CHAPTER 2

DIHYDROFOLATE REDUCTASE

2.1 Introduction

Dihydrofolate reductase [DHFR; 5,6,7,8-tetrahydrofolate-$NADP^+$ oxidoreductase (E.C. 1.5.1.3)] catalyzes the reduction of 7,8-dihydrofolate (DHF) to 5,6,7,8-tetrahydrofolate (THF) by nicotinamide adenine dinucleotidephosphate (NADPH) [47]. The Figure 2.1 is the schematic illustration of the reaction.

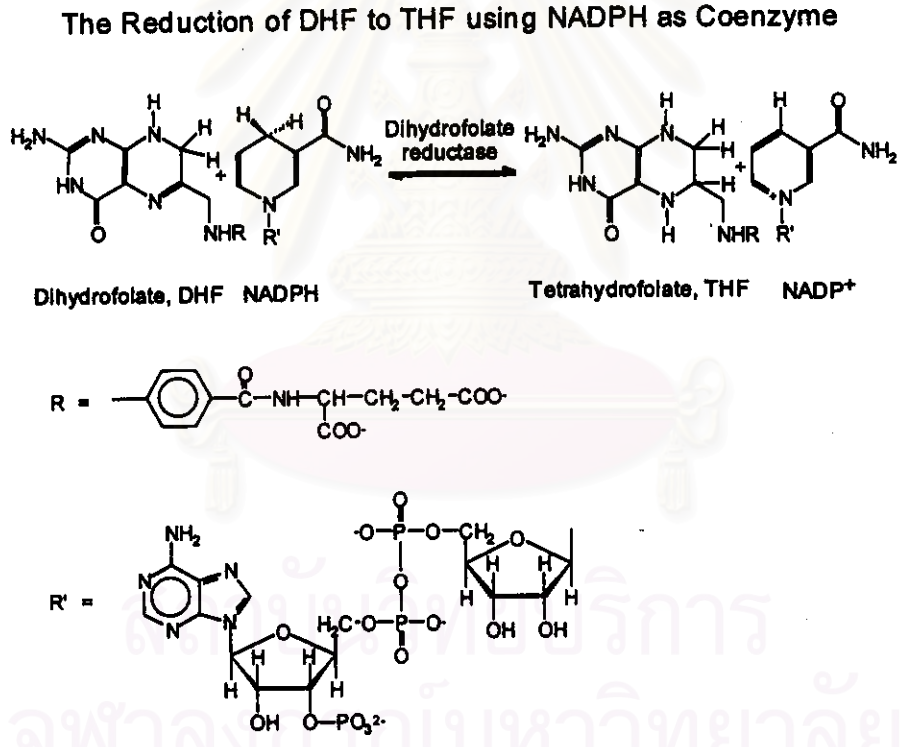

Figure 2.1 The reduction of DHF to THF using NADPH as coenzyme [47].

The THF and its derivatives involve in an essential step of the *de novo* synthesis of glycine, purines and deoxythymidine phosphate (the precursors of DNA synthesis), and are also of important in the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) as can be seen from Figure 2.2.

The enzyme is found in all cells. Its biological role is maintaining of folate levels in organism [47]. An inadequate level of THF causes to the decrease of nucleotide synthesis, leading to cell death. On the basis of the enzyme metabolic consequence, DHFR is a pharmacologically important intracellular target enzyme for a number of antifolate agents such as methotrexate (MTX) which is an anticancer agent, trimethoprim (TMP) which is an antibacterial agent, cycloguanil and pyrimethamine (CYC, PYR) which are an antimalarial agent [1].

In the process of designing novel drugs, one should know the action of the drug at the active site of the target. Recent interest has focused to the field of molecular biology. Details of atomic interactions between protein and drug molecules are the crucial information, which are meaningful in understanding the actions between drug and target molecules. Source of such information can be obtained from the three-dimensional structure incorporating with its behavior in solution as the real situation in human body.

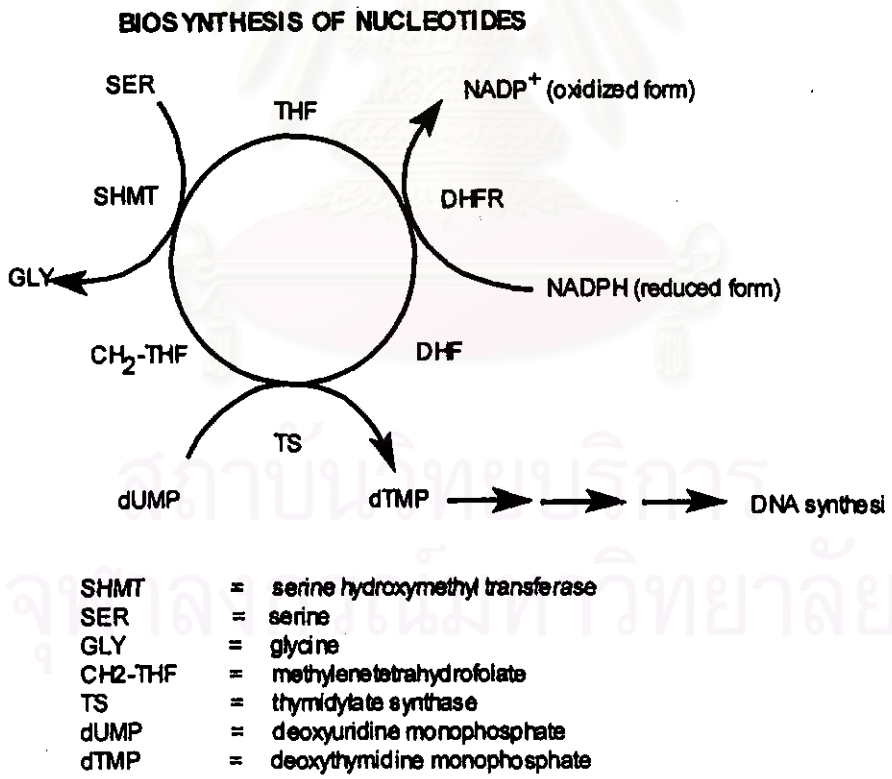

Figure 2.2 Biosynthesis of nucleotide [47].

DHFR was interesting and greatly stimulating because of its low molecular weight (18000-22000 daltons), and in all cases the enzyme is monomeric [50]. This makes this enzyme suited for structural studying. The x-ray crystallographic and NMR studies of a variety of protozoal, bacterial and vertebrate DHFRs, spanning all combinations of bound and unbound ligands, including *Leishmania major* [2], *Lactobacillus casei* [3-5], *Escherichia coli* [3;6;7], *Pneumocystis carinii* [8], chicken liver [9-13], and human [5;14-17] have provided useful structural information. Up to now, there are twenty-two entries of the three dimensional structure of DHFRs available in Brookhaven Protein Data Bank. They are listed in Table 2.1.

2.2 Structural studies on DHFR

Over the last decade, there has been an extensive study of DHFR from various sources. This made DHFR the best-understood enzyme structure. In order to elucidate the structure of the DHFR, many tools were used and the greatest advance in insight resulted from X-ray crystallography and sequencing studies.

2.2.1 X-ray diffraction results

2.2.1.1 Backbone structure

The first performance of x-ray diffraction studies is the work on crystals of the complex of MTX with DHFR from *E.coli* [28], and later on crystals of the ternary complex of *L.casei* DHFR with NADPH and MTX [29;30]. In both cases, a twisted β sheet that contains eight strands, all parallel except the last strand dominates the structure (Figure 2.3). These strands are designated as βA to βH, βA being nearest to the N-terminus and βH nearest to the C-terminus. The strand is in G-H-F-A-E-B-C-D order from left to right. Four α helices are packed against the β sheet and are designated corresponding to the strand; αB, αC, αE and αF. Loops that join the elements of secondary structure are the remainder of the residues.

Table 2.1 twenty-two PDB entries in database protein data bank [42].

ID	Source	Ligands	Resolution	Reference
1dhf	*Homo sapiens*	Folate	2.30	[18]
1dhi	*Escherichia coli*	MTX	1.90	[19]
1dhj	*Escherichia coli*	MTX	1.80	[19]
1dr1	*Gallus Gallus*	$NADP^+$ and Biopterin	2.20	[20]
1dr2	*Gallus Gallus*	Thionicotinamide adenine dinucleotide phosphate	2.30	[21]
1dr3	*Gallus Gallus*	Thionicotinamide adenine dinucleotide phosphate and Biopterin	2.30	[21]
1dr4	*Gallus Gallus*	NADPH and Biopterin	2.40	[22]
1dr5	*Gallus Gallus*	NADPH	2.40	[22]
1dr6	*Gallus Gallus*	NADPH and Biopterin	2.40	[22]
1dr7	*Gallus Gallus*	NADPH	2.40	[22]
1dra	*Escherichia coli*	-	1.90	[24]
1drb	*Escherichia coli*	-	1.90	[24]
1drf	*Homo sapiens*	Folate	2.0	[14]
2dhf	*Homo sapiens*	5-Deazafolate	2.30	[18]
2dre	*Escherichia coli*	-	1.90	[24]
3dfr	*Lactobacillus casei*	NADPH and MTX	1.70	[23]
3drc	*Escherichia coli*	-	1.90	[24]
4dfr	*Escherichia coli*	MTX	1.70	[23]
5dfr	*Escherichia coli*	-	2.30	[25]
6dfr	*Escherichia coli*	$NADP^+$	2.40	[26]
7dfr	*Escherichia coli*	Folate and $NADP^+$	2.50	[26]
8dfr	*Escherichia coli*	-	1.70	[27]

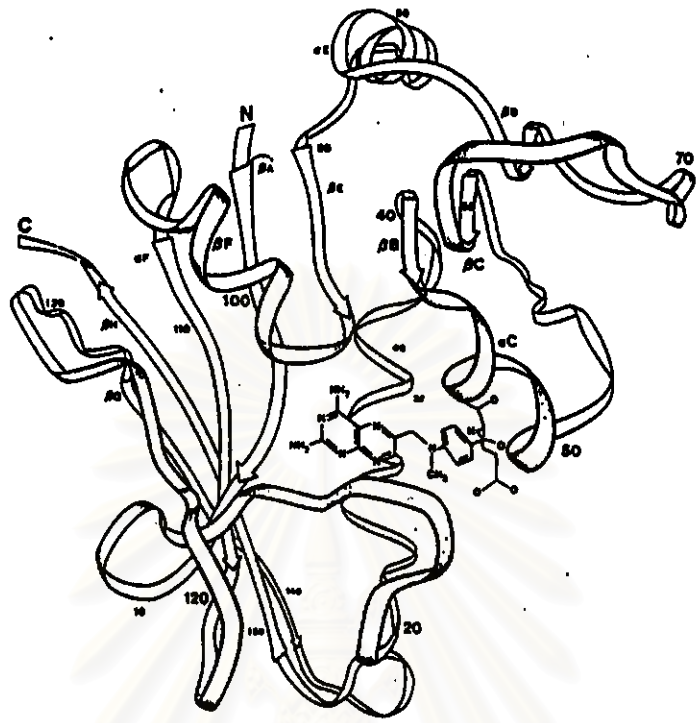

Figure 2.3 Backbone ribbon drawings of the *E.coli* DHFR-methotrexate binary complex. β-Strands (represented by arrow) and α-helices are labeled. The approximate position of every tenth residue is indicated [50].

2.2.1.2 The active site

The active site of the enzyme is lined by hydrophobic side chains and is pronounced 15Å deep cutting from its face [44]. The interactions that indicated the binding between the enzyme and substrate are hydrophobic and van der Waals. The studies of the binding of folate and MTX to DHFR indicated that although the substrate and inhibitor are similar in their structures, the pteridine ring of MTX binds to DHFR in the different orientation to that of folate and dihydrofolate as can be seen in Figure 2.4.

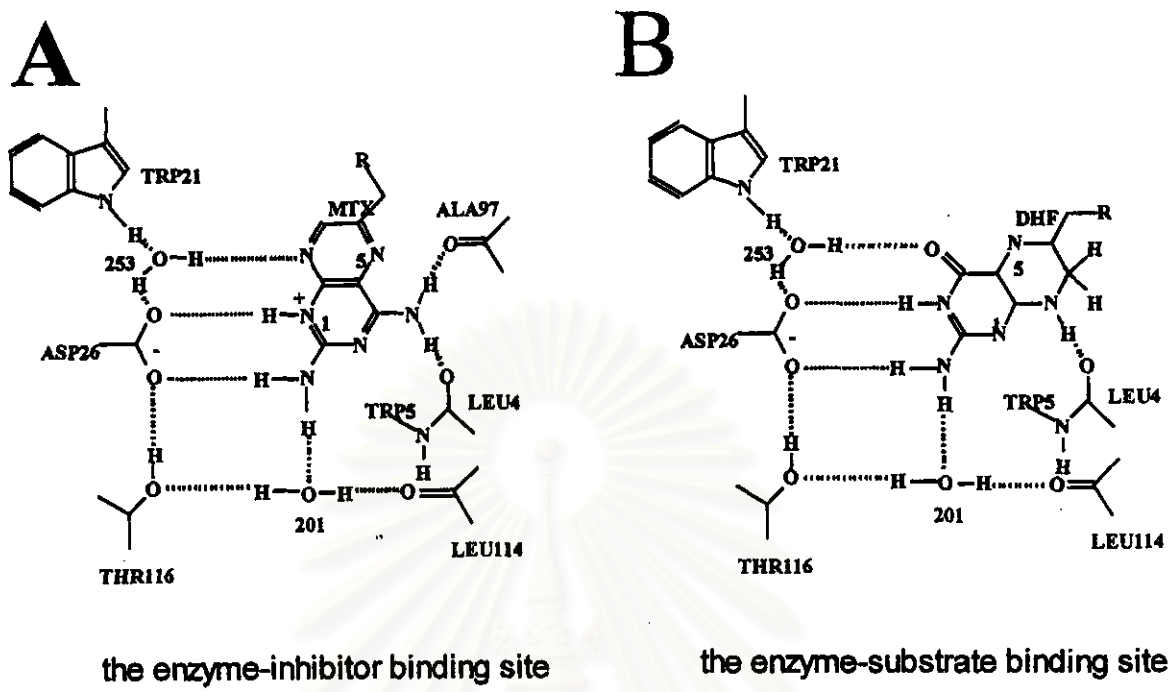

Figure 2.4 Schematic representation of the enzyme-inhibitor binding site and the enzyme-substrate binding site. Those drawing were based on the x-ray structure of *L. casei* DHFR [50].

2.2.2 Conformational transitions

There are several techniques that point out the conformational change in protein structure [50]. These include X-ray diffraction and NMR in which the evidence of conformational transitions and conformational changes induced by ligand binding. The X-ray studies provided the result that the backbone in the solvent exposed loops has the greatest flexibility, while in the various elements of secondary structure it is more rigid.

High field NMR was used to detect the equilibria between conformational states of DHFR and its complexes. Certain regions of the proton NMR spectrum corresponding to the aromatics residues are interpretable and the resonance of some specific residues in the sequence are assigned with a reasonable degree of confidence. There are some studies on DHFR from *L.casei*. Dynamic processes of protein are detected by observing its nuclei using NMR. It is indicated that side chains of each of the tyrosines in *L.casei*

DHFR undergo rapid interconversion between two conformers related by 180° rotation about the C-C bond [31]. This flipping of the aromatic rings appears to occur both in free enzyme and in complexes with inhibitors and coenzymes.

The binding process of ligand to protein, according to the induce-fit concept, will perturb the conformation of protein. There have been several reports indicated that the changes in NMR spectra of DHFR nuclei occur as a result of ligand binding. And this has been interpreted frequently as indicating induced conformational change.

2.3 DHFR from human

Human dihydrofolate reductase (hDHFR), with a molecular weight about 21,500 daltons, is a monomeric and monofunctional enzyme [42]. The hDHFR composes of 186 amino acid residues. Its amino acid sequences are as follows:

1				5					10			
VAL	GLY	SER	LEU	ASN	CYS	ILE	VAL	ALA	VAL	SER	GLN	ASN
MET	GLY	ILE	GLY	LYS	ASN	GLY	ASP	LEU	PRO	TRP	PRO	PRO
LEU	ARG	ASN	GLU	PHE	ARG	TYR	PHE	GLN	ARG	MET	THR	THR
THR	SER	SER	VAL	GLU	GLY	LYS	GLN	ASN	LEU	VAL	ILE	MET
GLY	LYS	LYS	THR	TRP	PHE	SER	ILE	PRO	GLU	LYS	ASN	ARG
PRO	LEU	LYS	GLY	ARG	ILE	ASN	LEU	VAL	LEU	SER	ARG	GLU
LEU	LYS	GLU	PRO	PRO	GLN	GLY	ALA	HIS	PHE	LEU	SER	ARG
SER	LEU	ASP	ASP	ALA	LEU	LYS	LEU	THR	GLU	GLN	PRO	GLU
LEU	ALA	ASN	LYS	VAL	ASP	MET	VAL	TRP	ILE	VAL	GLY	GLY
SER	SER	VAL	TYR	LYS	GLU	ALA	MET	ASN	HIS	PRO	GLY	HIS
LEU	LYS	LEU	PHE	VAL	THR	ARG	ILE	MET	GLN	ASP	PHE	GLU
SER	ASP	THR	PHE	PHE	PRO	GLU	ILE	ASP	LEU	GLU	LYS	TYR
LYS	LEU	LEU	PRO	GLU	TYR	PRO	GLY	VAL	LEU	SER	ASP	VAL
GLN	GLU	GLU	LYS	GLY	ILE	LYS	TYR	LYS	PHE	GLU	VAL	TYR
GLU	LYS	ASN	ASP									

Three dimensional structures of hDHFR have been solved by x-ray techniques with the resolution ranging from 2.0 to 2.5 Å [5;12;14-17]. However, the x-ray structure of the unligated enzyme has not yet been solved due to the poor stability of the protein. Multidimensional NMR spectroscopy was used to assign the resonance of the backbone, side-chain protons and a number of NOE's observed both from the hDHFR itself and

those complexed with methotrexate. Still the three dimensional solution structure of hDHFR has not been obtained.

2.4 Goal of this study

In this study, the molecular dynamics (MD) simulation was used to study the solution structure of the unbound DHFR from *Homo sapiens* at 300 and 310.5K. With the objectives to (1) extend our understanding of the x-ray structure of the hDHFR; (2) explore the dynamics of the apo-enzyme in solution at 300K and 310.5K; and (3) compare the dynamics properties of the apo-enzyme in solution at 300K and 310.5K. The structural and dynamics information obtained from this study may shed light into the relationships between protein structures and its biological function and lead to understanding in substrate-enzyme recognition.