AMINO ACID SEQUENCE HOMOLOGY IN ALCOHOL DEHYDROGENASE

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1. Introduction

Thiol groups have been implicated in the catalytic activity of a number of dehydrogenases (for recent review see [1]). Moreover, in the case of glyceraldehyde-3-phosphate dehydrogenase there is good evidence to show [2] that one cysteine residue per subunit (Cys-149 in the primary structure [3]) is directly involved in the catalytic reaction. The enzyme is inhibited by reaction of this cysteine with thiol group reagents and the amino acid sequence around the reactive cysteine is virtually identical in enzymes from a number a widely different species [4]. Alcohol dehydrogenase are also inhibited by reagents that react with thiol groups [5–7]. In the horse liver enzyme inhibition by iodoacetate has been shown to be due to its selective reaction with one cysteine residue [8] identified as cysteine-46 in the enzyme [9]. Alcohol dehydrogenase from yeast is inhibited in a similar manner and although there is some similarity in the amino acid sequence around the reactive cysteines in the two proteins [6] it is not as extensive as that found between the corresponding glyceraldehyde-3-phosphate dehydrogenases [4]. Moreover, differences in sequence close to the reactive cysteine in alcohol dehydrogenase have been shown to occur [10, 11] even within mammals.

Alcohol dehydrogenase from yeast and liver differ in several respects. For example, the latter is a dimer with a subunit consisting of 374 amino acids [8, 9] while the former is a tetrameric protein with a smaller subunit consisting of about 330 amino acids [6, 12]. Although there is a similarity in the amino acid sequence around the respective reactive cysteines [6] comparison of the compositions of other tryptic peptides from the yeast enzyme [12] with the known sequence of the horse liver enzyme [9] has not so far revealed that a high degree of sequence homology exists between the two proteins. Thus present evidence is not sufficient to establish the extent to which alcohol dehydrogenases from widely different sources form a homologous group of proteins containing essential thiol groups.

The reactive cysteine in liver alcohol dehydrogenase (Cysteine-46 [9]) is relatively close to the N-terminus of the protein so that a comparison of this part of the chain in the horse liver and yeast enzymes would have been within the scope of automated methods of sequence analysis [13] were it not for the presence in both proteins of blocked N-terminal residues [9, 12]. The isolation from *B. stearothermophilus* of an alcohol dehydrogenase that does possess a free N-terminal amino acid [14] has now enabled us to apply automated methods to determine its N-terminal sequence and thus to compare the N-terminal sequences of the bacterial and horse liver enzymes, a comparison that is of particular interest in view of the complete stability of the bacterial alcohol dehydrogenase towards thiol group reagents [14].

2. Materials and methods

Alcohol dehydrogenase was isolated in pure form from extracts of *B. stearothermophilus* as described by Kolb and Harris [14]. The enzyme was carboxymethylated with [2-^{14}C] iodoacetic acid in the presence of 6 M guanidine HCl and the S-[2-^{14}C] carboxymethylated protein (8 mg) was submitted to sequence analysis by automatic Edman degradation in a

Beckman 390B Sequencer. Phenylthiohydantoin (PTH) derivatives were identified and estimated by gas chromatography, and by amino acid analysis following regeneration of the amino acid by hydrolysis with hydrogen iodide [15].

3. Results and discussion

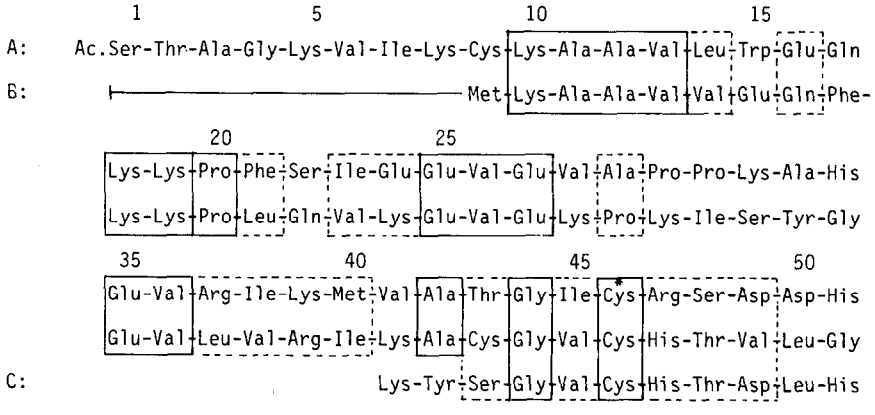
Sequence analysis of *B. stearothermophilus* alcohol dehydrogenase was carried out through 45 successive cycles and the N-terminal sequence was established to be:

Met–Lys–Ala–Ala–Val–Val–Glu–Gln–Phe–Lys–Lys–Pro–Leu–Gln–Val–Lys–Glu–Val–Glu–Lys–Pro–Ile–Ser–Tyr–Gly–Glu–Val–Leu–Val–Arg–Ile–Ala–Cys–Gly–Val–Cys–His–Thr–Val–Leu–Gly–Ala–Ala–

A repetitive yield of 96.5% was calculated from the yields of PTH-alanine at positions 4 and 34, and from the yields of PTH-valine at positions 5 and 37. Cysteine residues at positions 35 and 38 were identified as PTH-S-[2-^{14}C]carboxymethylcysteine.

At first sight no sequence homology could be detected by direct comparison of this sequence with the N-terminal sequence of the horse liver enzyme. However, closer inspection revealed a significant similarity in sequence between the two chains if they were aligned so that cysteine-38 in the bacterial enzyme corresponded to cysteine-46 in the horse liver enzyme (fig. 1). The N-terminal residue in the former then corresponds to residue 9 in the latter and from this point of reference 16 of the first 40 residues (40%) are identical in the two chains; in addition 15 of the amino acid differences could have arisen as the result of single point mutations. It may also be noted that the sequence of a heptapeptide, –Gly–Val–Cys–His–Thr–Asp–Leu–, in the immediate vicinity of the reactive cysteine in the yeast enzyme, is identical (apart from the replacement of Asp by Val in position 49, fig. 1) to the sequence around cysteine-38 in the bacterial enzyme. This establishes an even closer similarity of this part of the chain between the yeast and *B. stearothermophilus* enzymes than is manifest between the yeast and horse liver, or *B. stearothermophilus* and horse liver enzymes, respectively. Nevertheless, in spite of this common sequence, cysteine-38 in the bacterial enzyme, unlike its counterparts in the yeast and mammalian enzymes, is not reactive towards thiol group reagents and this appears to be the first known example of a normal wild type alcohol dehydrogenase that is not inhibited by iodoacetic acid.

Unprotected SH groups are potential sites for oxi-



ALCOHOL DEHYDROGENASES: N-terminal sequences

Fig. 1. Comparison of N-terminal amino acid sequences in alcohol dehydrogenase from horse liver (A) and *B. stearothermophilus* (B). The sequences are aligned so that cysteine-46 in A (ref. [9]) corresponds to cysteine-38 in B and to the reactive-cysteine in yeast alcohol dehydrogenase (C, ref. [6]). Residues shown within solid boxes are identical, and those within dotted boxes are related by single point mutation.

dative inactivation of enzymes and in this connection it is of interest that several enzymes from thermophilic sources, including glyceraldehyde-3-phosphate dehydrogenase, aldolase and phosphofructokinase have been shown to contain fewer SH groups than their mesophilic counterparts (unpublished results of this Laboratory). It is highly significant, therefore, that cysteine-38 has been retained (and that cysteine-35 has been introduced, three residues distant) in a conserved part of the primary structure of *B. stearothermophilus* alcohol dehydrogenase despite the necessity for it to retain activity under aerobic conditions and at temperatures of up to 65°C. Moreover it is also significant that cysteine-38 in the thermophilic enzyme (that corresponds to the 'active-site' cysteine in the mammalian and yeast enzymes) is totally unreactive towards a variety of thiol group reagents. Thus the enzyme is completely stable in the presence of iodoacetic acid or iodoacetamide and this marked change in the reactivity of the thiol group has been brought about, not by protection with coenzyme as in the mammalian and yeast enzymes [7, 5], or by an amino acid substitution at or close to it in the primary structure, but presumably as the result of a conformational change that confers stability in the thermophilic environment without affecting adversely the ability of the enzyme to form the catalytic ternary complex with NADH and substrate [16, 17]. In this connection it would be of interest to establish the nature of the structural change that has occurred in the iodoacetic acid resistant alcohol dehydrogenase that has been found in a chemically induced mutant of *E. coli* [18].

Jörnvall and co-workers have shown [10, 11] that the sequence of alcohol dehydrogenase has been highly conserved (to the extent of 80–85%) within mammals. They have also shown that sequence changes close to the "essential" thiol group (Cys-46) in the horse, human and rat enzymes can be tolerated without apparent effect on the activity of the enzyme. The present study shows that although a very similar sequence around a cysteine residue is found in the *B. stearothermophilus* enzyme its reactivity within the protein has changed, possibly in response to environmental factors. Are SH groups directly involved in the activity of alcohol dehydrogenase, or is it their chemical modification that interferes indirectly with the catalytic process [16, 19]. While a definitive answer to this question may require a knowledge of the three-dimensional structure the retention of cysteine in a homologous sequence in the thermophilic alcohol dehydrogenase does suggest that it is, in all probability, required to fulfil an essential role even though it occurs in a different environment in the tertiary structure of the thermophilic enzyme.

The amino acid sequence results also indicate a common evolutionary origin for alcohol dehydrogenases from yeast, *B. stearothermophilus* and mammalian liver. In this respect alcohol dehydrogenase (although evolving at a faster rate, cf. [11]) resembles glyceraldehyde-3-phosphate dehydrogenase [4] and not aldolase where the sequence of the thermophilic enzyme appears to be unrelated to that of its class II counterpart from yeast [20].

References

[1] J.L. Webb, in: Enzyme and metabolic inhibitors (Academic Press, New York, 1970) Vol. III, pp. 18–52.
[2] J.I. Harris, B.P. Meriwether and J.H. Park, Nature 197 (1963) 154.
[3] J.I. Harris and R.N. Perham, Nature 219 (1968) 1025.
[4] G.M.T. Jones and J.I. Harris, FEBS Letters 22 (1972) 185.
[5] E.P. Whitehead and B.R. Rabin, Biochem. J. 90 (1964) 133.
[6] J.I. Harris, Nature 203 (1964) 30.
[7] T.K. Li and B.L. Vallee, Biochemistry 3 (1964) 869.
[8] H. Jörnvall and J.I. Harris, European J. Biochem. 13 (1970) 565.
[9] H. Jörnvall, European J. Biochem. 16 (1970) 25.
[10] H. Jörnvall and R. Pietruszko, European J. Biochem. 25 (1972) 283.
[11] H. Jörnvall and O. Markovic, European J. Biochem. 29 (1972) 167.
[12] P.J.G. Butler, Ph.D. Thesis (University of Cambridge, 1967).
[13] P. Edman and G. Begg, European J. Biochem. 1 (1967) 80.
[14] E. Kolb and J.I. Harris, Biochem. J. 124 (1971) 76P, and unpublished results.
[15] J. Bridgen and D. Secher, FEBS Letters 29 (1972) 55.
[16] T.K. Li and B.L. Vallee, Biochemistry 4 (1965) 1195.
[17] F.M. Dickinson, Biochem. J. 126 (1972) 133.
[18] B. Meyhack, G. Pfleiderer and H.D. Mennigmann, European J. Biochem. 33 (1973) 19.
[19] J.S. KcKinley-McKee, D.L. Morris and C.H. Reynolds, in: Structure and function of oxidation–reduction enzymes, eds. A. Åkeson and A. Ehrenberg (Pergamon Press, 1972) pp. 613–618.
[20] R.S. Jack and J.I. Harris, Manuscript in preparation.