FEBS Letters 366 (1995) 170–172 FEBS 15590

Lack of correlation between mRNA expression and enzymatic activity of the aspartate aminotransferase isoenzymes in various tissues of the rat

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Received 24 April 1995

Abstract Little is known about control of expression of basal levels of the aspartate aminotransferases which are ubiquitous 'house keeping' enzymes in vertebrates. We have measured both mRNA and activity levels for both isoenzymes in various rat tissues as a function of age. Patterns of mRNA expression for the two isoenzymes were similar in a particular tissue about differed widely between tissues. Surprisingly, there was no simple correlation between mRNA levels and specific activities of the enzyme products. We conclude that translation for mRNA for these two isoenzymes is subject to tissue-specific, and in some cases age-related, regulation.

Key words: Aspartate aminotransferase; Isoenzyme; Enzyme regulation; mRNA regulation; mRNA expression

1. Introduction

Aspartate aminotransferase (AspAT) (EC 2.6.1.1.) exists in all tissues of vertebrates in two isoenzymic forms, one in the cytosol (cAspAT) and the other in the mitochondria (mAspAT) [1].The isoenzymes both participate in amino acid metabolism, in the link between the urea and citric acid cycles and, along with the malate dehydrogenases, in the malate-aspartate shuttle [2,3]. The cytosolic isoenzyme alone of the two is involved in the process of gluconeogenesis [4]. The two isoenzymes are both coded by the nuclear genome and synthesised in the cell cytosol after which the mitochondrial form is imported into the organelles [5]. Much work has been done on the control of regulation of expression of the cAspAT by hormones and in response to nutritional status and it has been shown that expression of the isoenzyme in liver and kidney but not in other tissues is increased by glucocorticoids consistent with its role in gluconeogenesis [6]. Conversely it seems that the mitochondrial isoenzyme is not subject to hormonal regulation [7]. Because of these tissue specific and isoenzyme specific regulatory effects it seemed to us of interest to investigate the correlation between mRNA and enzymatic activity levels in various rat tissues as a function of age. The results are reported here.

2. Materials and methods

Male Wistar rats of different ages were used. The animals were fed ad libitum on standard diet with free access to water. After sacrifice by decapitation the required tissues were excised and used immediately for enzyme assays or stored at −80°C until required for mRNA extraction. At all times RNAse-free materials were used.

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The probes used were cloned cDNAs for the isoenzymes from chicken. For mAspAT a 990 bp probe was cleaved from the recombinant plasmid pOTS-pmAspAT [8] using AvaI. For cAspAT a 1.45 kbp probe was cleaved from the recombinant plasmid pUC18-cAspAT [9] using *Nco*I and *Pst*I. The cleaved inserts were purified using a Qiaex Gel kit (Genenco) and then labelled using the random primer method with [α-^{32}P]dCTP (3,000 Ci/mmol, Amersham). Specific activities of 1×10^8 to 1×10^9 cpm/μg DNA were obtained. The human β-actin cDNA probe was obtained from the recombinant plasmid pBR322-β-actin linearised with *Eco*RI [10]

Total RNA was isolated from tissues as described by Chirgwin et al. [11] and purified by sedimentation through 5.7 M CsCl. Integrity of the product was established by electrophoresis on 1% agarose /2.2 M formaldehyde gel as described by Lehrach et al. [12]. For hybridisation, 40 μg of RNA was electrophoresed and then transferred to nitrocellulose membrane (Hybond C extra, Amersham). The membrane was dried for 2 h at 80°C under vacuum and then prehybridised for 4 h at 42°C in 50% formamide, 5 × Denhardt's (0.02% Ficoll, 0.02% polyvinylpyrolidone, 0.02% bovine serum albumin), 5 × SSPE (0.17 M NaCl, 10 mM Phosphate buffer pH 7.2, 1 mM EDTA), 0.5% sodium dodecyl sulphate containing 0.2 mg/ml denatured salmon sperm DNA. Subsequently hybridisation was carried out for 16 h at 42°C with 2–3 × 10^6 cpm/ml of appropriate probe. Filters were washed once with 2 × SSPE/0.5% SDS for 10 min at room temperature, once with 2 × SSPE/0.1% SDS for 10 min at room temperature, and finally with 0.1 × SSPE/0.5% SDS for 10 min at 37°C. After hybridisation, filters were autoradiographed at −80°C using X-Omat AR Kodak film with intensifying screen. Densitometry of the autoradiography was carried out using an LKB Ultroscan XL laser densitometer.

Differential assay of the AspAT isoenzymes was done using a method [13] that depends on the thermal instability of mAspAT at 70°C under conditions where the cAspAT is stable. Tissues were homogenised in 10 vol 250 mM sucrose, 1 mM EGTA, 20 mM Tris-HCl, pH 7.25. Triton X-100 (0.1%) and 2-oxoglutarate (3.7 mM) were added with Tris-acetate (pH 7.4) to a final concentration of 100 mM. One aliquot of this was incubated at 37°C for 15 min and another at 70°C for 15 min. The samples were centrifuged at 10,000 × g for 20 min.

Pyridoxal 5'-phosphate (0.1 mM) was added and the enzymatic activity determined at 25°C using the coupled assay of Karmen as described [14]. The activity of the sample pre-incubated at 37°C was taken to be that of both isoenzymes together, whereas that of the sample preincubated at 70°C was assumed to be due solely to the cytosolic isoenzyme [13]; the activity of the mAspAT was obtained by difference. Activities were expressed as Units (μmol of product/min) per mg of total protein. Protein concentrations were determined by the Biuret method [15].

Graphs (Figs. 2–5) were done by using GraFit (Erithacus Software) program for IBM computer.

3. Results and discussion

Availability of clones containing both the cytosolic and the mitochondrial [8,9] AspATs from chicken has allowed us to study the expression of the corresponding isoenzymes in rat tissue. Labelled probes were hybridised with total RNA obtained from rat tissues and subjected to autoradiography. The Northern blots showed bands of 2.1 and 2.4 kbp as expected for the cAspAT and mAspAT mRNAs, respectively. An example is shown in Fig. 1 for heart as a function of the age of the

SSDI 0014-5793(95)00518-8

animal. For quantitative work, the human β-actin cDNA was used as a control to allow for possible incomplete extraction of RNA and losses due to degradation or other causes; expression of this gene is essentially tissue and age independent [10]. After hybridisation and autoradiography the bands were measured by densitometry and the results expressed as a ratio of the areas of the band for the AspAT to that of β-actin. The results obtained with the probe for the mAspAT-RNA for each tissue as a function of age of the animal are given in Fig. 2 and those for the cAspAT in Fig. 3. In each case the results are expressed as a percentage of the highest value obtained for heart taken as 100% and are, in all cases, the average of three determinations.

The tissues clearly fall into two distinct groups with the same general features for both isoenzymes. In the case of heart and muscle, the expression at 4 weeks of age is low (comparable with that in the other tissues) but increases very markedly (up to 10-fold) in the period up to 10 weeks. Then, after puberty (about 10 weeks) the levels start to drop again but by 2 years have only decreased to 70–80% of their maximum levels. With liver, kidney and brain, the levels are low at the beginning and increase only slightly (brain and kidney cytosolic, brain mitochondrial) or not at all between 4 and 10 weeks; furthermore the levels do not decrease significantly in rats of 2 years of age. In terms of the relative levels of expression of the mAspAT (heart ≫ liver > brain) this order is the same as that observed in adult rats for mitochondrial malate dehydrogenase and medium chain acyl-CoA dehydrogenase [16].

It is tempting to interpret these results in terms of the requirements of the particular tissues for enzymatic activity but it turns out that this would be completely misleading as shown by the results in Figs. 4 and 5. In these figures are reported the specific activities in terms of Units/mg tissue for the two isoenzymes at corresponding ages for the same tissues. Differential assay of the two was done by making use of the heat resistance of the cytosolic form. Again the patterns for the two isoenzymes are similar but there are remarkable differences when enzyme activity is compared with mRNA expression.

In the case of heart, the increase in mRNA is mirrored in a corresponding increase in enzymic activity although the increase is proportionally somewhat greater for mAspAT.

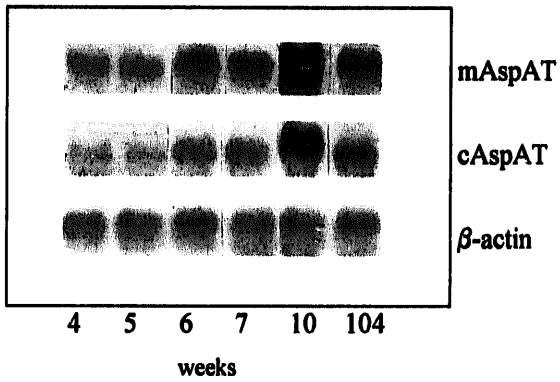

Fig. 1. Northern blot analysis of mRNA for mAspAT and cAspAT in rat heart tissue as a function of age. Total RNA (40 μg) was subjected to electrophoresis on formaldehyde gels and than transfered to nitrocellulose membranes. The membrane-bound RNA was successively hybridised to radiolabelled probes for mAspAT, cAspAT and β-actin and the membranes subjected to autoradiography. The β-actin probe was used as a control for quantitation purposes (see text).

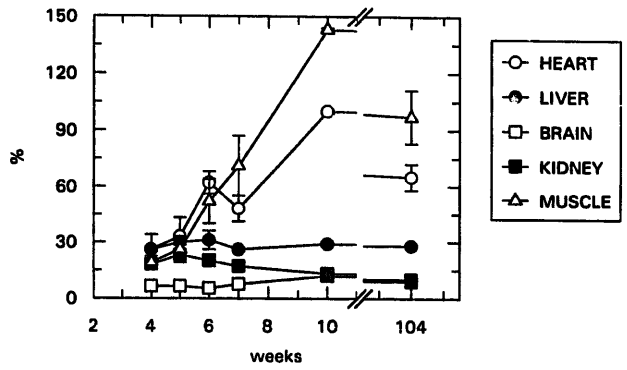

Fig. 2. Levels of mRNA for mAspAT in various rat tissues as a function of age. Autoradiographs obtained as described in Fig. 1 were subjected to densitometry and for each point the ratio was obtained of the area of the band arising from hybridization with the mAspAt probe to that from hybridisation with a probe for β-actin. This ratio was taken as a measure of the level of expression of the mRNA for mAspAT. For comparative purposes the results for all tissues and all ages are expressed as a percentage of the highest value obtained for the heart taken as 100%.

With muscle, the level of enzymatic activity was essentially constant with time in spite of the substantial increase in mRNA levels. Moreover, the specific activity values were low compared with those in heart; for example the specific activity of the mAspAT in heart reached 0.6 Units/mg compared with about 0.15 Units/mg in muscle in spite of the fact that mRNA levels were higher in muscle than in heart. With liver, the mitochondrial isoenzyme levels remained constant with age within the limits of accuracy of the determinations; in the case of the cytosolic form it is possible that there was a small decrease between 5 and 6 weeks followed by an increase but the change, if real, was small. The central point with this tissue is that levels of enzymatic activity were high for both isoenzymes, and particularly for the mitochondrial isoenzyme where the level was approximately the same as in heart, whereas reference to Figs. 2 and 3 shows that at all times the level of expression of the mRNA was constant and low, indeed with the cytosolic isoenzyme, expression of the liver mRNA was lowest of all the tissues. With the remaining tissues (kidney and brain) enzyme levels remained constant with time. The levels were relatively

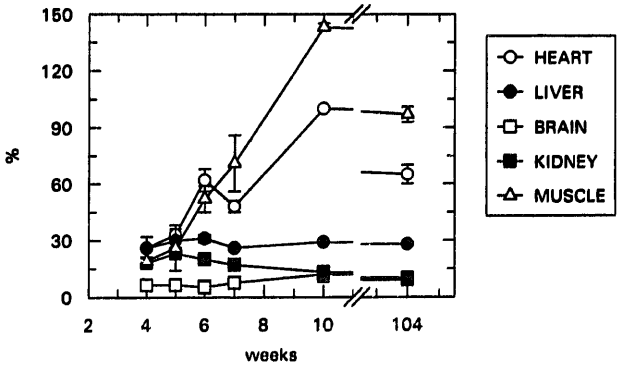

Fig. 3. Levels of mRNA for cAspAT in various rat tissues as a function of age. The experiments were carried out and the results analysed precisely as described in Fig. 2.

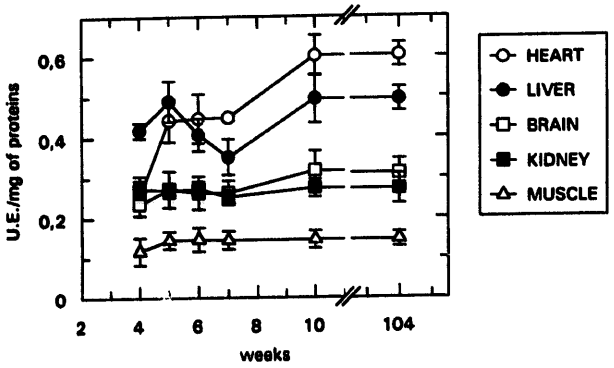

Fig. 4. Specific activity of mAspAT in various rat tissues as a function of age. Enzymatic activities were measured by the linked assay method of Karmen [14]. Assays were carried out on samples preincubated at 30°C and at 70°C for 15 min. Activity of the mitochondrial isoenzyme was taken as the difference between these two values [13]. Total soluble protein was measured using the Biuret method [15].

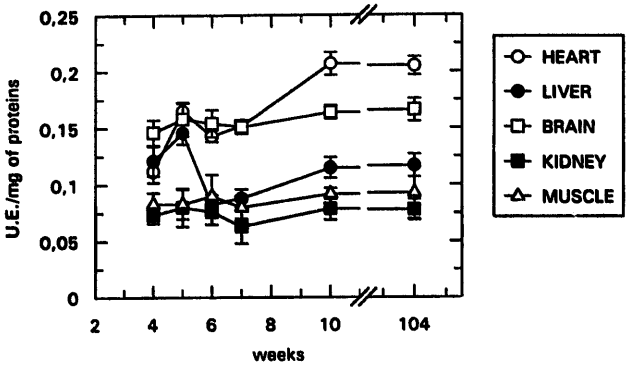

Fig. 5. Specific activity of cAspAT in various rat tissues as a function of age. Measurements were carried out as described in Fig. 4. The activity of the cytosolic isoenzyme was taken as the residual activity after incubation of homogenates at 70°C.

high for mAspAT in spite of having the lowest levels of mRNA expression. In the case of the cytosolic isoenzyme, levels in the brain were relatively high (comparable with liver) but significantly lower in kidney (comparable with muscle) despite the close similarity in levels of mRNA expression in the two tissues.

The first question that arises from these results is that of the metabolic rationale for the variations in enzymatic activity levels between tissues. The high levels in heart are probably related to the fact that this tissue carries out oxidative metabolism under all conditions and high AspAT activities are required for supply of metabolites for the citric acid cycle. The increases with age can then be related to increases in demand for this type of oxidative metabolism. With muscle, on the other hand, lower levels can be tolerated because metabolism is not strictly oxidative. The high level of mAspAT in liver (and to a lesser extend of cAspAT) is notable and may be connected to the biosynthetic role of this organ. Elevated levels of cytosolic isoenzyme in kidney and liver would not be expected in our experiments given that the rats had adequate dietary intakes.

More difficult to account for is the apparent lack of correlation between levels of enzymatic activity and levels of mRNA expression. This could be explained either in terms of tissue and age differences in rates of translation of mRNA or alternatively it might be the result of differences in rates of protein turn-over. The latter possibility does not seem likely. For example in liver, where proteolytic activity is generally high, we find a low level of mRNA for both isoenzymes but a high level of mitochondrial AspAT activity and a moderately high level of the cytosolic isoenzyme. On the other hand in muscle where the levels of proteolytic enzymes are low, the mRNA levels are very high, particularly at 10 weeks of age, but the AspAT activities are the lowest of any tissue examined. In addition it seems unlikely that protein turn-over could account for the observation that in muscle the mRNA levels increase with time but the levels of enzymatic activity do not.

Hence, in the absence of any alternative hypothesis, we are forced to conclude that the translation of mRNAs for the AspATs is subject to regulation that is both tissue specific and which varies, in some tissues, with age. That this may be so for what are generally considered, in most circumstances, to be 'house-keeping' enzymes clearly merits further investigation and indeed evidence has recently been reported for tissue specific initiation of transcription for the cytosolic isoenzyme in testis [17].

Acknowledgements: This work was partially supported by Italian National Research Council Target Project 'FATMA'.

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