PRIMARY STRUCTURE OF MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE FROM TURKEY LIVER

Cysteine-containing peptides

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1. Introduction

The determination of the complete primary structures of both cytoplasmic [1,2] and mitochondrial [3,4] isoenzymes of aspartate aminotransferase (EC 2.6.1.1) from pig heart coincides with a renewed interest in this system, as well documented by recent papers aimed at unraveling the three dimensional structures of these enzymes [5–7], their evolutionary history [8,9] and the structural basis and molecular mechanism of their intracellular compartmentation [10–12]. In this context we have studied some structural features of aspartate aminotransferase isoenzymes purified from various sources. Here we report results obtained with the mitochondrial isoenzyme from the liver of turkey, an animal evolutionarily rather distant from pig. This is of interest since the homotopic isoenzyme from another avian source, chicken, is being successfully subjected to crystallographic studies [7]. In particular, we have investigated the reactivity of sulphydryl groups in the turkey liver enzyme and have carried out primary structure studies of the enzyme labeled with radioactive iodoacetate. The latter studies allowed determination of 75% of the sequence of the polypeptide chain. The results are compared with those obtained from the homotopic isoenzyme from pig heart.

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2. Materials and methods

The enzyme was prepared from turkey liver by a modification of the purification procedure described for pig heart [13]. Titration of sulphydryl groups with 5, 5′-dithiobis (2-nitrobenzoic acid) in sodium dodecyl sulphate (SDS) and determination of their reactivity in the presence or absence of the substrate pair was performed according to [14]. An aliquot (350 mg) of holoenzyme was reduced with $NaBH_4$ and then carboxymethylated with iodo[2-^{14}C]acetate (Radiochemical Centre, Amersham) according to usual procedures [15]. The reduced, carboxymethylated protein was digested with 3.5 mg pepsin (Sigma) in 5% formic acid, for 1.5 h at room temperatures. The completely soluble digest was fractionated on a Sephadex G-25 fine column (4 × 130 cm) equilibrated and eluted with 5% acetic acid. Techniques employed for further purification and subsequent analysis of peptides were as reported in [15].

3. Results and discussion

The reaction of the mitochondrial isoenzyme from turkey liver with 5, 5′-dithiobis (2-nitrobenzoic acid) was very similar to that observed for the homotopic pig and chicken proteins [14]. Only 1 sulphydryl group/monomer of enzyme in the pyridoxal form was modified under native conditions following pseudo first-order kinetics and the rate of reaction

($28.9\ M^{-1}\ .\ min^{-1}$), which is of the same order of magniture as that measured for the other two proteins, was increased 2.4-fold in the presence of the substrate pair glutamate plus 2-oxoglutarate. This chemical modification leaves the catalytic activity of the enzyme practically unaffected. The total number of sulphydryl groups titratable in the turkey enzyme in the presence of SDS is 5/monomer; this value is identical to that found for the chicken enzyme [14], as might be expected from the evolutionary proximity of these two proteins. However, in this respect both avian enzymes are dissimilar to the pig enzyme, which contains 7 sulphydryl groups/subunit [3,4]. This observation, as well as recent experimental evidence on the possible role of sulphydryl groups in the intracellular localization of mitochondrial aspartate aminotransferase [12], stimulated us to undertake a comparative analysis of the sequences around cysteine residues in the turkey and pig enzymes.

From the peptic digest of the carboxymethylated enzyme from turkey liver, 5 radioactive peptides (P-3, P-12, P-14, P-21 and P-30) were isolated and analyzed. They accounted for the 5 cysteine residues/subunit expected since their sequences were established to be:

P-3:
Tyr–Arg–Asx–Asx–Asx–Gly–Lys–Pro–Tyr–Val–Leu–Asn–CmCys

P-12:
Arg–Tyr–Tyr–Asp–Pro–Lys–Thr–CmCys–Ser–Leu–Asp

P-14:
His–Ala–CmCys–Ala–His–Asx–Pro–Thr–Gly–Val(Asx, Pro 2, Arg, Glx 2, Trp)

P-21:
Thr–Val–Ile–CmCys–Arg–Asp–Ala–Glu–Glu

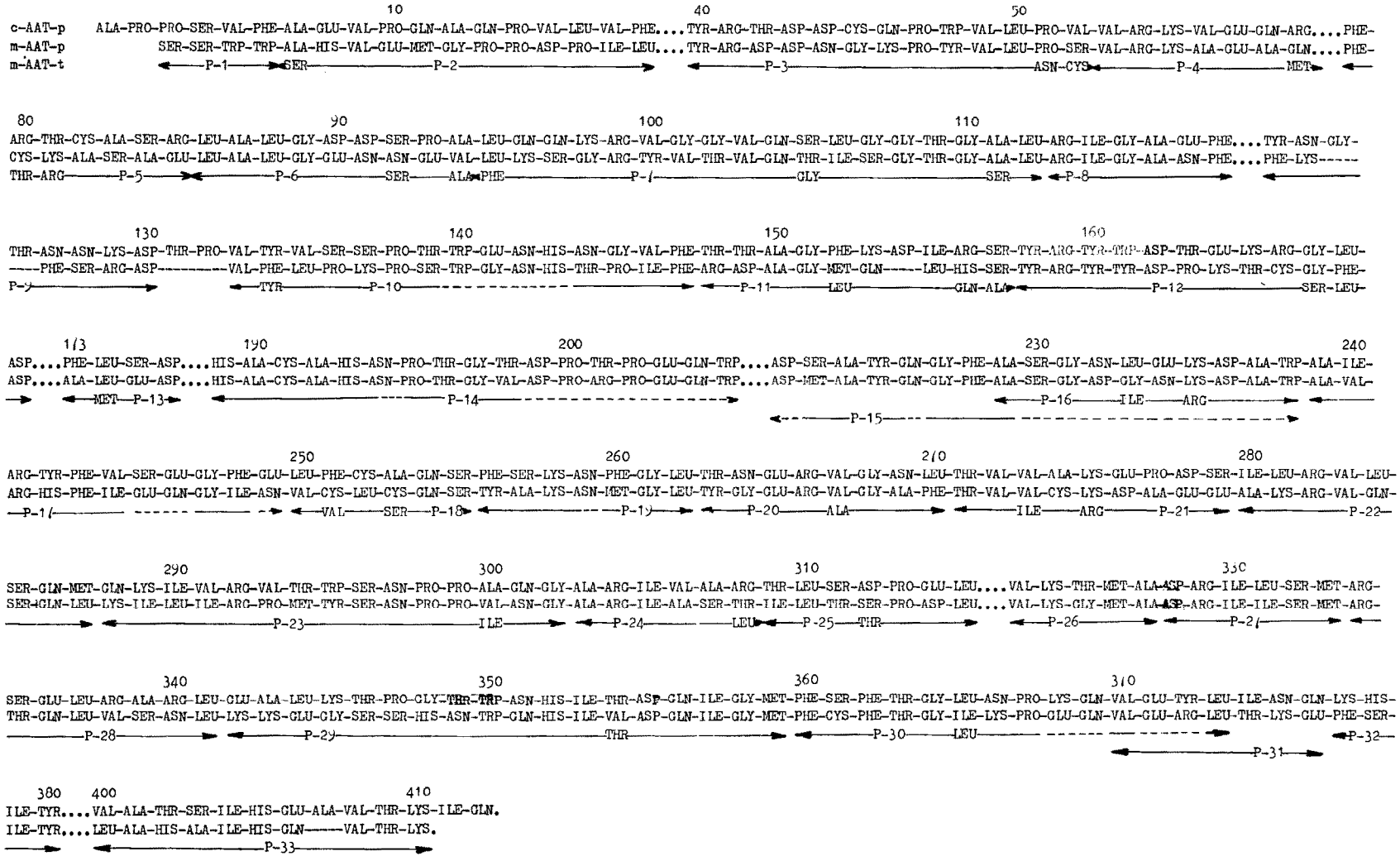
P-30:
Phe–CmCys–Phe–Thr–Gly–Leu–Lys–Pro–Glx–Glx–Val–(Glx, Arg)Leu

These sequences are reported in fig.1, aligned by homology along the polypeptide chain of the pig isoenzymes [1–4]. The numbering of residues refers to the sequence of the cytoplasmic isoenzyme from pig, which is 11 residues longer than the mitochondrial form. Comparison of sequences shows that Cys 80, 251 and 253 are absent in the turkey enzyme being substituted, respectively, by Thr, Val and Ser.

Cys 166, 191, 274 and 361 are conserved and a Cys substitutes for a Ser residue in position 52. It seems of some relevance to note conservation of Cys 166 and substitution of Cys 80; Cys 166 was identified as the residue subject to syncatalytic alterations of the rate of reaction both in pig and chicken mitochondrial enzymes [16] while Cys 80 was reported to be the 'syncatalytic' residue of the porcine enzyme under different experimental conditions [17]. The observed absence of Cys 80 in our avian enzyme seems to rule out conclusively any possible general role for this residue.

Purification and analysis of a number of non-radioactive peptic peptides was also performed; their structures were easily aligned, on the basis of homology considerations, along the sequence of the corresponding pig isoenzyme, as indicated in fig.1. Data on ~75% of the structure are now available (results using SDS–polyacrylamide gel electrophoresis, to be reported elsewhere, indicate that the molecular weight of the subunit is indistinguishable from that of the pig enzyme). These structural data, though not complete, allow some general observations to be made, since the peptides originate from different portions of the molecule, including N- and C-terminal segments and regions around active site-lysine and cysteine residues. Only 10% of residues are substituted relative to the pig protein; most of the substitutions can be considered conservative in nature and could result

Fig.1. Comparison of the primary structures of pig (m-AAT-p) and turkey (m-AAT-t) mitochondrial aspartate aminotransferase. Peptic peptides from the turkey isoenzyme are indicated by arrows; only the amino acids substituting for corresponding residues in the mitochondrial pig isoenzyme sequence are specified, inserted in the arrows. Dashed arrows denote sequences or amidation states inferred from amino acid compositions and comparison with sequences of the pig protein. The corresponding stretches of sequence of the cytoplasmic pig heart isoenzyme (c-AAT-p) are also reported for comparison [1,2].


c-AAT-p
m-AAT-p
m-AAT-t
P-1
SER
P-2
P-3
ASN-CYS
P-4
MET
P-5
P-6
SER
ALA
PHE
P-7
GLY
SER
P-8
TYR
P-10
P-11
LEU
GLN-ALA
P-12
SER-LEU-
MET
P-13
P-14
P-15
P-16
ILE
ARG
VAL
SER
P-18
P-19
P-20
ALA
ILE
ARG
P-21
P-22
P-23
ILE
P-24
LEU
P-25
THR
P-26
P-27
P-28
P-29
THR
P-30
LEU
P-31
P-32
P-33

from single base mutations. This indicates a high extent of conservation of the structure in the course of evolution within the class of the mitochondrial isoenzymes, as analysis of the N-terminal sequence of the chicken enzyme [18] and immunochemical studies [8,9] have already suggested. A greater extent of structure conservation with mitochondrial than with cytoplasmic isoenzymes may be explained on the basis that the mitochondrial isoenzymes should have conserved in the course of their evolution the structural features connected with their translocation into the mitochondria, besides those essential for the catalytic action.

It is also interesting that for 6 out of 30 substitutions observed, the replacing residue is identical to that found in the corresponding position of the sequence of the cytoplasmic pig isoenzyme (positions 92, 94, 134, 168, 354 and 365). This suggests that in these positions mutation (involving, however, interchange between a very restricted range of residues) is compatible with maintenance of the biological functions of the protein.

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