A proton n.m.r. study of iminodipeptide transport and hydrolysis in the human erythrocyte

Possible physiological roles for the coupled system

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The first description of a saturable iminodipeptide transport system present in human erythrocytes is given. The ^1H -n.m.r. spectra of glycyl-L-proline and those of free glycine and L-proline are significantly different. This enabled the non-invasive monitoring by ^1H -n.m.r. spectroscopy of the hydrolysis of the dipeptide in human erythrocytes and their lysates. The concentration-dependence of the rate of glycyl-L-proline hydrolysis by haemolysates was described by the Michaelis–Menten expression with $K_m = 14.1 \pm 2.4$ mmol/litre and $V_{\max} = 130 \pm 10$ mmol/h per litre of cell water. At concentrations of the dipeptide that saturated prolidase, hydrolysis of glycyl-L-proline by whole cells was ~ 130 times slower than by lysates. This rate difference indicated that transport is the rate-determining step in peptide hydrolysis by whole cells, and thus the concentration-dependence of the transport rate was determined. The membrane transport system was found to be saturable and could be described by the Michaelis–Menten expression with $K_t = 4.7 \pm 0.4$ mmol/litre and $V_{\max} = 0.997 \pm 0.026$ mmol/h per litre of cell water. Numerical integration of a consistent set of differential rate equations that described a minimal model of the coupled transport–hydrolysis system successfully described prolonged time courses of peptide hydrolysis by whole cells. The simulations showed very low steady-state levels of dipeptide in the erythrocyte and very small lag periods (< 5 min) in the progress curve describing the appearance of free amino acid inside the cells. The rates of transport of glycyl-L-proline into erythrocytes and kidney proximal-tubular epithelium were compared and the possible importance of erythrocyte prolidase in whole-body prolyl-peptide turnover is discussed.

A diverse range of exopeptidase activities reside in the cytosol of the mature human erythrocyte. Peptidases that have been purified and at least partially characterized include aminopeptidase B (arginine aminopeptidase, EC 3.4.11.6) (Söderling, 1982), aminotripeptidase (tripeptide aminopeptidase, EC 3.4.11.4) (Adams *et al.*, 1952), dipeptidylaminopeptidases II (dipeptidyl peptidase II, EC 3.4.14.2) (Pontremoli *et al.*, 1980) and III (dipeptidyl peptidase III) (Vitale *et al.*, 1981), *N*-acetylalanine aminopeptidase (Schönberger & Tschesche, 1981) and prolidase (proline dipeptidase, EC 3.4.13.9) (Endo *et al.*, 1982).

The physiological roles of these enzymes remain to be elucidated. There is no protein turnover in the red cell (Yoshikawa, 1974), hence typical peptide

Abbreviation used: DSS, sodium 2,2-dimethyl-2-silapentane 5-sulphonate.

substrates for these enzymes are not normally products of erythrocyte metabolism. It is likely that peptidases have an important physiological role in the erythrocyte only if an efficient membrane transport system for their substrates exists. A peptide-transport system has only recently been detected in mature human erythrocytes (King *et al.*, 1983).

Prolidase is an extremely specific exopeptidase, cleaving only dipeptides with a C-terminal proline or hydroxyproline residue (Adams & Smith, 1952). It is found in leucocytes (Haschen & Krug, 1966), brain (Hui & Lajtha, 1978), intestine (Heizer & Laster, 1969) and kidney (Davis & Smith, 1957), as well as in erythrocytes. Prolidase deficiency is a rare disorder inherited through an autosomal recessive trait and is characterized by a general clinical syndrome consisting of chronic skin

ulceration, moderate mental retardation, splenomegaly, recurrent respiratory-tract infection and iminodipeptiduria (Arata *et al.*, 1979; Ogata *et al.*, 1981). The role of deficient prolidase and the relative importance of the deficiency of the enzyme in each tissue in the aetiology of these clinical signs is at present unknown.

We have shown previously that proton spin-echo n.m.r. can be used to study the kinetics of dipeptidase activity *in situ* in intact human erythrocytes or haemolysates, and that the rate of influx of peptide substrates into intact cells can also be monitored (King *et al.*, 1983). By using an extension of this method we show that human erythrocyte prolidase displays simple Michaelis-Menten kinetics *in situ* and that substrates enter the cell through a saturable membrane transport system that can also be described by Michaelis-Menten kinetics. The coupled transport-enzyme system was computer-simulated and is discussed with regard to the physiological functions of human erythrocyte prolidase and its possible role in the clinical manifestations of prolidase deficiency.

Experimental

Materials

Nicotinamide was from Calbiochem, San Diego, CA, U.S.A. [2,3,4,6,6-²H]Glucose and DSS were from Merck, Sharp and Dohme, Point Claire-Dorval, Que., Canada. Glycine, glycyl-L-proline and L-proline were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Pyrogen-free cottonwool was supplied by Tuta Laboratories Pty. Ltd., Lane Cove, N.S.W., Australia. All other reagents were AR grade. Carbogen was from Commonwealth Industrial Gases, Alexandria, N.S.W., Australia.

Preparation of erythrocytes

Human erythrocytes were prepared from freshly drawn venous blood by repeated centrifugal washing with 3 vol. of ice-cold iso-osmotic saline (York *et al.*, 1982). Packed erythrocytes were then filtered through a cottonwool column (2 cm × 6 cm) which had been pre-equilibrated at room temperature with washing solution (0.9% NaCl). Absence of leucocytes was confirmed by a standard leucocyte-chamber-counting technique (Dacie & Lewis, 1975). Cells were repacked by centrifugation, then washed twice in Krebs bicarbonate buffer (Krebs & Henseleit, 1932) which had been gassed with Carbogen (O₂/CO₂, 19:1). The haematocrits of the cell suspensions were always between 0.75 and 0.85. Haemolysates were prepared by a single freeze-thaw cycle (−12°C/25°C).

Before ¹H-n.m.r. measurements, haemolysates

(430 μl) or cell suspensions (440 μl) were placed into 5 mm-outer-diameter n.m.r. tubes and heated to 37°C. Cellular energy requirements for the duration of the n.m.r. experiment were maintained by addition to cell suspensions of 20 μl of 0.25 M-[2,3,4,6,6-²H]glucose. To haemolysates were added 10 μl of the same solution plus 10 μl of 0.5 M-nicotinamide to competitively inhibit NAD(P)⁺ glycohydrolase [NAD(P)⁺ nucleosidase, EC 3.2.2.5] and thus prevent depletion of nicotinamide nucleotides (Kuchel *et al.*, 1982).

Kinetic measurements were made as follows: substrates were added from concentrated stock solutions to n.m.r. tubes to give the desired final concentration. The pH of all stock solutions was adjusted to between 7.2 and 7.4. Krebs bicarbonate buffer was subsequently added so that all tubes achieved a final volume of 500 μl; after thorough mixing, the tubes were inserted into the spectrometer. Zero reaction time was taken as the time of substrate addition and n.m.r. measurements were normally begun within 3 min.

N.m.r. measurements

A small time (~2.0 min) was allowed for thermal equilibration of the sample in the spectrometer before spectral acquisition. ¹H-n.m.r. spectra were recorded at 37°C and 400 MHz in the pulsed Fourier-transform mode with a Bruker WM 400 spectrometer. The spin-echo pulse sequence with 60 ms delay times (Brown *et al.*, 1977) was used with a low-power water saturation pulse of 1.0 s duration for cell suspensions and 0.5 s for lysates.

For measurement of prolidase activity in haemolysates, 20 consecutive spectra consisting of 32 transients each were acquired. The repetition time was 1.44 s; thus spectra were obtained every 46 s. For the transport experiments, nine spectra, each consisting of 128 transients, were obtained for each substrate concentration. These spectra were not accumulated consecutively; because of the extended time course, tubes were cycled so that consecutive spectra for a particular concentration were obtained ~40 min apart. Spectra were accumulated in 8 kilobytes of memory with a spectral width of 5 kHz, and processed by using 1 Hz line-broadening. DSS (0.5% in ²H₂O), which was present in a small capillary within the n.m.r. tube, was used as the chemical shift reference at 0.000 p.p.m.

Glycine concentrations were determined from the intensity ratio of the glycine H^α resonance to the DSS resonance (lysates) or the ergothioneine *N*-methyl resonance (cells) by using calibration curves. These were prepared by addition of known amounts of glycine to a lysate (Beilharz *et al.*, 1983).

Numerical methods

Linear regression of a straight line or polynomial was performed on a Tektronix 4052 graphics computer programmed in BASIC. Weighted linear regression of the Lineweaver-Burk equation on to data was used with v_{0i}^4/σ_i^2 as weights, where v_{0i} is the initial velocity and σ_i its s.d. (Roberts, 1977).

Non-linear regression was performed on a CYBER 720 computer using a program developed by Miller (1981), which is based on a modification of the Levenberg-Morrison-Marquardt algorithm (Osborne, 1976).

Numerical integration for the biochemical simulations was performed by using the program BIOSSIM (Roman & Garfinkel, 1978), which was obtained from the SHARE program library, Triangle Universities Computation Centre, Research Triangle Park, NC, U.S.A. This program converts the chemical reaction scheme of a given model into the corresponding set of differential equations and solves them by using the advanced predictor-corrector integration algorithm described by Gear (1971).

Simulations were executed on the CYBER 720 computer in FORTRAN IV and outputs plotted using the Tektronix 4052 graphics computer.

Results

Interpretation of ^1H -n.m.r. spectra

Figs. 1(a) and 1(b) show the homogated spin-echo (h.g.s.e.) proton-n.m.r. spectra of aqueous solutions of glycyl-L-proline and an equimolar mixture of the free amino acids L-proline and glycine respectively. In both spectra, resonances from the highly coupled protons on the imino ring have negligible amplitude, owing to phase modulation of the multiplets during the spin-echo pulse sequence (Freeman & Hill, 1975). The peptide spectrum is thus dominated by the uncoupled glycyl H^α resonance (b') at 3.92 p.p.m. and the spectrum of the amino acid mixture by the free glycine H^α resonance (b) at 3.53 p.p.m. Prolidase-catalysed hydrolysis of glycyl-L-proline can thus be readily monitored by the decrease in amplitude of the glycyl H^α resonance and the concomitant increase in amplitude of the free glycine H^α resonance. This highlights the efficacy of the ^1H -n.m.r. technique to monitor simultaneously both substrates and products of peptidases and other metabolic reactions; other similar cases have been reported previously (King *et al.*, 1983; Kuchel *et al.*, 1984).

Fig. 2 shows a typical series of spectra obtained when a lysate of human erythrocytes (haematocrit = 0.77) was incubated with glycyl-L-proline (6.2 mmol/litre of cell water). The decrease in amplitude of the glycyl H^α resonance (a) and the

Fig. 1. ^1H -n.m.r. homogated spin-echo spectra of aqueous solutions (100 mmol/litre) of (a) glycyl-L-proline and (b) glycine and L-proline

N.m.r.: 128 transients/spectrum in 16384 (16 kilobyte) data locations, 0.5 s homogated water saturation pulse. Spectral assignments: a, proline H^α ; b, glycine H^α ; c, proline H^δ ; d, proline H^β ; e, proline H^γ ; b', glycyl H^α . In this and succeeding Figures, chemical shifts (δ) are shown relative to DSS ($\delta = 0.000$ p.p.m.).

simultaneous rise in amplitude of the free glycine H^α resonance (d) is evidence of prolidase activity. Assignment of other relevant haemolysate resonances are given in the legend to Fig. 2 and are in accordance with those of Kuchel (1981). The small 1 Hz line-broadening [cf. 20 Hz used by Simpson *et al.* (1982) in studies on erythrocytes] was necessary to resolve the glycyl H^α resonance (a) from that of the creatine methylene (b).

Fig. 3 shows a series of spectra obtained when a suspension of intact erythrocytes (haematocrit = 0.76) was incubated with glycyl-L-proline (2.3 mmol/litre of extracellular fluid). Note the time course is far more protracted compared with hydrolysis in a haemolysate (Fig. 2). This result indicates that flux of substrate across the membrane is the rate-limiting step during hydrolysis of glycyl-L-proline by intact cells. Since the rate of hydrolysis in the haemolysate appeared to be at least two orders of magnitude higher than in intact cells, it can be considered that the rate of appearance of free glycine in intact cell suspensions is a good approximation to the rate of substrate influx.

The reduction in the amplitude of the H^5 resonance of the [2,3,4,6,6- ^2H]glucose (d) during the ex-

Fig. 2. ^1H -n.m.r. homogenated spin-echo spectra of a haemolysate (haematocrit = 0.77) to which glycyl-L-proline ($5\ \mu\text{l}$ of a $0.496\ \text{mol/litre}$ solution) was added at zero time. Other experimental conditions were as explained in the text. Spectral assignments: a, glycyl H^α ; b, creatine methylene protons; c, glycyl H^α of glutathione; d, glycine H^α ; e, N -methyl protons of ergothioneine.

Fig. 3. ^1H -n.m.r. homogenated spin-echo spectra of a suspension of intact human erythrocytes (haematocrit = 0.76) to which glycyl-L-proline ($2\ \mu\text{l}$ of a $0.200\ \text{mol/litre}$ solution) was added at zero time.

Other experimental conditions were as explained in the text. Spectral assignments: a, glycyl H^α ; b, creatine methylene protons; c, glycine H^α ; d, H^5 of $[2,3,4,6,6\text{-}^2\text{H}]\text{glucose}$; e, pyruvate H^β .

tended time course indicates that there was considerable glucose consumption. This resulted in the production of a large amount of lactate and consequently a resonance due to the methyl protons of pyruvate (f) increased in amplitude as a result of the lactate dehydrogenase-catalysed equilibrium.

Kinetic analysis of results

Plots of the amplitude of the free glycine H^α resonance versus time were used to estimate initial velocities of hydrolysis of glycyl-L-proline in lysates or cell suspensions. For lysates this was achieved by regression of the most appropriate

Fig. 4. Concentration-dependence of the rate of glycyl-L-proline hydrolysis by a lysate of human erythrocytes at 37°C .

The continuous line is the Michaelis plot obtained by using the parameters from regression analysis. Error bars denote 1 S.D.

Fig. 5. Concentration-dependence of the rate of glycyl-L-proline hydrolysis by a suspension of human erythrocytes at 37°C .

The continuous line is the Michaelis plot obtained by using the parameters from regression analysis. Error bars denote 1 S.D.

low-degree polynomial (i.e. lowest variance of parameters, or absence of systematic errors as evidenced by a plot of residuals versus independent variable) on to the product-versus-time data. The initial velocity was subsequently obtained from the first derivative of the polynomial at zero time.

Product-versus-time curves from experiments with intact cells were linear over a large extent of the reaction. Initial velocities were thus obtained from regression of a straight line on to the data.

Figs. 4 and 5 show the dependence of the initial rate of free glycine release on the glycyl-L-proline concentration in haemolysates and intact cells respectively. Note that the glycyl-L-proline concentrations are expressed differently in each diagram and that the ordinate scale is two orders of magnitude higher in Fig. 4. Both data sets were fitted by weighted linear regression using the Lineweaver-Burk equation or non-linear regression using the Michaelis-Menten equation. Parameter estimates by these methods were routinely within 3% (K_m) or 1% (V_{max}) of each other. The steady-state kinetic parameters obtained from the linear-regression analysis are shown in Table 1.

Simulation of coupled enzyme system

The coupled glycyl-L-proline-transporter-prolidase-enzyme system was computer-modelled by using the BIOSSIM program (see under

'Numerical methods' in the Experimental section) so that the response of the system to various physiological conditions could be predicted. For the purposes of the model the glycyl-L-proline transporter and prolidase were considered to be single-intermediate, irreversible, Michaelis-Menten-type systems catalysing the consecutive reactions. Product inhibition was not incorporated into the computer model, since this was not evident from analysis of progress curves using the integrated Michaelis-Menten equation. A set of unitary rate constants consistent with the experimentally derived kinetic parameters (Table 1) were obtained by setting all 'on' rate constants to $1 \times 10^6 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$ and assuming the concentrations of transporter and prolidase to be 1.0 nmol/litre and $1.0 \mu\text{mol/litre}$ respectively. The transport-hydrolysis scheme is depicted in Fig. 6, with the rate constants given in the legend.

Fig. 7 compares the experimental data with computer simulations using the derived kinetic parameters. Only three different concentrations of glycine are shown here, but it can be seen that the model of the coupled enzyme system accurately simulates the experimental data over the entire time course. It was thus considered reasonable to use the model to predict the response of the system to physiological concentrations of glycyl-L-proline (micromolar range), which are not detectable by n.m.r.

The simulations (see Figs. 7 and 8) reinforce two aspects of the method of analysis: (a) the intracellular glycyl-L-proline concentration reaches only a small steady-state value, confirming the assumption that intracellular hydrolysis virtually instantaneously succeeds transport of peptide across the membrane; thus the production of free glycine accurately reflects the transport rate. (b) The hydrolysis of glycyl-L-proline and the production of glycine and L-proline, at experimental concentra-

Table 1. *Michaelis-Menten kinetic parameters for hydrolysis of glycyl-L-proline by human erythrocyte lysates and whole cells*

Sample	V_{max} (mmol/h per litre of cell water)	Michaelis constant (mmol/litre)
Haemolysate	130 ± 10	14.1 ± 2.4
Intact erythrocytes	0.997 ± 0.026	4.7 ± 0.4

Fig. 6. *Chemical reaction scheme used for computer simulation of the coupled transport-hydrolysis system.*

The abbreviations of the various species are as follows: Intracellular: GP_i , glycyl-L-proline; G_i , glycine; P_i , proline; X_2 , prolidase-iminodipeptide complex; P , free prolidase. Extracellular: GP_0 , glycyl-L-proline. Membrane: X_1 , transport protein-iminodipeptide complex; C , free transport protein. The values of the parameters were calculated as explained in the text: k_{+1} , $1.00 \times 10^6 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$; k_{-1} , $4.44 \times 10^3 \text{ s}^{-1}$; K_{+2} , 277 s^{-1} ; k_{+3} , $1.00 \times 10^6 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$; k_{-3} , $1.41 \times 10^4 \text{ s}^{-1}$; $[C]_0$, $1.00 \times 10^{-9} \text{ mol} \cdot \text{litre}^{-1}$; $[P]_0$, $1.00 \times 10^{-6} \text{ mol} \cdot \text{litre}^{-1}$. This consistent set of unitary rate constants yielded $K_t = 4.7 \text{ mmol} \cdot \text{litre}^{-1}$, V_{max} (for transport) = $2.77 \times 10^{-4} \text{ mmol} \cdot \text{litre}^{-1} \cdot \text{s}^{-1}$, $K_m = 14.1 \text{ mmol} \cdot \text{litre}^{-1}$ and V_{max} (for hydrolysis) = $3.51 \times 10^{-2} \text{ mmol} \cdot \text{litre}^{-1} \cdot \text{s}^{-1}$.

Fig. 7. Free glycine concentration as a function of time for three different experimental extracellular concentrations of glycyl-L-proline

Glycyl-L-proline concentrations were: ■, 19.4 mmol/litre; ▲, 10.3 mmol/litre; ●, 4.56 mmol/litre. The computer simulations using the reaction scheme of Fig. 6 are shown by continuous lines.

Fig. 8. Computer simulation (using the scheme of Fig. 6) of the physiological response of human erythrocytes to an extracellular glycyl-L-proline concentration of 10 $\mu\text{mol/litre}$, assuming the dipeptide is not metabolized elsewhere. Chemical species are: a, extracellular glycyl-L-proline; b, intracellular glycine; c, intracellular glycyl-L-proline.

tions of the dipeptide, are linear with time, confirming regression of a straight line on to the data as an appropriate method of rate analysis.

Discussion

Human erythrocyte prolidase was only quite recently isolated in a homogeneous form and physicochemically characterized (Endo *et al.*, 1982). Until now, no detailed measurements of the kinetics of the enzyme either *in vitro* or *in situ* had been undertaken. Furthermore, the permeability of red cells to iminodipeptides and the process by which they entered the red cell was unknown.

The results presented here indicate that glycyl-L-proline enters the intact human erythrocyte via a saturable membrane-transport system. This system displays Michaelis-Menten kinetics with a Michaelis constant (K_t) of 4.7 ± 0.4 mmol/litre and a $V_{\max}$ of 0.997 ± 0.026 mmol of glycyl-L-proline transported/h per litre of cell water. The K_t is significantly higher than for similar transport systems in the small intestine and kidney of the rabbit, which exhibit apparent Michaelis constants for glycyl-L-proline of 0.9 and 1.1 mmol/litre respectively (Ganapathy *et al.*, 1981b). To our knowledge this is the first demonstration of a saturable peptide transport system in the human erythrocyte.

Glycine uptake by human erythrocytes consists of two components (Winter & Christensen, 1964): a low-capacity saturable system describable by Michaelis-Menten kinetics ($K_t = 0.3$ mmol/litre and $V_{\max} = 1.2 \mu\text{mol/min}$ per litre of cell water) and a high-capacity non-saturable system described by an apparent diffusion coefficient ($K_D = 0.004/\text{min}$). From these values it can be calculated that, for a plasma glycine concentration of 0.34 mmol/litre (the mean level in human adults; Dickinson *et al.*, 1965), the total flux of glycine into human red cells is $\sim 2.0 \mu\text{mol/min}$ per litre of cell water (i.e. $\sim 1.36 \mu\text{mol/min}$ per litre of cell water by the non-saturable component and $\sim 0.64 \mu\text{mol/min}$ per litre of cell water by the saturable system). On the basis of the present work, for a similar concentration the rate of uptake of glycyl-L-proline would be $\sim 1.1 \mu\text{mol/min}$ per litre of cell water. Absorption of glycine by human erythrocytes is thus slightly more rapid for the free amino acid than when it is in the form of an iminodipeptide. This is unlike the phenomenon of increased rates of glycine absorption by human intestinal epithelium, when the glycine is in the form of a dipeptide (for a review, see Matthews, 1975).

Although complete kinetic data are not available for L-proline uptake by human erythrocytes, the rate of uptake at a plasma concentration of 1.0 mmol/litre was shown to be $21.4 \mu\text{mol/min}$ per litre of cell water (Young & Ellory, 1977). Since the rate of glycyl-L-proline transport at this concentration can be calculated from our data to be $2.9 \mu\text{mol/min}$ per litre of cell water, it appears that L-proline uptake by human red cells is, as with glycine, more rapid when it is in the free form.

Hydrolysis of glycyl-L-proline in haemolysates obeyed Michaelis-Menten kinetics, with a K_m of 14.1 ± 2.4 mmol/litre and a $V_{\max}$ of 130 ± 10 mmol hydrolysed/h per litre of cell water. Comparison with the kinetic parameters for transport indicates that glycyl-L-proline, at saturating concentrations for the enzyme, is hydrolysed ~ 130 times slower by intact cells than by haemolysates. This rate estimate for whole cells is significantly greater than

the 1000-fold difference between the rate in whole cells and lysates reported by Endo *et al.* (1982). A possible explanation for the large difference in the two sets of results is that Endo *et al.* (1982) may have underestimated the extent of hydrolysis in whole cells by not allowing for the efflux of L-proline during their extended incubation (1 h) and washing period (~0.5 h). On the other hand, the total (intracellular and extracellular) glycine concentration was monitored in our proton-n.m.r. experiments.

An alternative suggestion for the very different observed rates of hydrolysis in cells and lysates might be that there is a change in catalytic behaviour of the enzyme on lysis rather than from rate-limiting transport. However, we consider this to be unlikely, firstly because haemolysates were prepared from cell suspensions of high haematocrit, ensuring that their ionic strength and composition would be very similar to that of the intracellular milieu (especially since human red cells lack organelles). Secondly, the macromolecular organization of prolidase, which is known to be cytosolic, is unlikely to be significant, since minimal heterogeneous association with the major cytosolic protein, haemoglobin, is indicated by their ready separation by ion-exchange chromatography at pH 7.0 (Endo *et al.*, 1982).

Glycyl-L-proline is one of the three major iminodipeptides excreted in excess in the urine of patients with prolidase deficiency (Isemura *et al.*, 1979). Goodman *et al.* (1968) detected the dipeptide in the plasma under such conditions, but the concentrations were not reported. The restoration of ~35% of normal peripheral erythrocyte prolidase activity by infusion of normal erythrocytes into a prolidase-deficient person led to no reduction in urinary iminodipeptides (Endo *et al.*, 1982).

The latter result is somewhat surprising, since simulations based on the kinetic data presented here clearly show that physiological concentrations of glycyl-L-proline are rapidly absorbed and metabolized by circulating red blood cells (Fig. 8). The role of the kidney in the metabolism of plasma iminodipeptides in humans is unclear. Ganapathy *et al.* (1981a,b) have fairly recently shown that rabbit renal brush-border vesicles possess a saturable glycyl-L-proline transport system with a K_t of 1.1 mmol/litre and a V_{max} of 0.81 nmol/min per mg of protein. Assuming that the human kidney displays similar transport kinetics and that a normal human adult has a total kidney mass of 300 g consisting of ~0.4% proximal-tubule membrane protein, then it can be calculated that human erythrocytes have a significantly larger glycyl-L-proline influx than do the kidneys; an alteration in the amount of erythrocyte enzyme should thus lead to a change in urinary prolyl-peptide levels.

Absence of *erythrocyte* prolidase may thus be a contributing factor in the aetiology of the clinical manifestations of prolidase deficiency, more so than a deficiency in the kidney enzyme. As suggested by Ogata *et al.* (1981), the dermatological manifestations are most probably linked to deficient recycling of L-proline for collagen biosynthesis; this may be imposed in part by a deficiency in red-blood-cell prolidase.

The glycyl-L-proline transport system needs further study to determine whether it represents a common route for influx of iminodipeptides into the human erythrocyte. Larger, and perhaps biologically active, C-terminal prolyl-peptides [such as thyrotropin-releasing hormone (thyroliberin)] may also enter the red cell via this transport system. In view of this, and perhaps other peptide transporters, existing in the mature human erythrocyte, it is possible that the principal role of the amino-acid-transport systems is not to enable influx of amino acids *into* a cell that cannot utilize them, but rather the *efflux* of amino acids resulting from the hydrolysis of absorbed peptides.

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