THE AMINO ACID SEQUENCE OF RABBIT MUSCLE TRIOSE PHOSPHATE ISOMERASE

P.H. CORRAN and S.G. WALEY
Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, U.K.

Received 5 January 1973

1. Introduction

Triose phosphate isomerase (EC 5.3.1.1) (TIM) is the enzyme in glycolysis that interconverts *D*-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. The enzyme is widely-distributed and highly active [1], and has special appeal to those studying mechanism because the reaction catalysed is, chemically, rather simple, and because both substrates are bound by the enzyme. Work on the active site [2–6], the mechanism [7, 8] and the three-dimensional structure [9] is in progress, and the wide distribution permits comparison of the properties and sequences of the enzymes from muscle [10–12], yeast [13] and bacteria [14]. Below we report the sequence of rabbit muscle TIM.

The crystallographic results on rabbit TIM showed it to be a dimer; the sub-units, related by a two-fold rotation axis, had a molecular weight of 26,000 [15]. Structural work showed that the sub-units were polypeptide chains, and established the sequences around the five cysteine residues [16, 17]. We now find that the polypeptide chain has 248 amino acid residues (fig. 1) and that the molecular weight of the dimer is 53,257.

2. Materials and methods

Rabbit muscle TIM was from Boehringer Corporation. Dipeptidyl aminopeptidase [18] was a kind gift from Dr. J.K. McDonald. Other enzymes and their use were as previously described [17]. Thiol groups were carboxymethylated, oxidised with performic acid or aminoethylated with ethyleneimine [19]. The methods used for peptide purification, amino acid analysis and sequence analysis were essentially as previously described [17]. Dipeptidyl aminopeptidase proved especially useful in placing amide groups and in work on tryptophan peptides.

3. Results and discussion

3.1. *Tryptic peptides*

All of the expected tryptic cleavages occurred, although one Lys–Leu bond (54–55) was incompletely cleaved. Lysyl–lysine was present in the tryptic digest, but this dipeptide is only slowly hydrolysed by trypsin [20]. There was some fission after tyrosine-47 and, in the earlier work [16], after tryptophan-90. The fission of the Asn–Ala (29–30) bond by trypsin is unusual, but not without precedent [21]. The tryptic peptides accounted for 248 amino acid residues, in agreement with the value expected from the amino acid composition. We believe that all the tryptic products have been recognised and sequenced.

3.2. *Overlapping of tryptic peptides*

Peptide maps of tryptic digests of the three cyanogen bromide fragments [17] enabled, in nearly all cases, a tryptic peptide to be assigned to a CNBr fragment. Overlapping peptides were isolated from peptic, thermolytic and chymotryptic digests of the whole protein and the CNBr fragments, and their evidence enabled the sequence in fig. 1 to be suggested. The evidence for the order of a given pair of tryptic peptides was, in general, drawn from the compositions and N-terminal residues of several peptides that overlapped the junction of the pair. However, in three in-

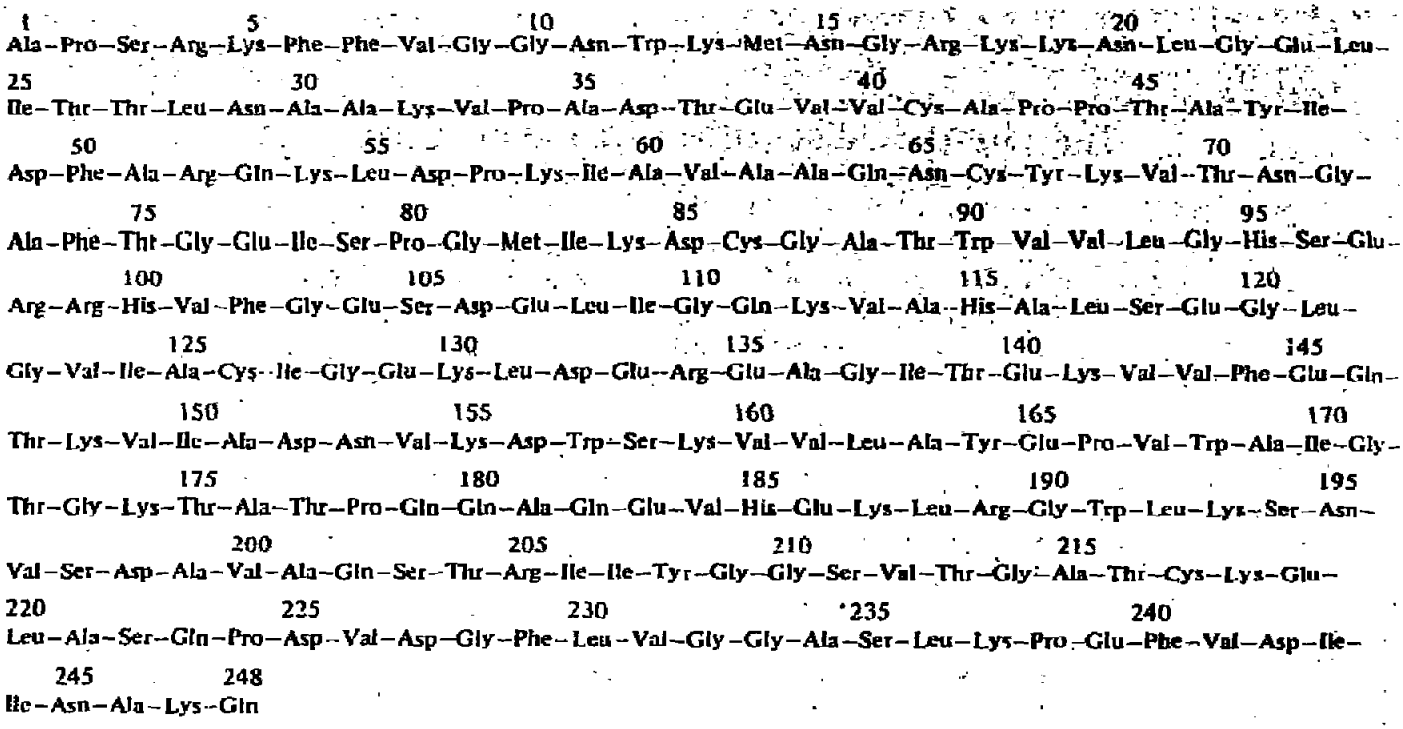

Fig. 1. Amino acid sequence of rabbit muscle triose phosphate isomerase.

stances, only one overlap peptide has been isolated: the junctions at residues 148–149, 155–156 and 159–160 are thus less firmly established than the other junctions.

There were several chymotryptic cleavages of glutaminyl bonds, and surprisingly, the Arg–Arg bond (98–99) was cleaved.

The sequence of the first 20 residues (83–102) of the C-terminal CNBr fragment was checked with the Beckman Sequencer [22]; our results were confirmed with one exception, namely that we had misplaced a histidine residue (now residue 95). The sequence of one tryptic peptide, residues 160–174, has been reported by Hartman [2]; our results agree, except that we believe that residue 160 is valine, not tryptophan. Of the 248 residues, glutamic acid – 165 is the only one so far known to be in the active site [2–4].

The only variant forms of the peptides that we encountered were due to partial deamidation of asparaginyl or glutaminyl residues; the asparaginyl residues 15 and 71 are in ....Asn–Gly... sequences, and these are particularly prone to deamidation [23]. We do not know if deamidation occurred only during isolation of the peptides, or whether there are variant forms of the protein, perhaps corresponding to the electrophoretically resolvable isoenzymes [24].

Acknowledgements

The support of the Medical Research Council is gratefully acknowledged; as is the technical assistance of Mrs. C. Moss and Miss R. Sammons. We thank Dr. R.E. Offord for much helpful information on the tryptic peptides from chicken TIM, and Dr. J.I. Harris for the results with the sequencer. This paper is a contribution from the Oxford Enzyme Group.

References

[1] P.M. Burton and S.G. Waley, Biochim. Biophys. Acta 151 (1968) 714.
[2] F.C. Hartman, Biochemistry 10 (1971) 146.
[3] J.C. Miller and S.G. Waley, Biochem. J. 123 (1971) 163.
[4] S. de la Mare, A.F.W. Coulson, J.R. Knowles, J.D. Priddle and R.E. Offord, Biochem. J. 129 (1972) 321.

[5] I.A. Rose and E.L. O'Connell, J. Biol. Chem. 244 (1969) 6548.
[6] S.G. Waley, Biochem. J. 126 (1972) 255.
[7] J.R. Knowles, P.F. Leadlay and S.G. Malster, Cold Spring Harbor Symp. Quant. Biol. 36 (1971) 157.
[8] D. Plaut and J.R. Knowles, Biochem. J. 129 (1972) 311.
[9] D.W. Banner, A.C. Bloomer, G.A. Petsko, D.C. Phillips and C.I. Pogson, Cold Spring Harbor Symp. Quant. Biol. 36 (1971) 151.
[10] I.L. Norton, P. Pfluderer, C.D. Stringer and F.C. Hartman, Biochemistry 9 (1970) 4952.
[11] S.J. Putman, A.F.W. Coulson, I.R.T. Farley, B. Riddleston and J.R. Knowles, Biochem. J. 129 (1972) 301.
[12] J.D. McVittie, M.P. Esnouf and A.R. Peacocke, European J. Biochem. 29 (1972) 67.
[13] W.G. Krietsch, P.G. Pentchev, J. Klingenburg, T. Hofstatter and Th. Bücher, European J. Biochem. 14 (1970) 289.
[14] R.C. Fahey, E. Kolb and J.I. Harris, Biochem. J. 124 (1971) 77P.
[15] L.N. Johnson and S.G. Waley, J. Mol. Biol. 29 (1967) 321.
[16] P.M. Burton and S.G. Waley, Biochem. J. 107 (1968) 737.
[17] J.C. Miller and S.G. Waley, Biochem. J. 122 (1971) 209.
[18] P.X. Callahan, J.K. McDonald and S. Ellis, in: Methods in Enzymology, Vol. 25, Part B, eds. C.H.W. Hirs and S.N. Timasheff (Academic Press, New York, 1972) p. 282.
[19] R.D. Cole, in: Methods in Enzymology, Vol. 11, ed. C.H.W. Hirs (Academic Press, New York, 1967) p. 315.
[20] S.G. Waley and J. Watson, Biochem. J. 55 (1953) 328.
[21] R.P. Ambler and L.H. Brown, Biochem. J. 104 (1967) 784.
[22] J. Bridgen, E. Kolb and J.I. Harris, personal communication.
[23] D.M. Shotton and B.S. Hartley, Nature 225 (1970) 802.
[24] W.K.G. Krietsch, P.G. Pentchev and H. Klingenburg, European J. Biochem. 23 (1971) 77.