MODULATION OF SPECIFIC BINDING OF *LACTOBACILLUS CASEI* DIHYDROFOLATE REDUCTASE TO DNA BY FOLINIC ACID

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1. Introduction

Dihydrofolate reductase (DHFR) plays an essential role in intermediary metabolism, catalysing the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate, the latter serving as a carrier of one-carbon fragments in the biosynthesis of amino acids, thymidylate and purines [1]. DHFR has been the subject of intense investigations as it is the target of important anti-bacterial and anti-neoplastic drugs such as trimethoprim and methotrexate. It has been demonstrated that DHFR from *Lactobacillus casei* has affinity for double-stranded DNA [2]. A small difference was observed in the nitrocellulose filter binding curves obtained for the binding of DHFR to pBR 322 and pWDLcB1 DNA, the latter differing from the former only in the presence of a 2.9 kb insert containing the DHFR structural gene from a methotrexate-resistant strain of *L. casei*, suggesting that DHFR binds specifically to a site at or near its own structural gene. Here, we demonstrate that specific binding of DHFR to DNA is modulated by the tetrahydrofolate analogue folinic acid but not by the coenzyme NADPH, in contrast to non-specific binding which is unaffected by either folinic acid or NADPH.

2. Materials and methods

DHFR was purified exactly as in [3] and its concentration determined by assaying its catalytic activity. NADPH and folinic acid were obtained from Sigma Co. Ltd., restriction endonucleases *Bam*HI and *Bgl*II from Boehringer Corp. and d[α-^{32}P]ATP (2000–3000 Ci/mmol) from Amersham. All other chemicals used were of the highest purity commercially available and were used without further purification.

Two plasmids were employed: pBR 322 [4] (length 4.36 kb [5]) and pWDLcB1 which was constructed by inserting a 2.9 kb fragment of DNA from a methotrexate-resistant strain of *L. casei* containing the DHFR structural gene into pBR 322 [6]. End-labelling of plasmid DNA with ^{32}P was carried out as in [7].

Equilibrium binding studies of DHFR to pBR 322 and pWDLcB1 DNA were carried out using the nitrocellular filter binding technique as in [2]. Aliquots (29 ng) of labelled plasmid DNA were incubated with various amounts of DHFR in 100 μl 100 mM Tris–HCl, 100 mM KCl, pH 7.5 at 22°C for 20 min.

Folinic acid and NADPH was employed in these experiments at 10^{-4} M, which is sufficient to ensure complete saturation of DHFR with folinic acid and/or NADPH at the highest concentration of DHFR employed (2×10^{-6} M) [8].

Abbreviation: DHFR, dihydrofolate reductase

3. Results and discussion

The experimental equilibrium filter binding curves for the binding of DHFR alone, the DHFR–folinic acid, DHFR–NADPH and DHFR–NADPH–folinic acid complexes are shown in fig.1. We observe (fig.1) that the filter binding curves may be grouped into 3 sets based on the apparent affinities of DHFR and its complexes with DNA:

(i) Those curves obtained for the binding of DHFR alone or in the presence of folinic acid and/or NADPH (either as binary or ternary complexes)

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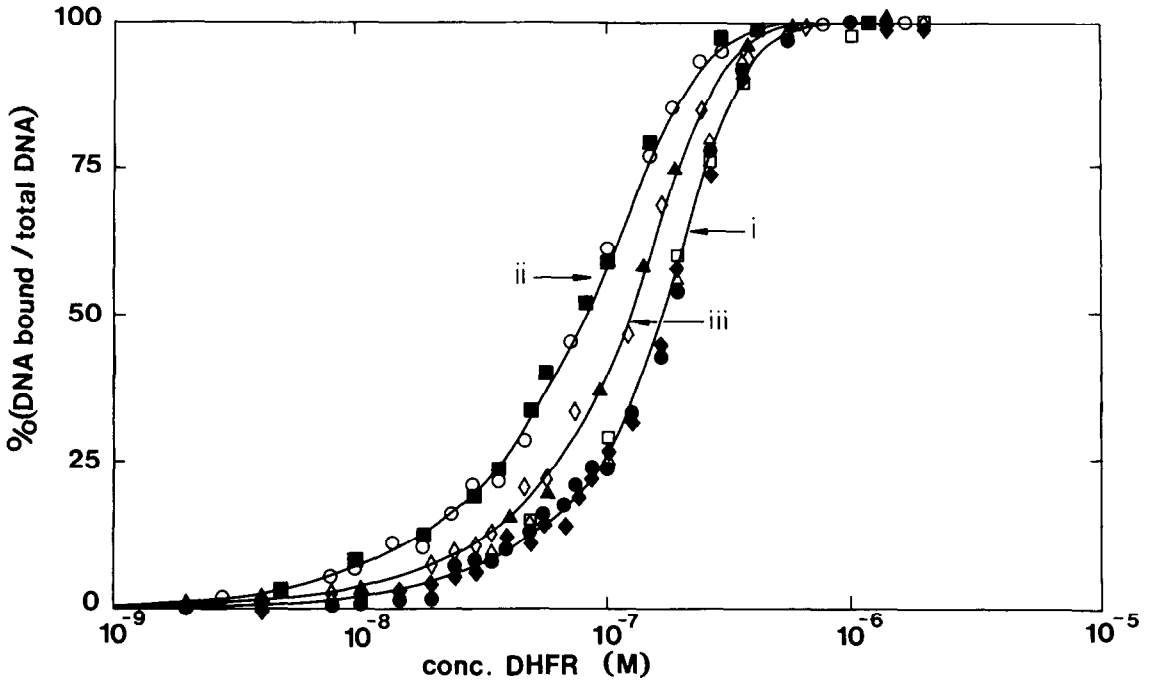

Fig.1. Filter binding curves for the binding of DHFR to DNA from plasmids pBR 322 and pWDLcB1: (i) binding of DHFR alone (•) and the DHFR–NADPH (▫), DHFR–folinic acid (△) and DHFR–NADPH–folinic acid (♦) complexes to pBR 322 DNA; (ii) binding of DHFR alone (○) and the DHFR–NADPH complex (▪) to pWDLcB1 DNA; (iii) binding of the DHFR–folinic acid (▲) and DHFR–NADPH–folinic acid (◇) complexes to pWDLcB1 DNA. The symbols are the means of the actual data points obtained from three separate experiments. All experimental details are given in section 2.

to pBR 322 DNA which have a value of L_{50} = 1.8×10^{-7} M (L_{50} is defined as the concentration of DHFR at half-saturation).

(ii) Those curves obtained for the binding of the DHFR alone and the DHFR–NADPH complex to pWDLcB1 DNA which have a value of L_{50} = 8×10^{-8} M.

(iii) Those curves obtained for the binding of the DHFR–folinic acid and DHFR–NADPH–folinic acid complexes to pWDLcB1 DNA which have a value of $L_{50} = 1.2 \times 10^{-7}$ M.

Under the assumption that the binding of DHFR and its complexes with NADPH and folinic acid to pBR 322 DNA only reflects non-specific binding, the observation that the corresponding filter binding curves are identical within experimental error, indicates that the affinity of the non-specific sites for DHFR is unaffected by either folinic acid or NADPH. The observation that the affinity of pWDLcB1 DNA for DHFR alone and the DHFR–NADPH complex is significantly greater than that for the DHFR–folinic acid and DHFR–NADPH–folinic acid complexes therefore strongly suggests that:

(i) DHFR in the presence or absence of NADPH binds to a specific site in pWDLcB1 DNA at or near the DHFR structural gene;

(ii) The affinity of the specific site for DHFR is significantly reduced in the presence of folinic acid.

The small difference between the filter binding curves for the binding of the DHFR–folinic acid and DHFR–NADPH–folinic acid complexes to pWDLcB1 DNA and the filter binding curves for the binding of DHFR alone or in the presence of NADPH and/or folinic acid to pBR 322 DNA could be accounted for by the longer length of pWDLcB1 DNA (7.26 kb [6]) compared to pBR 322 DNA (4.36 kb [5]).

These results suggest that specific binding of DHFR to DNA might be of physiological relevance to the regulation of bacterial DHFR synthesis in vivo. Such a hypothesis is supported by genetic data from *Diplococcus pneumoniae* and *Escherichia coli* where some mutants resistant to either trimethoprim or metho-

trexate result in increased DHFR levels due to increased mRNA synthesis and map in or near the DHFR structural gene [9–12]. One possible model for the regulation of bacterial DHFR synthesis which accounts for our binding data and the genetic data is as follows:

> DHFR alone or as a binary complex with coenzyme binds specifically to a site in or near the DHFR structural gene preventing transcription of the structural gene; when DHFR is in the form of a ternary complex the affinity of the specific site for DHFR is reduced resulting in a decreased level of repression.

This model predicts that a mutation in the specific site resulting in a decrease in its affinity for DHFR will result in an increase in the intracellular concentration of DHFR.

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