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pH-profile of cystine and glutamate transport in normal and cystinotic human fibroblasts

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In the human recessive condition cystinosis, cystine transport has been reported to be normal in the plasma membrane but defective in the lysosome membrane. A possible explanation is that the transport systems at the two cellular sites are identical and that the defect in cystinosis affects the porter's ability to operate at the low pH of the lysosome. To test this hypothesis the uptake of ^{3}H-labelled cystine and glutamate by normal and cystinotic human skin fibroblasts has been measured in vitro at pH 5.8, 6.5, 7.0, 7.4 and 8.0. Uptake of glutamate was more rapid than that of cystine. Uptake of cystine increased with increasing pH, but uptake of glutamate showed no marked pH-dependence. Transport in cystinotic cells was similar to that in normal cells, and similarly affected by pH. This finding is incompatible with the hypothesis proposed above. It is concluded that the cystine porters of the plasma membrane and the lysosome membrane are probably genetically distinct.

Cystinosis is a recessively inherited human metabolic disorder characterized biochemically by abnormally high levels of free cystine in the lysosomes of many body cells. Recent evidence indicates that the lysosome membrane of normal cells contains a cystine transport system and that this porter is deficient in cystinotic cells (for a review, see Ref. 1).

The plasma membrane also contains a transport system for cystine. Cystine transport into human fibroblasts has been extensively studied [2–4], the same porter mediating the entry of L-cystine and L-glutamate, which show mutual competition. Uptake of cystine, but not of glutamate, increases with pH in the pH range 5.8–8.0.

Cystine uptake by cystinotic cells has been studied in peripheral leucocytes [5], lymphoblasts [6], and skin fibroblasts [7]. No consistent differences from normal cells were apparent. This may be because the cystine porters in the plasma membrane and the lysosome membrane are genetically distinct, but an alternative explanation is possible. The two porters could be identical or related, and the defect in cystinosis an alteration in the pH-characteristics. If the mutation led to a loss of activity at low pH, the porter might be ineffective in the lysosome membrane but operate normally in the plasma membrane.

We report here the effect of pH on the uptake of cystine and glutamate by human skin fibroblasts from two normal control patients and a patient with nephropathic cystinosis. Cells were maintained in culture as described previously [8] and experiments were performed on confluent cells between the 6th and 17th doublings. Cells were trypsinized and subcultured in flat-bottomed wells, growing area 2 cm^2, in plastic tissue-culture multidishes (Flow Laboratories Inc., Irvine, Ayrshire, U.K.). After 96 h of culture, amino acid uptake was measured by the technique of Bannai and

Kitamura [2]. Cells were rinsed three times with prewarmed (to 37°C) phosphate-buffered saline (10 mM, containing 0.9 mM $CaCl_2$, 1.1 mM $MgCl_2$, 5.6 mM glucose, 137 mM NaCl and 3.0 mM KCl) of the pH of the subsequent incubation. They were then incubated for 0.5, 1, 2 or 5 min at 37°C in 150 μl of phosphate-buffered saline containing labelled and unlabelled cystine or glutamate. L-[3,3′-^{3}H]Cystine was from Amersham International plc., Amersham, U.K.; L-[2,3-^{3}H]glutamate was from New England Nuclear, Southampton, U.K.; L-cystine and L-glutamate were from Sigma, Poole, Dorset, U.K. The pH of the incubation medium was readjusted with sodium hydroxide following addition of the amino acid, and the final concentration of amino acid was 4 μCi and 0.05 μmol/ml. Incubations were terminated by aspirating the radiolabelled incubation medium and rinsing the cells three times in ice-cold phosphate-buffered saline of the same pH. The cells were then dissolved in 150 μl of 0.5 M NaOH, and 50 μl portions assayed for radioactivity using Lumagel scintillation fluid (Lumac Systems Inc., Titusville, U.S.A.) and a Packard 2425 liquid scintillation spectrometer, and for protein content by the method of Lowry et al. [9].

The uptake of both cystine and glutamate was linear with time between 0.5 and 5 min of incubation, at each of the five pH values. Consequently it was possible to calculate a rate of uptake for each amino acid at each pH. Fig. 1 shows these rates, for two normal and one cystinotic cell lines. At all pH values the rate of uptake of glutamate by normal fibroblasts was greater than that of cystine. Uptake of glutamate showed little pH-dependence, whereas uptake of cystine increased with pH, being some four times more rapid at pH 8.0 than at pH 5.8.

The measured rates of uptake of glutamate and cystine by normal skin fibroblasts were about two-thirds and one-fifth, respectively, of the rates reported by Bannai and Kitamura [3] for normal fetal lung fibroblasts. The effects of pH on the uptake of these amino acids was broadly the same in fibroblasts from both sources. Bannai and Kitamura [4] measured the uptake of glutamate and cystine in normal human skin fibroblasts at pH 7.4, their reported rates averaging 0.67 and 0.21 nmol/mg protein per min, respectively. The

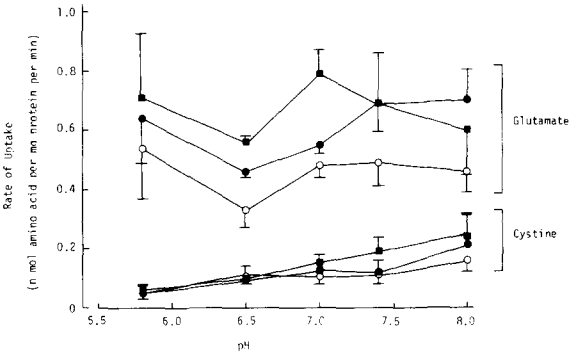

Fig. 1. Effect of pH on the rate of uptake of glutamate and cystine by normal and cystinotic fibroblasts. Fibroblasts from one cystinotic (○) and two normal (●, ■) cell lines were each incubated in medium containing [^{3}H]glutamate and 0.05 μmol unlabelled glutamate per ml, or [^{3}H]cystine and 0.05 μmol unlabelled cystine per ml. Duplicate incubations of between 0.5 and 5 min were conducted at each of five different pH values. Rates of uptake from two to four experiments, (mean ± S.E.) are expressed as nmol of amino acid detected per mg fibroblast protein per min.

data in Fig. 1 are fully compatible with these values.

There was no difference between normal and cystinotic skin fibroblasts in their ability to take up glutamate and cystine (Fig. 1). This confirms and extends earlier indications that cystine uptake is not abnormal in cystinotic cells. The new finding in the present investigation is that the plasma membrane cystine porter is not defective in cystinotic fibroblasts at low pH. It is therefore no longer possible to argue that the cystine porters in the lysosomal and the plasma membranes are identical and that the defect in cystinosis is merely in the porter's capacity to operate at low pH.

One must conclude either that the cystine porters in the lysosome membrane and the plasma membrane are genetically distinct, or that they are identical and the mutation in cystinosis one that adversely affects insertion into the lysosome membrane. Although the plasma membrane of cystinotic cells contains a cystine porter shown to be capable of operating at low pH, the massive accumulation of cystine in the lysosomes of such cells indicates that incorporation of plasma membrane into the vacuolar system (by pinocytosis) fails to lead to the presence in the lysosome membrane of a functional plasma membrane-derived

cystine porter. This is more readily explicable if the two porters are genetically distinct. Observations on the substrate specificity of the cystine porter in leucocyte lysosomes [10] also support this conclusion.

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