturation along the crypt-villus axis.

Cell and microvillus membrane characterization

The technique used for isolating cells from along the crypt-villus axis has been previously shown to produce fractions relatively enriched in cells from the villus-tip, mid-villus and crypt regions of the intestine [10]. To assess the relative purity of the current fractions we utilized markers for both mature villus-tip cells and the rapidly dividing crypt population; sucrase activity and incorporation of BrdU respectively. BrdU was administered 3 h before killing at a dose of 100 mg/kg iv. The results of these studies are summarized in Table II. Sucrase activity was highest in the villus tip fraction and declined in both the mid-villus and crypt populations. Conversely, BrdU labelled cells were prominent in the crypt fraction, scarce in the mid-villus and undetectable in the villus-tip population. Thus, the three cell fractions represent a gradient of increasing enterocyte maturity. Despite the gradient in total sucrase specific activity along the crypt-villus axis it is also apparent from Table II that microvillus membrane prepared from these fractions demonstrated equal purification as judged by the equivalent increase in su-

TABLE III

Membrane physical properties along the crypt-villus axis

Data represent the mean ± S.E. values for measurements performed in quadruplicate for the membrane isolates depicted in Fig. 4.

Membrane	Probe	Anisotropy parameter (r_s)	Limiting hindered anisotropy (r_∞)	Order parameter (S_{DPH})	Total fluorescence
Villus tip	2-AS	0.161 ± 0.002	–	–	4.49 ± 0.36
	6-AS	0.175 ± 0.004	–	–	8.69 ± 0.59
	9-AS	0.161 ± 0.002	–	–	9.12 ± 0.34
	12-AS	0.139 ± 0.002	–	–	8.75 ± 0.79
	16-AP	0.095 ± 0.008	–	–	11.45 ± 1.0
	DPH	0.233 ± 0.003	0.211 ± 0.004	0.763 ± 0.007	8.29 ± 0.38
Mid-villus	2-AS	0.172 ± 0.003	–	–	5.40 ± 0.28
	6-AS	0.180 ± 0.004	–	–	8.33 ± 1.40
	9-AS	0.163 ± 0.003	–	–	10.4 ± 1.30
	12-AS	0.138 ± 0.003	–	–	8.68 ± 0.70
	16-AP	0.083 ± 0.001	–	–	11.1 ± 1.08
	DPH	0.235 ± 0.006	0.213 ± 0.008	0.766 ± 0.015	8.51 ± 0.23
Crypt	2-AS	0.154 ± 0.003 *	–	–	4.21 ± 0.38
	6-AS	0.152 ± 0.005 *	–	–	7.47 ± 1.63
	9-AS	0.142 ± 0.003 *	–	–	7.94 ± 0.48
	12-AS	0.115 ± 0.004 *	–	–	6.67 ± 0.61
	16-AP	0.072 ± 0.001 *	–	–	7.46 ± 1.17 *
	DPH	0.213 ± 0.004 *	0.184 ± 0.006 *	0.712 ± 0.012 *	6.55 ± 0.47 *

* $P < 0.001$ vs. villus tip.

TABLE II

Marker characteristics of cell isolates

Sucrase activity represents the mean ± S.E. for determinations in five membrane preparations. Bromodeoxyuridine (BrdU) activity was determined from the initial cytospots following a 3 h exposure *in vivo*.

Cell isolate	Sucrase			BrdU nuclear incorporation (%)
	homogenate activity (U/mg protein)	MVM activity (U/mg protein)	purification (-fold)	
Villus tip	0.079 ± 0.019	1.88 ± 0.21	23.8 ± 6.5	0
Mid-villus	0.043 ± 0.006 *	1.41 ± 0.09 *	32.8 ± 7.7	0.88 ± 0.8
Crypt	0.012 ± 0.005 *	0.32 ± 0.05 *	26.7 ± 13.3	10.9 ± 1.0 *

* $P < 0.05$ vs. villus tip.

crase specific activity in the microvillus membrane fractions.

As well as demonstrating a gradient of increasing cell maturity the microvillus membrane isolated from these cells undergoes a decline in relative motional freedom as the enterocyte matures along the crypt-villus axis. These data are presented in Table III. For a variety of fluorescent probes sensitive to differing types of molecular motion the microvillus membrane of the mature villus tip enterocyte was significantly less fluid than observed in the crypt enterocyte. In terms of membrane physical properties vesicles from the mid-villus enterocytes appeared very similar to those obtained from the mature villus tip enterocytes. The observed alterations in steady-state anisotropy parameters are unlikely to be secondary to an increased fluorescent lifetime of the probes in the crypt microvillus membrane. This parameter cannot be directly obtained with steady-state techniques, however, since total fluorescence is directly proportional to fluorescent lifetime and this parameter was either the same or lower in the crypt preparations (Table III) it is difficult to postulate an increase in fluorescent lifetime. Furthermore, in the studies of Brasitus et al. [1] no differences in this parameter were observed when lifetime was directly measured. Although no studies of membrane lipids were performed with these vesicles we and others have previously demonstrated that these alterations in membrane physical properties can be accounted for by alterations in the cholesterol/phospholipid ratio, phospholipid subclasses and the fatty acid composition of these lipids [1,9,10].

Rates of L-proline uptake into these vesicles are presented in Fig. 4 with the corresponding parameter estimates in Table IV. In a fashion similar to membrane physical properties little difference could be observed between vesicles from the mid-villus and villus tip fraction. However, a 10-fold reduction in the J^{max} was observed in the microvillus membrane from

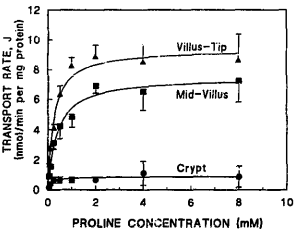

Fig. 4. Rates of microvillus membrane L-proline uptake in enterocytes from along the crypt-villus axis. Sodium-dependent rates of L-proline uptake in at least five separate membrane preparation from the cell fractions characterized in Table II. Rates of transport in the villus tip vesicles were all significantly different from those observed in the crypt fraction.

TABLE IV

Kinetic parameters for L-proline transport along the crypt-villus axis

Each value represents best fit parameter estimate from the experiments depicted in Fig. 4. In brackets are the 95% confidence intervals for each determination. Also included are the steady-state anisotropy parameter values for the membranes in which L-proline transport rates were determined

Membrane preparation	J^{max} (nmol/min per mg)	K_m (μM)	DPH anisotropy (r_s)
Villus tip	9.4 (7.2, 11.4)	252 (164, 340)	0.233 ± 0.003
Mid-villus	7.9 (5.4, 10.4)	405 (216, 592)	0.235 ± 0.006
Crypt	0.8 (0.2, 1.4) *	182 (−51, 415)	0.213 ± 0.004 *

* $P < 0.001$ vs. villus tips.

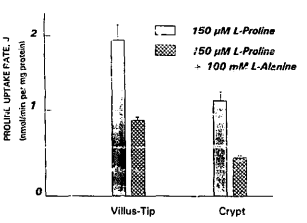

Fig. 5. Contribution of the imino system along the crypt-villus axis. Total rates of L-proline uptake in either the presence or absence of L-alanine are illustrated. At least five membrane preparations were used and the results represent mean values ± S.E. Villus tip vesicles have higher total rates of L-proline uptake than crypt vesicles but the relative contribution from the imino system is identical.

the crypt fraction when compared to the villus tip without any appreciable difference in the K_m. This would suggest that as the enterocyte matures and migrates up the villus, the affinity of the transporters for substrate is not changed but that there is a change in either transporter number or functional activity.

Finally, in order to assess the functional maturation of proline transport systems, in terms of the relative contribution between the imino and neutral brush border systems, we quantitated uptake of proline by the imino pathway in both crypt and villus tip microvillus membrane. These data are shown in Fig 5, and demonstrate that although total rates of L-proline transport are much lower in crypt enterocytes the relative contribution of both transport systems are similar along the crypt-villus axis.

Discussion

The study of amino acid transport in the small intestine is complicated by species variation, multiple transport systems for the same amino acid and multiple transport mechanisms [16]. However, proline lends itself to the current kinetic analysis since the imino carrier has been well defined in specificity and no other naturally occurring amino acids appear to be transported by this system [18,23]. Wright et al. [18] have previously demonstrated that proline uptake in rabbit brush border vesicles occurs by two Na^+-dependent means: the neutral brush border system (accounting for 35% of total uptake) and the imino system (60% of total uptake). A small amount of passive Na^+-independent uptake (less than 5% of total) is also observed [18]. Thus, in the current studies we examined the relationship between the functional activity of these transport systems and the physical characteristics of the surrounding lipid environment. Membrane physical properties in this paper refer only to those assessed as 'membrane fluidity'. We obviously cannot draw conclusions about other significant biophysical variables. It must be emphasized once again that two distinct transport systems exist for L-proline. The data presented here provides kinetic information for both pathways as a unit. From a mathematical perspective both the imino and NBB system appear to have similar affinities for L-proline and, therefore, they cannot be kinetically dissected when only L-proline is utilized as a substrate. Thus, the single K_m gleaned from our data represents the 'average' value for the two systems present while the single J^{max} reflects the combined transport capabilities of both systems.

In preliminary studies (Fig. 1) it appeared that total rates of L-proline transport were quite sensitive to in vitro modification of membrane physical properties. By incubating microvillus membrane vesicles with a homologous series of *n*-alkanols we observed a marked reduction in rates of L-proline uptake that coincided with an increase in motional freedom of probes inserted in the bilayer. Such experiments have numerous pitfalls, the most prevalent being that certain membrane fluidizing agents collapse the transmembrane sodium gradient required for efficient function of sodium-dependent transport processes. However, we have previously demonstrated that, unlike benzyl alcohol, the linear alcohols provide efficient fluidization of native microvillus membrane without increasing sodium permeability in the concentrations used for these studies [10]. The mechanism by which these alcohols fluidize membranes probably relates to random intercalation between native membrane lipids and the disruption of hydrophobic bonds. Since they were used in relatively high concentrations many membrane domains were likely to be influenced making it probable that the lipid environment in the immediate vicinity of the transporter was involved, the so-called annular lipids. In the current experiments the effect of these alcohols on both proline transport rates and membrane physical properties were similar for three alcohols (butanol, pentanol and hexanol) despite the structural differences between them. Thus, we conclude that the L-proline transport system(s) are sensitive to the surrounding membrane lipid physical properties, and require a minimal degree of rigidity for proper function.

In the case of the sodium-dependent glucose transporter membrane physical properties appear to play a role in the conformational change that accompanies sodium binding seen experimentally as a difference in the K_m of this transport system for glucose [10]. However, the situation for L-proline appears to be quite different. When we examined rates of L-proline uptake over a wide concentration gradient we were able to demonstrate that increasing the fluidity of the microvillus membrane decreased the maximal transport rate of proline, J^{max}, without an appreciable effect on the K_m for this transport system. Furthermore, this effect appeared to involve both the neutral brush border transport system as well as the imino system. From a variety of kinetic experiments Wright et al. have proposed a kinetic model for the imino carrier in which two Na^+ ions bind to the carrier on the *cis* membrane side. The resultant conformational change allows the binding of one proline molecule. This complex isomerizes to a configuration in which the binding sites are now facing the *trans* side. Release of Na^+ and proline then occur followed by reisomerization of the binding sites to the *cis* side of the membrane [14,15,23]. From kinetic data Wright has suggested that the rate-limiting step is the reisomerization of the unloaded carrier from the *trans* to the *cis* side of the membrane. This is in agreement with studies of the rat erythrocyte glucose transporter

where the conformational change of the empty Sugar-binding site from the *trans* to *cis* orientation is the rate-limiting step for sugar entry [21]. Our data would suggest that the interaction between protein conformational changes and membrane physical properties differ between the Sodium-dependent glucose transporter and the proline transport systems. In the case of the former sodium binding initiates a conformational change that is dependent upon a certain degree of membrane fluidity [10,11], while for the latter the rate of one (or both) isomerization steps may be reduced when the membrane environment becomes too fluid. Thus, alterations in membrane physical properties interact with membrane transport proteins in a fashion that is specific for each transport system.

Since membrane physical properties appeared to play a major role in the functional expression of the proline transport systems, we next examined the maturation of these systems along the crypt-villus axis of the rabbit jejunum. In this species we have previously demonstrated that the microvillus membrane becomes progressively more rigid as the enterocyte matures and moves onto the villus. These observations are species dependent and true for both the Sherman rat [1] and the rabbit, while in the Lewis rat the opposite seems to hold [5]. However, the current studies once again confirmed these observations for the rabbit jejunum (Table III). In terms of proline transport a maturation process was also evident along the crypt-villus axis (Fig. 2). Maximal rates of L-proline transport were 10-fold higher in the villus tip microvillus membrane than observed in the crypt without any appreciable alteration of the K_m for these transport systems. The explanation for these observations is open for debate. It could be argued that the proline transport systems are not expressed in the immature crypt microvillus membrane and only become evident after enough time has passed for the proteins to be synthesized and inserted into the apical membrane. In the absence of probes for either mRNA or antibodies for the membrane proteins this question cannot be reliably addressed. However, from the data presented in this study functional activity of these membrane proteins in the very fluid crypt microvillus membrane (if they exist) would appear unlikely, unless the local microenvironment of the protein was radically different from that measured in the bulk phase. Finally, it appears that the relative activity of the imino transport system in the crypt is the same as at the villus tip. The implications of this might be that this protein is regulated in concert with the neutral brush border transport system or once again that the difference between villus tip and crypt regions is entirely due to differing membrane physical properties, since both systems appear to be equally sensitive to alterations in membrane environments. Studies with an animal model such as the Lewis rat where a different pattern of apical membrane maturation exists would help to resolve this question.

In summary we have demonstrated that brush border proline transport systems are sensitive to alterations in membrane physical properties. Furthermore, these interactions are distinctly different from those observed for the sodium-dependent glucose transport system. Thus, it appears that membrane transport proteins can be added to the list of integral membrane proteins sensitive to surrounding membrane lipid composition and physical properties. However, it does not appear possible to predict these interactions from observations with other transporters.

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References

1 Brasitus, T.A. and Dudeja, P.K. (1985) Arch. Biochem. Biophys. 240, 483–488.
2 Carriere, B. and Le Grimellec, C. (1986) Biochim. Biophys. Acta 857, 131–187.
3 Dahlqvist, A. (1964) Anal. Biochem. 7, 18–25.
4 Dinda, P.K. and Beck, I.T. (1981) Dig. Dis. Sci. 26, 23–32.
5 Dudeja, P.K., Wali, R.K., Klitzke, A. and Brasitus, T.A. (1990) Am. J. Physiol. 259, G571–G577.
6 Fernandez, Y.I., Boigegrain, R.A.M., Cambon Gros, C.D. and Mirjavilia, S.E. (1984) Biochim. Biophys Acta 770, 171–177
7 Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 806, 136–154.
8 Meddings, J.B., Scott, R.B. and Fick, G.H. (1990) Am. J Physiol. 257, G982–G989.
9 Meddings, J.B. and Theisen, S. (1990) Am. J. Physiol. 256, G931–G940.
10 Meddings, J.B., DeSouza, D., Goel, M. and Theisen, S (1990) J. Clin. Invest. 85, 1099–1107.
11 Peerce, B.E. and Wright, E.M. (1984) Proc. Natl. Acad. Sci. USA 81, 2223–2226.
12 Rowling, P.J.E. and Sepulveda, F.V. (1984) Biochim. Biophys. Acta 771, 35–41.
13 Schachter, D. and Shinitzky, M. (1977) J. Clin. Invest. 59, 536–548.
14 Stevens, B.R. and Wright, E.M. (1985) Ann. N.Y. Acad. Sci. 456, 115–117.
15 Stevens, B.R. and Wright, E.M. (1987) J. Biol Chem. 262, 65466450.
16 Stevens, B.R., Ross, H.J. and Wright, E.M. (1982) J. Membr. Biol. 66, 213–225.
17 Stevens, B.R., Wright, S.H., Hirayama, B.S., Gunther, R.D., Ross, H.J., Harms, V., Nord, E., Kippen, I. and Wright, E.M. (1982) Membr. Biochem. 4, 271–282.
18 Stevens, B.R. and Wright, E.M. (1985) J. Membr. Biol. 87, 27–34
19 Thulborn, K.R. (1981) in Fluorescent Probes (Beddard, G.S. and West, M.A., eds.), pp. 113–141, Academic Press, London.

20 Van Blitterswijk, W.J., Van Hoeven, R.P. and Van der Meer, B.W. (1981) Biochim. Biophys. Acta 644, 323–332.
21 Whitesell, R.R., Regen, D.M., Beth, A.H., Pelletier, D.K. and Abumrad, N.A. (1989) Biochemistry 28, 5618–5625.
22 Wright, E.M. and Peerce, B.E. (1984) J. Biol. Chem. 259, 14993–14996.
23 Wright, E.M., Schell, R.E. and Stevens, B.R. (1985) Biochim. Biophys. Acta 818, 271–274.
24 Wynford-Thomas, D. and Williams, E.D. (1980) Cell Tissue Kinet. 19, 179–182.