THE AMINO ACID SEQUENCE OF DIHYDROFOLATE REDUCTASE FROM L1210 CELLS

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1. Introduction

Dihydrofolate reductase (5678 tetrahydrofolate: $NADP^+$-oxidoreductase, E C 1.5.1.3) has been intensively studied because of its role as a target enzyme for antibacterial drugs. The antibacterial activity of these drugs, particularly trimethoprim, has been shown to depend on the much higher affinity with which they bind to the bacterial enzymes compared to the mammalian enzymes [1]. This difference in susceptibility to inhibitors has stimulated interest in the structures of dihydrofolate reductases from both bacterial and mammalian sources, although work has generally focussed on the bacterial enzymes because of their greater availability. Thus the complete amino acid sequences of the dihydrofolate reductases from *E.coli* MB1428 [2], *E.coli* RT500 [3] and *S.faecium* [4] have been established while, in contrast, the only data for mammalian enzymes are two reports of the amino terminal 34 and 19 residues of the bovine liver enzymes [5,6] and the amino terminal 25 residues of the mouse sarcoma 180 enzyme [7].

This present paper reports the amino acid sequence of the dihydrofolate reductase from a Methotrexate-resistant line of the mouse lymphoma L1210 and compares this sequence with those of other dihydrofolate reductases.

2. Experimental

L1210 cells were grown in DBA/2 mice and the dihydrofolate reductase was isolated essentially as described by Whiteley *et al.*[8].

Dihydrofolate reductase in which the cysteine residues had been alkylated with iodo [^{14}C] acetic acid was cleaved with cyanogen bromide and the resulting fragments separated into three fractions by gel-filtration on Sephadex G-75. The largest fragment was eluted as a single component. Further purification of the intermediate and low molecular weight fractions was carried out by chromatography on DEAE-cellulose and high-voltage electrophoresis respectively. A total of 6 fragments were isolated by these procedures.

The amino acid sequence of the three fragments comprising the low molecular weight fraction were determined by the dansyl-Edman procedure. The major parts of the sequences of the three larger fragments were determined by automatic sequencer analysis in the Beckman 890C automatic sequencer using the peptide programme number 102974. The information required to complete these sequences was obtained from manual dansyl-Edman sequence determinations of peptides isolated from either tryptic or staphylococcal protease digests of the fragments.

The alignment of the three amino terminal CNBr fragments was determined by automatic sequencer analysis of the intact protein in the Beckman 890C using the protein programme number 122974. In order to obtain overlap data for the remaining fragments, tryptic peptides were isolated from dihydrofolate reductase in which the methionines had been alkylated with iodo [2-^{14}C] acetic acid. These radioactive peptides were isolated by gel-filtration on Sephadex G-25 followed by high-voltage electrophoresis and their sequences determined either by automatic sequencer analysis or by the dansyl-Edman procedure. Full details of these procedures will be published elsewhere.

The methods for CNBr cleavage, enzymic digestion, amino acid analysis, high-voltage electrophoresis, the

dansyl-Edman procedure and automatic sequencer analysis are as described previously [3].

3. Results and discussion

The sequence of L1210 dihydrofolate reductase is shown in fig.1. No particular problems were encountered in the sequence determination although the Met_{37} Thr_{38} bond failed to cleave with CNBr. The failure of Met–Thr bonds to cleave with CNBr has been reported for other proteins [9,10] although in these cases partial cleavage occurred. In the present case however, no detectable cleavage occurred and the separation of the CNBr fragments was not complicated by the presence of partial cleavage products. Consequently only six CNBr fragments were isolated instead of the seven anticipated.

The sequence determined contains 186 residues and corresponds to mol 21 458. This value is in fairly good agreement with the value of 22 500 determined by SDS–polyacrylamide gel electrophoresis (J. Raper, unpublished results) and the value of 20 000 reported by Neef and Huennekens [11] for the enzyme from L1210/R6 cells. The L1210 enzyme is therefore longer than the *S.faecium* and *E.coli* enzymes by 18 and 26 residues respectively. As shown in fig.1, all these enzymes show a considerable number of areas of identical sequence, the degree of identity between the L1210 enzyme and both bacterial enzymes being 29%. The most extended regions of homology between all three enzymes are those between residues 49 and 60 and 138 and 149, although the following 4 regions also contain a high proportion of identical residues: 16–30, 65–78, 91–98, 111–122. On the basis of a comparison of the sequences of the *S.faecium* and

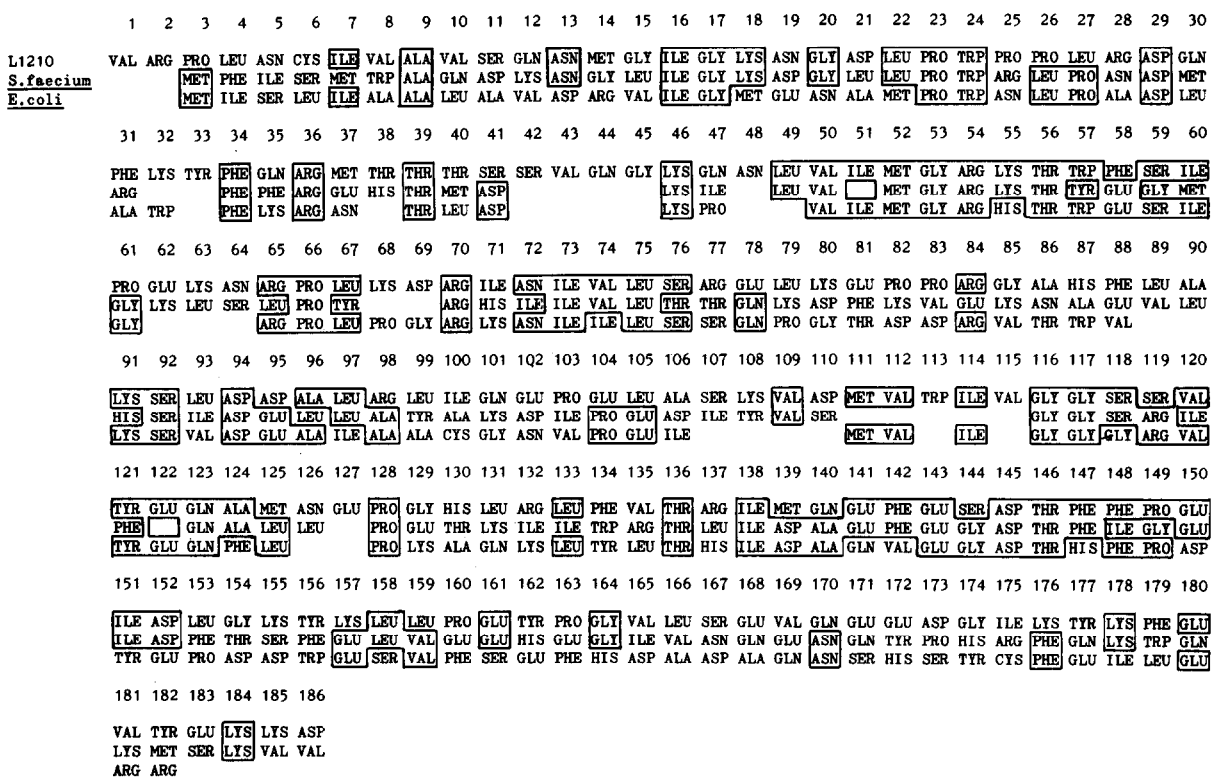

Fig.1. Comparison of the amino acid sequences of dihydrofolate reductases from L1210, *E.coli, S.faecium.* The numbering is that for the L1210 enzyme. The sequences have been aligned to maximise the homology between each of the bacterial enzymes and the L1210 enzyme without introducing gaps into the latter sequence.

E.coli enzymes, Gleisner *et al.* [4] drew attention to two domains of homology which exist between the two molecules and it is interesting to note that the regions of those enzymes which show the most extensive homology with the L1210 reductase also fall within these domains.

A comparison of the N-terminal part of the L1210 enzyme with the N-terminal portions of other mammalian dihydrofolate reductases shows very close similarity. The first 30 residues are identical with the pig liver enzyme (our unpublished results) with the possible exception that in the latter case residue 6 is either Cys or Ser. The sequence of the first 25 residues is identical to that of the mouse sarcoma 180 [7]. However the sequence of the first 19 residues of a bovine liver enzyme reported by Bauman and Wilson [5] differs in having Ser at position 6 while the sequence of the first 35 residues of a bovine liver enzyme reported by Peterson *et al.* [6] differs from L1210 at positions 6 (Ala for Cys), 21 (Tyr for Asp) and 32 (Lys for Glu).

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