BIOORGANIC CHEMISTRY **24,** 10–18 (1996)
ARTICLE NO. 0002

On Transition Structures for Hydride Transfer Step: A Theoretical Study of the Reaction Catalyzed by Dihydrofolate Reductase Enzyme

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Received February 14, 1995

A theoretical study is presented of the catalytic mechanism of dihydrofolate reductase (DHFR) enzyme based upon the characterization of the transition structure (TS) for the hydride transfer step. Analytical gradients at AM1 and PM3 semiempirical levels have been used to characterize the saddle point of index one (SPi-1) on global energy hypersurface for the hydride transfer in the active site of DHFR enzyme. The geometry, stereochemistry, electronic structure, and transition vector (TV) components associated to SPi-1 are qualitatively computational level independent. The TV amplitudes show primary and secondary isotope effects to be strongly coupled. The geometrical arrangement of the TS results in optimal frontier orbital interaction. Comparison of the TS geometry with the X-ray coordinates shows that the TS can be fitted without any stress at the active site. By comparison of the TS for the hydride transfer step in models of related enzymes, the geometries and the components of TV for TSs in hydride transfer steps are transferable and invariant. The results of this study suggest that the primary function of the enzyme is to constrain the reactants in the endo configuration, situating the system in a neighborhood of a SPi-1. This fact is in accordance with Pauling's postulate for enzyme catalysis.

INTRODUCTION

The enzyme dihydrofolate reductase (5, 6, 7, 8-tetrahydrofolate: NADP oxidoreductase, E.C. 1.5.1.3) (DHFR) catalyzes the NADPH-dependent reduction of 7,8-dihydrofolate (DHF) to 5, 6, 7, 8-tetrahydrofolate (THF) in both bacterial and vertebrate cells (*1*) (Scheme 1). DHFR is necessary for maintaining intracellular pools of THF and its derivatives, which are essential cofactors in the biosynthesis of several amino acids, are an important target for drug (inhibitor) therapy, and have been extensively studied from many points of view (*2*). Nevertheless, the details of the enzymatic molecular reaction mechanism are still unclear and can only be hypothesized at present (*3–5*). The mechanism of the reaction requires the addition of both a proton and a hydride ion (from NADPH) to dihydrofolate, and it is generally accepted that the protonation precedes hydride ion transfer and activates the substrate (*6*).

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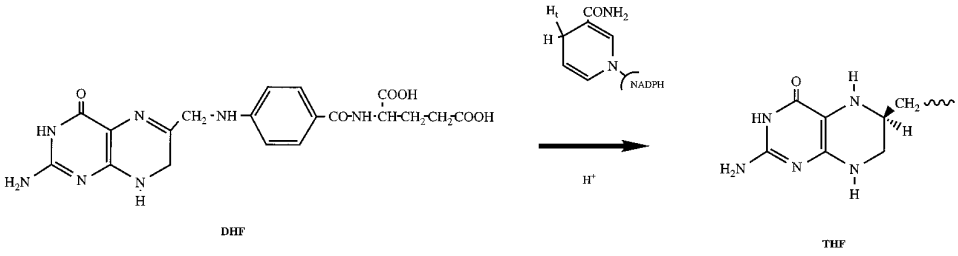
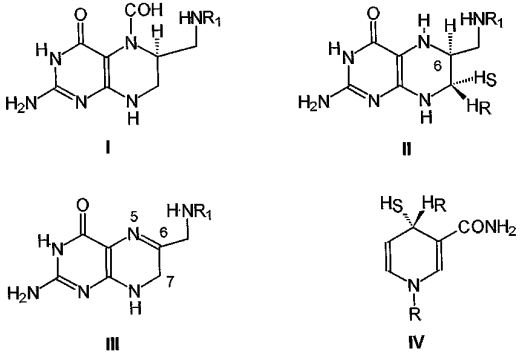


SCHEME 1

The stereochemistry on C6 of the biologically active diastereoisomer of the folinic acid, **I** in Scheme 2, has been determined like *S* based on X-ray findings on 5,10-metenyltetrahydrofolate (*7*). The interconversion of all the tetrahydrofolic acid derivates in the biological systems by means of enzymatic reactions retains the stereochemistry on C6. Therefore, it is possible to determine the configuration of this carbon atom in the tetrahydrofolic acid (*8*). The purification of the tetrahydrofolate, **II,** followed by the conversion to folinic acid, **I,** rents the active stereoisomer of THF, thus defining the stereochemistry on C6 of tetrahydrofolate and the reduction enzymatic reaction like (*S*). In this sense, the attack of the NADPH takes place on the reface of the 7,8-dihydrofolate, **III.** On the other hand, it is well established that the enzymatic reduction of **III** requires the transfer of the hydrogen 4-pro-R from the NADPH, **IV** (*1, 9*); this hydride transfer takes place from the lower face of NADPH with the amide group pointing in the direction of C7 of the dihydrofolate, thus defining the stereochemistry of the reaction.

The key question to understanding the global transfer of a hydride ion between C4 of dihydropyridine ring (NADPH) and C6 of the pteridine ring (DHF) is the nature of transition state (TS). The concept of TS plays a central role in describing mechanistic aspects of chemical reactions and its theoretical characterization is



SCHEME 2

based on the theory of rate processes (*10–14*). While in the past TS were difficult to obtain, contemporary computational quantum chemistry offers almost the unique way to determine them with high accuracy. This provides an independent source of information concerning the geometry, stereochemistry, charge distribution, and the reactive fluctuation patterns. The value of TS for understanding enzyme catalysis is widely used (*15*). The TS for the hydride transfer step in the DHFR active center was assembled as shown in Fig. 1, where a schematic view of the geometry and nomenclature is depicted.

In quantum chemical terms this stationary point on the potential energy hypersurface corresponds to a saddle point of index one (SPi-1), where the eigenvector or transition vector (TV) (*16*) associated with the unique negative eigenvalue describes those atomic infinitesimal displacements that are related to the interconversion step for a given molecular mechanism.

The geometry of this TS *in vacuum* is determined with quantum chemical techniques including analytical gradients and Hessians. One of the interesting results obtained is a sort of geometric invariance of the fragments participating in a SPi-1, for a given interconversion step the corresponding transition state structure is invariant. Recently we have located the TSs for hydride transfer for models of different enzymes, liver alcohol dehydrogenase (*17, 18*), formate dehydrogenase (*19*), lactate dehydrogenase (*20*), and DHFR, using a reduced molecular model (*21*). Furthermore, for the enzyme-catalyzed reactions studied so far (*22*), it is only the geometric arrangement of the SPi-1 *in vacuum* which can be docked at the active site of the corresponding enzyme.

Following the analysis of important reactions, we have suggested (*23, 24*) that the phenomenon of catalysis can be highlighted from the characterization of SPi-1 *in vacuum*. The enzyme catalysis phenomena can be described as a specific binding process. The enzyme would have evolved to trap the reactants by molding them into a geometry reassembling, as much as possible, one of the SPi-1 *in vacuum* (*22–24*), and then a molecular deformation is introduced to adapt them at the corresponding active site surface. These results may be transposed to the DHFR reaction subject to the restrictions imposed by stereochemical considerations, so that a model emerges as a possibility for the chemical reduction step (*25*).

In the present study, AM1 and PM3 semiempirical calculations have been carried out in order to investigate the hydride transfer step in DHFR enzyme, with special emphasis on the characterization of the TS. The comparative analysis of this work and the results presented in related studies (*21*) could prove the basic hypothesis for a typical hydride transfer reaction in enzyme catalysis. Under Computing Methods and Models, computing methods are briefly described. Results are presented and analyzed and a general discussion closes the paper under Results and Discussion.

COMPUTING METHODS AND MODEL

Semiempirical calculations, AM1 (*26*) and PM3 (*27*), have been performed with the GAUSSIAN 92/DFT series of programs (*28*). The molecular geometries were optimized using Berny analytical gradients optimization routines (*29, 30*). The

request convergence on the density matrix was 10^{-9} a.u., and the threshold value of maximum displacement was 0.0018 Å and that of maximum force was 0.00045 hartree/bohr.

Examination of the TS has been achieved by the evaluation of the Hessian matrix; the nature of these stationary points was established by calculating analytically and diagonalizing this matrix of energy second derivatives to determine the unique imaginary frequency. Once the stationary points are obtained, the Hessian is recalculated and the zero-order atom dynamics are obtained with a calculation of normal modes.

Computational chemical models are playing an ever increasing role in chemical research. During the last decades quantum mechanical calculations have been gradually recognized as a useful tool in research related to elucidation of molecular reaction mechanisms. Semiempirical methods are intended for studying large molecular systems of chemical or biological interest. The different features of the various semiempirical methods arise from the different approximations introduced, the different parametrization procedures, and the different sets of molecules used to obtain the parameters. Semiempirical methods have progressed over the past few years to a surprising level of accuracy and reliability, considering the limitations of the underlying approximations (*31, 32*).

The elements of the molecular model used in the calculations are the following: the THF is represented by a pteridine ring and the coenzyme NADP is represented by the N-methylated nicotinamide ring reduced at C4. Both rings appear oriented as the basic constituents belonging to the catalytic system. Atom numbering of the molecular model is depicted in Fig. 1.

RESULTS AND DISCUSSION

Figure 1 and Table 1 summarize the geometry and Table 2 the TV, i.e., the eigenvector associated to the unique negative eigenvalue of the force constant matrix, for SPi-1 obtained with AM1 and PM3 semiempirical methods. The completely optimized geometries are available from the authors on request. The results obtained with the different procedures are nearly identical.

From a geometrical point of view, the TS has an endo configuration and a bent structure, with a C6–H_t–C14 angle range of 163.01–170.64. The forming C6–H_t bond is shorter than the breaking C14–H_t. The factors influencing hydride transfer geometry have been well discussed by Williams *et al.* (*33*). The endo structure seems to be a general feature for hydride transfer steps (*19, 21, 34–38*).

The TV yields in a nutshell the essentials of the chemical process under study. The atomic displacements in the TV correspond to the hydride transfer step for the reaction catalyzed by DHFR. The motion corresponds to H_t from C14 and C6. The normal mode analysis of TS yields a relatively high imaginary frequency: 1469i and 1760i cm^{-1} for AM1 and PM3, respectively, corresponding to the breaking C6–H_t bond and the interatomic C6–C14 distance. For AM1 results, all force constants are positive and the unique negative eigenvalue results from the cross terms in the force constant matrix while for PM3 results, the negative curvature of