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Towards an Explanation of Carboxylation/Oxygenation Bifunctionality in Rubisco. Transition Structure for the Carboxylation Reaction of 2,3,4-Pentanetriol

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Abstract. We construct a theoretical model of the transition structure for the carboxylation reaction of ribulose-1,5-biphosphate catalyzed by Rubisco. This is a first-order saddle point on the energy hypersurface for the nucleophilic attack of carbon dioxide on CH_3—$(CHOH)_3$—CH_3 at the C2 center. *Ab initio* analytical gradients methods at a 4-31G basis set level are used.

The carbon framework and oxygens of the stationary structure superpose with the corresponding atoms of 2-carboxyarabinitol-1,5-biphosphate, which is a transition state analog that has recently been highly refined with X-ray methods. The hydroxyl group in C3 is *cis* to the C2 oxygen. The C3 center is somewhat pyramidized, the dienol O2–C2–C3–O3 is not planar.

The geometry of the transition state allows for simple explanations of both the enolization of Rubisco's substrate ribulose-1,5-biphosphate, O_3PO—CH2—CO—$(CHOH)_2$—CH_2—OPO_3 and oxygenation reaction. The former is due to the pyramidal deformation at C3 and out of plane of O2–C2–C3–O3 framework: the enoliation is intramolecular and is probably enhanced by proton tunnelling. The latter is related with the fact that a rotation around an ethylene-like bond brings the triplet state down in energy. The reactive skeleton has a stationary geometry in the triplet state not very different from the one obtained in the global transition structure. There, the triplet is only 9 kcal/mol above the singlet. The spin densities at C2 and C3 centers clearly indicate the place where oxygenation will take place.

Key words. Rubisco, carboxylation, oxygenation, transition structure, catalysis.

1. Introduction

Rubisco (Ribulose-biphosphate carboxylase-oxygenase) controls two competing physiological functions in green plants via its catalytic activity in the carboxylation reaction of photosynthetic carbon fixation and the competing oxygenase reaction of photorespiration [1–8]. Via photorespiration, stored energy is dissipated as heat, thereby limiting crop yield. It is therefore an important economical and ecological issue to change the carboxylase/oxygenase ratio in this type of plant and improve the efficiency of photosynthesis. For these and other scientific reasons, this molecule has become an important target for protein engineering studies. The changes carried on the enzyme designed to selectively modify the function requires a thorough knowledge of the catalytic mechanism, the 3-D structure of the molecule and how the structure of the active site is achieved. Essential progress along these lines has been made [1–8] but the nature of bifunctional catalysis remains an unsolved problem [8].

In this paper, the geometry of a transition state for the carboxylation step is

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worked out. The transition structure corresponds to a first order saddle point on the energy hypersurface of the enolized 2-ketoarabinitol interacting with carbon dioxide. Only the phosphate groups are missing in this model with respect to the real substrate RuBP (ribulose-1,5-biphosphate). The transition structure (TS) is compared with the crystallographically determined transition state analogue 2-carboxy-*d*-arabinitol-1,5-biphosphate (CABP), which has recently been refined.

Thereafter, the structure of the triol moiety found in the TS is optimized in its electronic triplet state. This new stationary structure is compared with a recently reported X-ray structure of the substrate bound to the enzyme.

2. Model System and TS Determination

According to the well accepted molecular mechanism, the transition state for the carboxylation reaction must be found on the enolized form of RuBP [1–8]. The starting structure corresponds to the 2-pentene-2,3,4-ol, which is the enolized form of 2-ketoarabinitol. The two phosphate groups present in RuBP are not present in our model; they are assumed to determine the substrate binding process.

The global internal geometry parameters are separated in two subspaces: (1) the control space; and (2) the complementary space. The former includes all chemically relevant degrees of freedom. They are those contributing with finite amplitudes to the transition vector. This latter is the eigenvector corresponding to the unique negative eigenvalue obtained after diagonalization of the Hessian matrix (force constant matrix) [9–11]. The complementary space gathers all other internal degrees of freedom that do not contribute with meaningful amplitudes to the transition vector.

The geomery optimizations are carried out alternatively on each subspace, one at a time, until a stationary structure is obtained (forces below a given, small threshold). In the control space, the subroutines based on the VA05 method [12] are used to bracket a first order saddle point. This method has been developed [11] and applied in our laboratory to determine the transition structures for a number of enzyme catalyzed reactions [14–16].

The geometry of the bent carbon dioxide moiety found in the transition structure for the zinc hydroxide mechanism in carbonic anhydrases [15] is used to seed the calculations. We assume that in the transition states, where carbon dioxide acts as a nucleophile, the corresponding structure is (almost) invariant [16].

Restricted and unrestricted Hartree–Fock SCF-MO calculations and the analytical gradients were calculated with MONSTERGAUSS program [17]. The standard split-valence 4-31G basis set was used.

3. Nature of the Saddle Point

(i) The structure of the transition state is depicted in Figure 1. Compared with the X-ray TS analog, both structures superimpose fairly well. This case can be considered as an example of Pauling's lemma [18], namely, that the structure of the active site is complementary in shape to the geometry of the transition state for the reaction catalyzed by the enzyme.

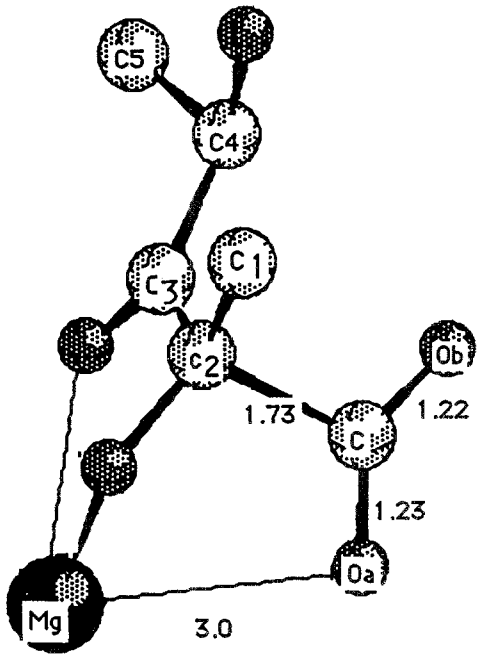

Fig. 1. Spatial disposition and geometrical parameters of the first order saddle point. The magnesium atom is depicted to help the view. As O2 and O3 were kept at the positions found in the CABP inhibitor structure by I. Andersson, all other atoms were free to reorganize. The carbon dioxide moiety is then farther away when compared with the inhibitor, as it should be. The hydrogen sitting at O2 is making an H-bond interaction with one oxygen of the carbon dioxide moiety. This is the hydrogen presumably transferred via an intramolecular rearrangement leading to a pyramidalized and deformed diol structure. Some relevant distances (Å) are indicated in the figure. Others are: C2C3 5 1.47; C3C4 5 1.53; C4C5 5 1.56; C1C2 5 1.56; C2O2 5 1.38; C3O3 5 1.26; O4C4 5 1.40. Angles: OaCOb 5 136.28; pyramidal angle at C3 (C3C2O3C4 5 11.48).

(ii) The transition vector (TV) yields, in a nutshell, the essentials of the chemical process under study. The degrees of freedom for carbon dioxide entering the TV describe the approach to (C–C2), bending the orientation of this moiety. For the substrate moiety, the C2–C3 distance enters antisymmetrically with respect to C–C2 distance, as well as the angles related with pyramidalization of C2 and C3 and a dihedral associated with O3. The negative eigenvalue results from the cross terms in the force constant matrix.

The antisymmetric combination of C–C2 and C2–C3 distance amplitudes in the TV clearly indicates that a shortening of the C–C2 distance will produce an increase in the C2–C3 bond. This can be achieved by weakening the double-bond character of the dienol, i.e., by forcing the structure out of planarity. This is what the TV tells us.

The keto–enol equilibrium required to change the substrate into an active intermediate is most likely achieved via intramolecular proton transfer. This can be seen from the structure of the transition state. There, pyramidalization and displacement out of plane in the *cis*-dienol provide a simple path for proton exchange between the C3 hydrogen and the C2 carbonyl oxygen in the substrate.

4. Substrate Binding and Oxygenation

The geometry of the triol moiety in its electronic triplet state shows to be very near the one found in the real substrate [5] and with our carboxylation TS in so far as the stereochemistry of the diol functional groups are concerned. Under these circumstances, the energy for the triplet state of the deformed 2,3-dienol structure moves down and approaches the electronic energy of the singlet state. The spin density is mainly located at the carbon centers C2 and C3 which are involved in the carboxylation reaction on the singlet energy hypersurface. Thus, the oxygen molecule, whose ground electronic state structure is a triplet, and therefore does not strongly interact with its surroundings, may proceed almost without a solvation–desolvation barrier along its reaction coordinate to attack these centers if carbon dioxide is not already there. In so doing, relatively small geometrical changes are required to get a low-lying triplet state on the C2–C3 dienol that will combine with the incoming oxygen triplet to form a low-lying singlet for the supermolecule. This theoretical fact, which is a general property of deformed diene bonds, explains the double functionality of Rubisco.

Actually, we have just calculated the first-order saddle point structure for the oxygenation reaction. The C2–C3 centers are involved in the reaction coordinate.

5. Conclusion

The theoretical results obtained by us and the experimental structural work on inhibitor and substrate binding [5,6,8], lead us to suggest that this enzyme binds the substrate with its reactive (control) subspace with a deformed structure which corresponds with, or is very near to, the transition state geometry *for the reaction in vacuo*. From this new perspective, the mechanisms of carboxylation and oxygenation reactions can be simultaneously discussed and a unified viewpoint emerges, thereby giving an explanation of the enzyme's dual behavior.

This new framework strongly suggests that the enzyme was initially designed to carry out the oxygenation reaction. Thereafter, the active site evolved to accommodate the transition structure for carboxylation which requires a great deal of fine-tuning [1–6,8] and might have happened latter on in evolution. The change in the oxygen content of the atmosphere may have prompted this re-adaptation of an enzyme that might initially be designed to keep oxygen levels low.

The initial oxygenation reaction could not be totally wiped out by evolution since the substrate deformation leading to enolization and a *cis*-deformed diol is intrinsically built into the transition vector for carboxylation. The 'inevitability' hypothesis (see Andrews and Lorimer's review [7]) is confirmed by the present theoretical work.

It will be shown in forthcoming papers that the basis theoretical facts reported here can be used to explain nearly all experimental data obtained from site-directed mutagenesis experiments.

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