AMINO ACID TRANSPORT INTO WOOL FOLLICLES

N. WILSON, P.I. HYND and D.R. TIVEY

Dept of Animal Science, University of Adelaide Waite Campus, Glen Osmond, S.A. SO64

It is well-recognised that wool growth is limited by the supply of amino acids to the follicle (Reis 1970) but to date no studies have been conducted into the mechanisms operating to transport the amino acids into the cells of the follicle.

To localise sites of amino acid uptake, skin strips (lmm x 10mm) were incubated in Krebs/Ringer phosphate buffer (3 10m OsM) containing ^{35}S-cysteine or ^{3}H-alanine, -lysine, or -leucine at 37°C in a humidified atmosphere of 95% air/5% CO_2 for 60 mins. After extensive washing in 5% TCA (4°C), strips were fixed in buffered formalin, embedded in paraffin, sectioned longitudinal to the follicles (8µm) and coated in gel film emulsion (Ilford LA). After appropriate exposure time (3-21 days), the films were developed and the sections stained using the Sacpic procedure. To provide preliminary information on the kinetic parameters for uptake of the labelled amino acids, skin strips (n = 6/treatment) were incubated (2h) in a range of concentrations of cold amino acid (0.005-15mM) and 185MBq of labelled amino acid/mL buffer. Uptake was quantified by scintillation counting. Saturable (Km, Vmax) and non-saturable (Md) parameters were estimated by analysis of the Michaelis Menten curves fitted to the data using a curve-fitting programme.

Cysteine was almost exclusively taken up by cells in the keratogenous zone of the follicle as has been previously shown. Lysine appeared predominantly in the lowermost cells of the germinative zone of the bulb and in the inner root sheath, in line with its role in histone and trichohyalin synthesis. Leucine was predominantly taken up in the germinative region of the bulb, the keratogenous zone and the inner root sheath, in keeping with the high leucine content of both wool and inner root sheath proteins. Alanine was evenly distributed throughout the follicle, in both the germinative bulb cells and the keratogenous zone. No radioactivity was ever detected in the dermal papilla.

Table 1. Kinetic parameters for amino acid uptake into cultured skin (means ± SEM)

Substrate	Km (mM)	Vmax (nmols/g.minute)	Kd (nmols/g.mM)
L-leucine	0.04±0.001	1.30±0.033	0.52
L-alanine	0.48±0.051	0.07±0.043	0.17
L-cysteine	1.01±0.442	3.54±0.384	0.03
L-lysine	0.50[A]	0.60[A]	0.15

[A] Approximate only, because there was evidence of multi site binding of this amino acid.

The smooth rectangular hyperbola fitted to the saturable component of the curves for alanine, cysteine and leucine is consistent with the operation of a single transporter for each of these amino acids. Leucine enters the follicle by a high affinity, high capacity transporter as well as diffusion, in contrast to alanine which enters by diffusion and a low affinity, low capacity carrier. Cysteine was transported by a low affinity, high capacity carrier with little diffusion, while lysine appeared to be transported by a low affinity, low capacity carrier.

The results strongly support the use of cultured skin as a means of investigating the kinetics of amino acid uptake into wool follicles. Not only were the sites of uptake consistent with those anticipated from cell function in different regions, but also the kinetic parameters were consistent with those likely to operate within the follicle. Some of the genetic differences in fibre growth may reside in differential expression of carrier transport proteins.

REIS, P.J. (1970). *Aust. J. Biol. Sci.* 23: 441-6.