ASPARAGINE METABOLISM IN THE BOVINE LENS: A COMPARISON WITH THE METABOLISM OF ASPARTIC ACID

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1. Introduction

The vertebrate lens is a relatively isolated tissue; it has no blood supply and is dependent upon the aqueous humour, a fluid secreted by the ciliary body, for its nutrition. Recent investigations have shown that the lens is able to take up, incorporate into protein, and catabolise a wide range of amino acids, and that amino acids may make a significant contribution to its energy needs [1,2]. Of the amino acids that have been studied the two dicarboxylic ones, glutamic and aspartic acids, appear to be the least readily taken up. In the lens, as in most tissues, there is a barrier to the entry of these amino acids [3]. The amide of glutamic acid, glutamine, has been shown to be the major source of the parent acid; it is more readily taken up and inside the lens it is largely deamidated to glutamate [2,4]. Animal tissues are, in general, more permeable to glutamine than to glutamate [5,6], but much less is known of the relationship between aspartic acid and its amide, asparagine.

The purpose of the present study was to investigate the metabolism of asparagine in the lens and to compare its metabolism with that of aspartate. The results indicate that asparagine is readily taken up and metabolised by the tissue, and that there appears to be a difference in the metabolism of exogenously and endogenously derived aspartate.

2. Materials and methods

Fresh adult bovine lenses (wet wt 1.9 to 2.1 g) were incubated aerobically for 22 hr at 35°C in Merriam and Kinsey lens culture tubes [7]. Each lens was incubated in 15 ml of a medium consisting of a modified Krebs-Ringer phosphate solution, 10 mM glucose, antibiotics, and 5 μCi of L-[U-^{14}C]asparagine [1]. The L-[U-^{14}C]asparagine (obtained from the Radiochemical Centre, Amersham, Bucks, U.K.) the radiochemical purity of which was checked and found to be $>99\%$, was used at the specific activity supplied, namely 151 mCi/mmol. After the incubation period radioactivity was collected and measured in CO_2, protein and trichloroacetic acid-soluble material as described previously [1].

3. Results

The uptake and distribution of radioactivity in CO_2, protein and trichloroacetic acid-soluble material are given in table 1. The results obtained in earlier experiments with [^{14}C]aspartate are included in the table for comparison. The radiochemical concentration of the two amino acids was identical (5 μCi/15 ml of medium) and the molar concentration was similar (2.2 mM for asparagine and 1.5 mM for aspartate). At the tracer levels employed, asparagine was readily taken up by the lens – much more readily than aspartate. Over one half of the radioactivity of the asparagine taken up was incorporated into protein and one seventh metabolised to CO_2, the remainder being present in the acid-soluble fraction (table 1). In percentage terms, less CO_2 was produced from asparagine than from aspartate, although the converse is true when the results are expressed in absolute amounts of radioactivity.

Nearly two thirds of the total radioactivity in the acid-soluble material was due to unchanged

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Table 1
The uptake and metabolism of L-[U-^{14}C]asparagine to CO_2, protein and trichloroacetic acid-soluble material

	Asparagine	Aspartic acid*
CO_2 (c.p.s./g of lens)	327 ± 27	126 ± 11
Trichloroacetic acid-soluble material (c.p.s./g of lens).	617 ± 47	94 ± 11
Protein (c.p.s./g of lens)	1466 ± 76	21 ± 5
Total radioactivity taken up (c.p.s./g of lens)	2410 ± 90	241 ± 19
Percent uptake by the whole lens	54.2 ± 2.6	5.3 ± 0.7
Percent uptake as CO_2	13.6 ± 1.2	52 ± 3
Percent uptake as trichloroacetic acid-soluble	25.6 ± 1.4	39 ± 4
Percent uptake as protein	60.9 ± 2.3	8.7 ± 1.5

The results for asparagine are the means ± the S.D. for four lenses and are adjusted to a value for the initial radioactivity in the medium of 9000 c.p.s./15 ml. The recovery of radioactivity from asparagine was > 95%; this is defined as the sum of radioactivity found after incubation in CO_2, the medium, protein, and trichloroacetic acid-soluble material, as a percentage of that initially in the medium.
* Data from [1].

asparagine (table 2). The major metabolite was glutamate, which was formed like the CO_2, by the entry of aspartate (presumably derived from asparagine by the action of an asparaginase) into the citric acid cycle. A small amount of the glutamate was incorporated into glutathione (table 2). Very little radioactive aspartate was present.

Table 2
The radioactivity in each compound detected in the trichloroacetic acid-soluble fraction from lenses incubated with [^{14}C]asparagine

	Asparagine	Aspartate*
Asparagine	62 ± 4	not detected
Aspartate	6 ± 4	14 ± 2
Glutamate	28 ± 7	43 ± 6
Glutathione (GSH and GSSG)	3 ± 1	21 ± 3
Glutamine	not detected	Trace
Proline	not detected	Trace
Alanine	not detected	15 ± 1
Lactate	not detected	7 ± 7
c.p.s./g of lens in the acid-soluble fraction	617 ± 47	94 ± 11

The results are expressed as a percentage of the total radioactivity in the trichloroacetic acid-soluble fraction and are the means ± the S.D. for three lenses.
* Data from [1].

The large incorporation of radioactivity from asparagine into protein was in contrast to the low incorporation from aspartate, both as a percentage of the uptake and in absolute amounts of radioactivity (table 1). An experiment was therefore done to see whether all the radioactivity in the protein following incubation with asparagine was in the form of asparagine/aspartate or whether some was present as glutamate, since much glutamate had been formed (table 2). A sample of the protein was hydrolysed in 6 M HCl for 20 hr at 108°C. The hydrolysate was then separated on paper by two-dimensional electrophoresis and chromatography, as for trichloroacetic acid-soluble lens extracts, and autoradiographed. Only radioactive aspartate was detected. In addition no radioactivity from [^{14}C]asparagine was found in the lipids.

4. Discussion

It appears that the bovine lens is able to take up asparagine much more readily than its parent acid, aspartic acid, to which there is a strong barrier. Once inside the tissue asparagine is broken down to aspart-

ate which is itself subsequently metabolised to oxaloacetate, an intermediate in the citric acid cycle. It seems probable that asparagine provides some or most of the aspartate required for protein synthesis. The relationship between asparagine and aspartate parallels that pertaining in the lens with glutamine and glutamic acid [2,4]. The amount of $^{14}CO_2$ derived from asparagine suggests that, again like glutamine, it may make a contribution to the energy needs of the lens. In this regard it is of interest that the amount of $^{14}CO_2$ produced from both these amino acids was approximately doubled when glucose was omitted from the incubation medium (P. Trayhurn, unpublished work).

It is noteworthy that neither alanine, lactate, proline nor glutamine were formed with [^{14}C]asparagine as the precursor amino acid while all these compounds were produced from [^{14}C]aspartate [1]. This difference cannot be explained by differences in the size of the acid-soluble radioactive fraction for that from asparagine was several times greater than that from aspartate (table 1). On the assumption that asparagine must be deamidated to aspartate in order to label the metabolites mentioned above, exogenously and endogenously derived aspartate is clearly metabolised in different ways: this suggests that the two forms of aspartate may be handled in different compartments.

The possibility that there are two (or more) aspartate compartments in the lens is reinforced by the fact that a far greater proportion of the glutamate derived from [^{14}C]aspartate was incorporated into glutathione than was the case with glutamate derived from [^{14}C]asparagine (table 2). This in turn indicates that there are at least two glutamate compartments,one of which is much more concerned than the other with the synthesis of glutathione. The concentration of glutathione in the lens, is greater than in most tissues [8].

The compartmentation of glutamate metabolism is a well established phenomenom in the cerebral cortex (see ref [9]) where it appears that the different compartments are associated with different cell types: the same situation has recently been described in the retina [10]. In the case of the lens it is more difficult to associate different metabolic compartments with particular cell types since the tissue is composed of only one type of cell (epithelial) at various stages of differentiation. Nevertheless, it is reasonable to associate different metabolic compartments with the epithelial cells, those cells that are actively differentiating and those cells that have differentiated into mature fibres. Clear metabolic differences between them must occur because, for example, differentiation of lens cells into mature fibres is accompanied by a loss of cell organelles.

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