16:56:28 OCA PAD INITIATION - PROJECT HEADER INFORMATION 03/30/89

Active

Project #: G-41-A01 Cost share #: G-41-379 Rev #: 0
Center # : R6481-1A0 Center shr #: F6481-1A0 OCA file #:
Work type : RES
Contract#: 5 R29 GM39779-02 Mod #: Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N
Main project #:

Project unit: PHYSICS Unit code: 02.010.152
Project director(s):
HUANG T-H PHYSICS (404)894-2821

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 890401 to 900331 (performance) 900630 (reports)

Sponsor amount	New this change	Total to date
Contract value	94,213.00	94,213.00
Funded	94,213.00	94,213.00
Cost sharing amount		16,441.00

Does subcontracting plan apply ?: N

Title: STRUCTURE, DYNAMICS AND FUNCTION OF DIHYDROFOLATE REDUCTASE

PROJECT ADMINISTRATION DATA

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Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A N/A supplemental sheet
Equipment title vests with: Sponsor GIT X
NONE PROPOSED

Administrative comments -
NOA DATED 3/15/89 AUTHORIZES $94,213 FOR YEAR 2 OF 3 YEAR AWARD

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 07/19/90

Project No. G-41-A01 Center No. R6481-1A0

Project Director HUANG T-H School/Lab PHYSICS

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

Contract/Grant No. 5 R29 GM39779-02 Contract Entity GTRC

Prime Contract No.

Title STRUCTURE, DYNAMICS AND FUNCTION OF DIHYDROFOLATE REDUCTASE

Effective Completion Date 900331 (Performance) 900630 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	Y	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments CONTINUED BY G-41-612.

Subproject Under Main Project No.

Continues Project No. G-41-679

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

NOTE: Final Patent Questionnaire sent to PDPI.

1. Plan for next year:

Isolation of R67 DHFR appears to be more difficult than initially envisioned. However we have obtained a new DHFR over-producing strain of E. coli from Dr. E. Howell of University of Tennessee. We were able to isolate 60 mg of chromosomal DHFR per liter of culture. We have obtained gram quantity of DHFR. Thus we decided to concentrate only on the chromosomal species for next year. In addition to the solid state NMR experiments that were initially proposed, we will also carried out some solution NMR experiments to characterize this enzyme more thoroughly. As reported below there is relatively little information known about the solution conformation of this enzyme. Thus solution work is crucial for future analysis of solid state results.

2. Studies conducted during the current year :

A. Characterization of basic solution properties of DHFR:

There has been no report on solution NMR study on chromosomal DHFR. Thus we decided to undertake some solution NMR studies to characterize our enzyme. The work proved to be worthwhile. So far the following results have been obtained:

(a) DHFR catalyzes the oxygen-dependent oxidation of NADPH:

While attempting to determine the solution conformation of NADPH in protein complex we were surprised to find that added NADPH was oxidized rapidly to $NADP^+$. The rate of oxidation is about 10^{-4} times that of the normal reductase activity. Further characterization revealed that this activity is oxygen-dependent and can be inhibited by normal DHFR inhibitors such as TMP and MTX. Four moles of NADPH was oxidized per mole of oxygen consumed. The specific activity is highly pH dependent. It increases from 9.6×10^{-3} units/mg protein at pD = 5.2 to 0.56×10^{-3} units/mg protein at pD = 8.0 at 21°C. The pH profile fit quite well to a titration curve of s single group with a pK of 6.5. The activation energy was determined to be 18 kcal/mol at pD = 6.8. NADP+ partially inhibit this reaction. Thus this oxidative activity of DHFR shares several similar characteristics of the normal reductase activity and suggests some common structural requirements. The significance of this newly discovered activity need to be further explored. However it is clear that one must be careful in attempting to determine the structure of DHFR/NADPH complex. The results have been submitted to Journal of Biological Chemistry for publication. Copies of the manuscript are attached (Appendix A).

(b) ^{31}P characterization of solution conformation:

We have conducted extensive ^{31}P NMR investigations of binary and ternary $NADP^+$ and NADPH enzyme complexes. These results are summarized and attached (Appendix B). The major findings are (i) Only single cofactor binding site was detected. This is different from that of the MB1428 species where the presence of multiple binding sites was reported. (ii) DHFR/$NADP^+$ and DHFR/NADPH exist in solution in two conformations of nearly equal populations at pD = 6.8 as characterized by the presence of two ^{31}P resonances for the 2'-phosphate in these complexes. (iii) The observation of two resonances of 0.22 ppm apart suggests that the kinetics of conformational equilibrium is slower than 100 s^{-1}. (iv) The two conformations have very different affinities toward cofactors bindings. No cofactor binds to the low affinity form (L) prior to the complete binding at the high affinity form (H). (v) The linewidth of the ^{31}P resonance corresponding to H form is narrower and is temperature independent whereas that of the L form is much broader and exhibit exchange broadening behavior. Thus the high affinity site is also the tighter binding site. (vi) The kinetics of $NADP^+$ binding to the L conformation agree well with that determine by Benkovic et al. Complete analysis of these data will take approximately one month.

B. Solid state NMR experiments:

Two major difficulties with solid state NMR studies of proteins are: (i) The need of large quantities of protein for each sample. We have solved this problem by being able to obtain gram quantities of protein. (ii) The need to maintain protein in proper state such that there is no overall rotation yet retain its native solution conformation. Thus samples for solid state NMR is usually in polycrystalline form. The question of whether a protein molecule is in its native solution conformation in the crystal form is an important one and has been a topic under intensive scrutiny. The answer appears to be positive. However growing protein crystal is a non-trivial task. While we are trying to grow protein crystals we decided to test two other approaches. The first method is to us partially hydrated sample. There are evidences that suggest that the presence of 20 to 30 wt% of water is sufficient to allow macromolecules to retain their native conformations. However there is no solid proof for this. The other possible form is ammonium sulfate precipitate. The mechanism of ammonium sulfate precipitate is believed to be a salt-out process and the conformation is widely regarded as in near native form. We are in the process in performing experiments to probe the conformation of DHFR in these two forms. We have obtain some deuterium NMR spectra of (3,4-d_6)TMP/DHFR and (2',6'-d_2)TMP/DHFR in either ammonium precipitate or in partially

hydrated forms (Appendix C). The purpose of this study is to determine the dynamics of TMP bound to the active site. The spectra of (3,4-d_6)TMP alone is typical of rigid methoxyl group with fast methyl group rotation even at temperature as high as 70°C. The spectra of (3,4-d_6)TMP/DHFR are apparently motionally averaged. The detailed dynamic behavior is yet to be deduced from lineshape simulation and relaxation measurements which are currently underway. This will take approximately one month to complete. The spectra of (2,6-d_2)TMP/DHFR is surprisingly rigid, a Pake pattern of breadth 120kHz. Thus the benzyl ring motion is likely to be small amplitude (< 10°). We are attempting to measure T_2 and T_1 to obtain more complete dynamic information on the benzyl ring.

In conclusion, during the last year we obtained the following results:

(i) We found that DHFR is capable of catalyzing oxygen-dependent oxidation of NADPH. The catalytic activity shares some common properties of normal DHFR reductase activity.

(ii) We have employed ^{31}P NMR to characterize the solution conformation of E. coli in binary or ternary complexes with cofactors and other ligands. The presence of multiple conformation is detected. The characteristics appears to be differ from that of the E. coli RT500 or L. casei species.

(iii) We have partially completed deuterium NMR experiments of (2',6'-d_2)TMP/DHFR and (3,4-d_6)TMP/DHFR complexes and is in the process of deducing the dynamic properties of these complexes.

3. Human subjects : Not applicable.

4. Vertibrate Animals : Not applicable.

5. Publications:

(i) "Dihydrofolate Reductase from E. coli catalyzes the oxygen-dependent oxidation of NADPH in the absence of folates" by F.-y Huang et al submitted to J. Biol. Chemistry.