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AMINO ACID SEQUENCE OF DIHYDROFOLATE REDUCTASE FROM AN AMETHOPTERIN-RESISTANT STRAIN OF *LACTOBACILLUS CASEI*

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1. Introduction

Dihydrofolate reductase (5,6,7,8-tetrahydrofolate: $NADP^+$-oxidoreductase, EC 1.5.1.3) appears to be the major intracellular receptor for the action of 4-amino analogs of folic acid, such as amethopterin (4-amino-10-methyl-4-deoxyfolate). Such analogs have been employed extensively in the chemotherapeutic treatment of certain leukemias, lymphomas and other clinical disorders [1,2]. In addition, a differential sensitivity to certain drugs, such as trimethoprim, by mammalian and bacterial reductases have lead to the development of a class of compounds with potent antibacterial activity [3]. The underlying differential inhibitory effects of certain classical drugs as trimethoprim must reside in differences in the three-dimensional architecture of the active centers of bacterial versus mammalian dihydrofolate reductases. Thus, a knowledge of the complete primary and tertiary structures of dihydrofolate reductases from both bacterial and mammalian sources in the presence and absence of inhibitors, coenzymes, and substrates should aid in our understanding of these differential inhibitory effects.

In the present communication the primary structure of dihydrofolate reductase from an amethopterin-resistant strain of *Lactobacillus casei* of 162 residues is reported and compared with two bacterial reductases whose sequences have been previously described.

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2. Experimental

Dihydrofolate reductase was purified from an amethopterin-resistant strain of *L. casei* by a modification [4] of the procedure of Gundersen et al. [5]. Automated sequence analysis was performed with the Beckman Sequencer (Model 890C) on the intact protein and on most of the derived peptides as previously described [6]. Digestion of the protein with CNBr at methionine residues and subsequent purification of the resulting peptides were done as described by Freisheim et al. [6]. Digestion of peptide fragments with 'TPCK-Trypsin' was performed according to conventional procedures. The digest was fractionated on Sephadex G-50 (superfine). Some of the smaller tryptic peptides were further purified either by gel filtration on Sephadex G-25 (fine), on Bio-Gel P-6 or by ion-exchange chromatography. Digestion of CNBr-II with *S. aureus* protease was done according to Houmard and Drapeau [7]. Separation of the resulting peptides was achieved on Sephadex G-50. Some of the lower molecular weight *S. aureus* protease peptides were purified by gel filtration on Bio-Gel P-6 or by electrophoresis (pH 6.5) followed by paper chromatography in the solvent system: butanol/glacial acetic acid/pyridine/water (15:3:10:12). Cleavage of peptides with the *Myxobacter* protease was performed as described by Wingard et al. [8]. Digestions employing carboxypeptidase C were performed according to Tschesche and Kupfer [9] and those employing carboxypeptidases A and B were performed essentially as determined by Morris et al. [12] employing mass

were performed using a Durrum Model D-500 amino acid analyzer after acid hydrolysis according to the general procedures of Moore and Stein [11].

3. Results and discussion

The alignment of the three CNBr fragments has been described [6]. Automated sequencer analyses were performed on the three CNBr fragments as well as on tryptic, *Myxobacter* protease *Al*-1 and *S. aureus* protease peptides to obtain overlap data. The sequence of the *L. casei* dihydrofolate reductase is shown in fig.1.

These results are in agreement with the sequence of most of the elastase peptides from an *L. casei* reductase as determined by Morris et al. [12] employing mass spectrometry.

The amino-acid sequence of dihydrofolate reductase from the amethopterin-resistant strain of *L. casei* described is based on present data and on those reported previously from this laboratory [6]. The molecular weight calculated from the sequence is approx. 18 300.

Comparison of the amino-acid sequence of the *L. casei* reductase with those obtained for enzymes from *S. faecium* [13] and *Escherichia coli* [14] are shown in fig.1. Optimal alignment of the three sequences required the assumption that the *L. casei* reductase is lacking the first residue which is Met in the other two enzymes. The *L. casei* reductase sequence shows a 29% identity with that of the *E. coli* enzyme and a 34% identity with that of the *S. faecium* enzyme with the alignments given in fig.1. A similar comparison of the *S. faecium* and *E. coli* reductases gives 32% identical residues. If minimum single-base changes are also included, the homology comparisons vary between 64% and 73% as indicated in table 1.

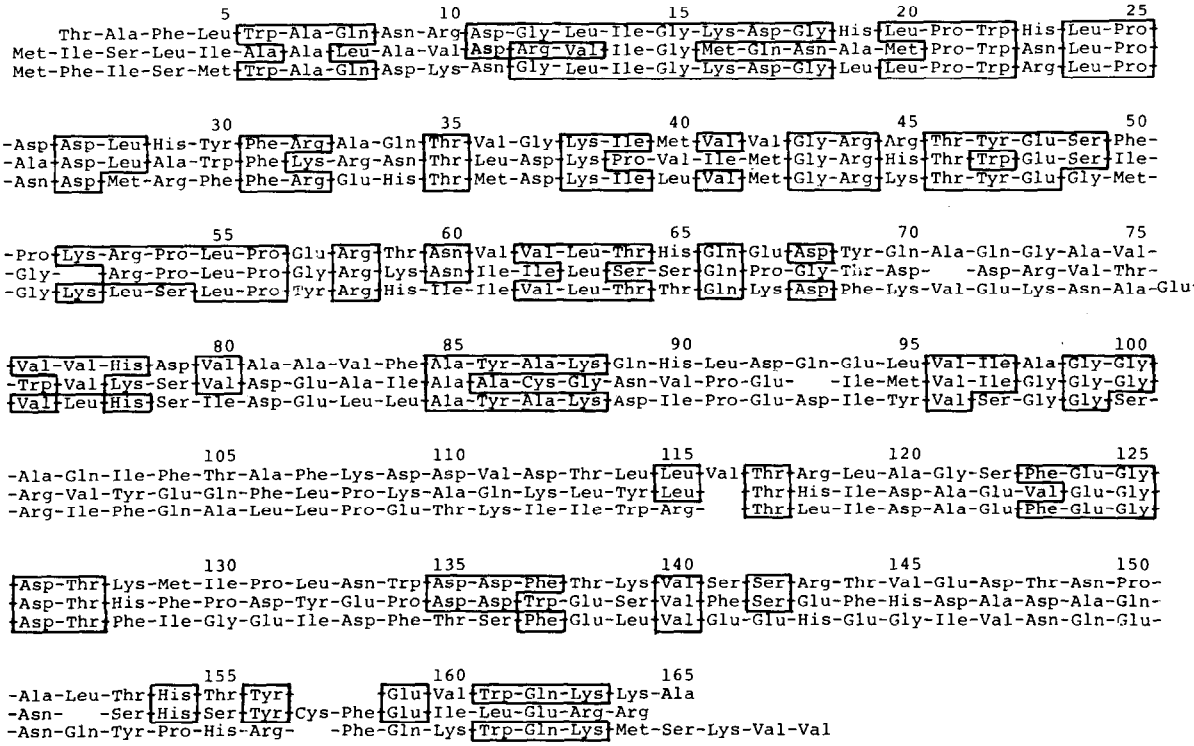

Fig.1. Comparison of the amino acid sequences of dihydrofolate reductase from *L. casei* (upper sequence), *E. coli* (middle sequence [14]), and *S. faecium* (lower sequence [13]). Positions at which the *L. casei* enzyme is identical to one or more of the other proteins are enclosed. Position numbers are based on the *E. coli* sequence. Gaps are introduced to achieve maximum homology.

Table 1
Comparison of the amino acid sequences of dihydrofolate reductases from *L. casei*, *E. coli* and *S. faecium*[a]

Sequences compared		Min. Base Changes, %[b]		Mean Base Change per/Codon
		0	0 + 1	
L. casei to *S. faecium*	res. 1–68	54	91	0.55
	total sequence	34	73	0.94
L. casei to *E. coli*	res. 1–68	39	79	0.82
	total sequence	29	64	1.08
E. coli to *S. faecium*	res. 1–68	37	75	0.88
	total sequence	32	72	0.98

[a]The comparisons are based on the sequence alignments indicated in fig.1
[b]The percentages are based on the minimum number of base changes necessary to change the amino acid residue in one sequence to that found at the same position in the sequence being compared

In agreement with the conclusions of Gleisner et al. [13] who compared the *S. faecium* and *E. coli* MB 1428 reductase sequences, it is evident that the amino-terminal regions of the three proteins are highly conserved. The first 68 residues of these amino-terminal regions constitute the longest set of clusters of identical residues (cf. fig.1). It is clear in comparing reductase sequences from *L. casei* and *S. faecium*, both folate-requiring organisms, that these two exhibit the greatest degree of homology in this region as shown in table 1. These two enzymes show a 54% identity in the first 68 residues with a mean base change per codon of 0.55 as compared with 0.82 and 0.88, respectively, when the *L. casei/E. coli* and *E. coli/S. faecium* reductase sequences are similarly examined. The high degree of conservation of amino acid residues in the amino-terminal portion of the dihydrofolate reductases examined suggests a functional involvement for certain residues in this region of the molecule.

Previous chemical modification studies [6] have implicated Trp-22 in the function of *L. casei* dihydrofolate reductase. The position of this tryptophan is conserved in enzymes from at least five different species [6]. The common sequence among bacterial reductases is: Leu/Met–Pro–Trp_{22}–*X*–Leu–Pro–*X*–Asp–Leu/Met ([6], cf. fig.1). The proline residue at positions 21 and 25 may serve to position Trp-22 into the appropriate configuration for optimum binding interactions. In addition, one lysine [15] and at least two arginine residues [16] have been implicated in either folate or NADPH binding, although these residues have not, as yet, been placed in the *L. casei* enzyme sequence.

Recently Matthews et al. [17] have described contact residues involved in the binding of Methotrexate (MTX) to the *E. coli* reductase by means of X-ray crystallographic analyses. Based in part on sequence alignments suggested by this laboratory (cf. fig.1), certain residues have been implicated in the binding of MTX to dihydrofolate reductase [17]. Based on the alignments indicated in fig.1, it appears that Arg-58 and either Arg- or Lys-32 are involved in the binding of the α- and β-carboxyl groups, respectively, of the L-Glu moiety of the MTX molecule. Residue Asp_{27}, which is identical in the three bacterial enzymes (cf. fig.1), may play an important role in binding of several atoms of the MTX molecule [17]. In addition, Arg-44, which is identical in all three bacterial species, may be involved in the binding of the 2′-phosphate in NADPH, as suggested by Matthews et al. [17].

The molecular details of the interaction of *L. casei* dihydrofolate reductase with NADPH in the presence and absence of MTX remain to be elucidated, but such studies are in progress (D. A. Matthews and J. Kraut, personal communication).

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