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Published in:
Journal of the American Chemical Society

DOI:
10.1021/ja034697a

2003

Document Version:
Peer reviewed version (aka post-print)

Link to publication

Citation for published version (APA):
Jensen, K., & Ryde, U. (2003). Conversion of homocysteine to methionine by methionine synthase: a density functional study. *Journal of the American Chemical Society*, *125*(46), 13970-13971. https://doi.org/10.1021/ja034697a

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Conversion of Homocysteine to Methionine by Methionine Synthase: A Density Functional Study

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Methionine synthase is a cobalamin-dependent enzyme that catalyzes the conversion of homocysteine to methionine.[1] This requires a methyl group, which is taken from the cofactor methylcobalamin (Scheme 1). Methylcobalamin is a 6-coordinate, low-spin cobalt complex, with a unique Co–C bond and a histidine from the enzyme constituting the *trans* axial ligand.[2] Upon transfer of the methyl cation, the cofactor is converted to a d^8 square-planar Co(I) intermediate.[3] Hence, methylcobalamin looses both its axial ligands during the reaction. In a remethylation step, cob(I)alamin obtains a methyl group from 5-methyltetrahydrofolate, returning to the resting state. The way in which the enzyme activates the Co–C bond for methyl transfer is not yet known, but the overall retention of methyl configuration indicates that a double S_N2 reaction may be active.[4]

We present here a theoretical study of this reaction, including a characterization of the transition state (TS). The results are based on density functional calculations with large basis sets, and they include thermodynamic, relativistic, and solvent effects. We find that the suggested S_N2 mechanism explains well the experimentally observed reaction rate. The results show that the reaction is highly polar, as reflected in the change of charge density along the reaction coordinate. It is enhanced in the protein by deprotonation of the substrate and desolvation.

The calculations were performed with the B3LYP method, which gives geometries in good agreement with experiment for model complexes of cobalamins: For methylcobalamin models in particular, calculated metal–ligand bond distances[5-8] agree with X-ray structures[9] to within 0.03 Å, except for the very flexible Co–N_{ax} bond, which is ~0.1 Å too long. All geometries were optimized with the 6–31G(d) basis set.[10] We used the Turbomole 5.3[11] and Gaussian98[12] software. The chemical model (63 atoms) included a corrin ring,[13] an imidazole as the axial ligand modeling histidine, methyl as the other axial ligand, and CH_3S^- as the substrate. The latter was assumed to be deprotonated by binding to a zinc ion, in accordance with experimental data.[4]

The optimized structures are shown in Figure 1. Accurate energies were calculated with the large triple-ζ 6–311+G(2d,2p) basis set.[14] This is important for the energy profile, increasing the barrier by 7 kJ/mol and making the reaction 10–12 kJ/mol less exothermic. The authenticity of the TS was confirmed from vibrational analysis: It displayed only one imaginary frequency of 94.3*i* cm^{-1}. The vibration coincided with a clean, axially directed S_N2 reaction coordinate, containing significant amounts of sulfur, methyl, cobalt, and axial nitrogen atom displacements.

The energies of the reaction are collected in Table 1. We estimated the solvent effect on the reaction profile from a conductor-like screening model both in water (ε = 80) and a more protein-like environment (ε = 4). In vacuum (ε = 1) the reaction is strongly exothermic (–408 kJ/mol for separated species) as would be expected for a charge neutralization reaction, but it is much less so in aqueous solution (–47 kJ/mol). The free energy of activation was calculated from the vibrations of reactants and TS by applying an ideal-gas approximation; the entropy of activation at room temperature ($T\Delta S^{\neq}$) is 12 kJ/mol relative to the reactant complex or –19 kJ/mol counting from separated reactants. This gives a total barrier $\Delta G^{\neq}$ = 104 kJ/mol at 298.15 K and 1 atm in aqueous solution (including zero-point energies). The experimental rate of product formation is 140 s^{-1} at 298 K in the enzyme,[15] which corresponds to a barrier of 61 kJ/mol. In aqueous solution, the reaction is $6{\cdot}10^6$ times slower,[16] implying a barrier of 100 kJ/mol, in excellent agreement with the calculated value.

Some structural parameters are collected in Table 2. They indicate that the TS is very early, i.e. similar to the reactant complex. Moreover, as the Co–C bond is stretched along the reaction coordinate, the Co–N_{ax} bond is also elongated by a strong *trans* electronic effect. This means that the proposed S_N2 reaction will lead to the experimentally observed square-planar d^8 Co(I) intermediate.[3] The corrin fold angle is similar in all three species. This indicates that there is no distortion of the corrin ring in the TS, which could be enhanced by the enzyme, as is suggested by the mechanochemical trigger mechanism.[17-18] A main feature of the reaction is the polarity and the large transport of charge along the *z*-axis, as seen from the atomic charges in Table 3. Already in the reactant complex, 0.37 *e* has been transferred from the substrate, explaining the strong electrostatic perturbation of the corrin and the long Co–N_{ax} bond. The imidazole group accepts 0.17 *e* during reaction, whereas the major part, 0.86 *e*, passes through the methyl and into the corrin ring.

Scheme 1. Reaction investigated in this work.

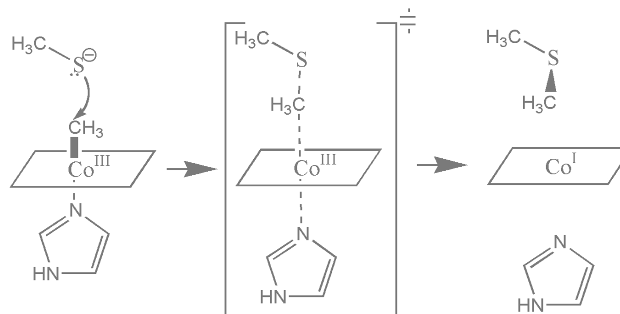

Table 1. Relative energies (kJ/mol) of species involved in the reaction mechanism (corrected for scalar relativistic effects).

System	E (ε=1)	E (ε=4)	E (ε=80)
Separated reactants	217.8	27.4	–47.9
Reactant complex	0	0	0
Transition state	20.5	29.1	34.5
Product complex	–192.5	–123.0	–72.0
Separated products	–190.3	–127.1	–94.7

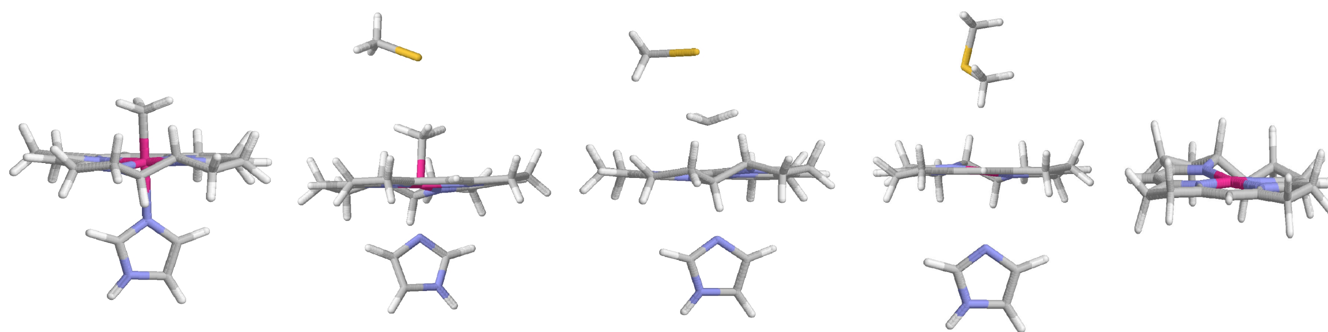

Figure 1. Optimized geometries. From left: Separated reactant, Reactant complex, Transition state, Product complex, and Separated product.

Table 2. Bond distances (Å), corrin fold angle (°), and C–Co–N_{ax} angle (°) of species involved in the reaction mechanism.

	Co–C	Co–N_{ax}	Co–N_{eq} (aver)	C–S	S–Co	Fold Angle	C–Co–N_{ax}
Separated reactants	1.97	2.25	1.92	---	---	5.3	177.5
Reactant complex	2.01	2.57	1.91	3.13	5.08	5.0	177.7
Transition state	2.08	2.70	1.91	2.88	4.96	5.1	177.6
Product complex	4.38	4.59	1.89	1.83	6.18	5.5	133.4
Separated products	---	---	1.88	1.83	---	5.8	---

Table 3. Mulliken charges of species involved in the reaction mechanism.

	N_{ax}	Co	CH_3	SCH_3	Corrin ring	Im	N_{eq} (aver)
Separated reactants	–0.39	0.54	–0.01	–1.00	0.30	0.17	–0.46
Reactant complex	–0.37	0.47	0.11	–0.63	–0.06	0.11	–0.47
Transition state	–0.37	0.49	–0.15	–0.62	0.19	0.09	–0.48
Product complex	–0.41	0.40	–0.02	0.02	–0.43	0.03	–0.50
Separated products	–0.59	0.56	(0.00)	(0.00)	–0.56	0.00	–0.53

In order to study the importance of zinc activation (partial deprotonation) of homocysteine,[19] we optimized the reactant complex and TS of a smaller (still fully conjugated) model with a protonated CH_3SH substrate. This gave a much higher activation barrier of 197 kJ/mol, so deprotonation of homocysteine is a primary and necessary step to obtain the favorable energies encountered both in the enzyme and in solution. This is in accordance with kinetic experiments in solution.[20] Moreover, it shows that an important role of the protein is to lower the acid constant of the bound substrate. This effect is not included in the $6 \cdot 10^6$ enhancement of the reaction, because the solution reaction was measured at pH = 11,[20] i.e. with a fully deprotonated substrate. However, the present calculations may underestimate the barrier in the enzyme, since a free CH_3S^- anion is probably more nucleophilic than when bound to a zinc ion.

A second role of the enzyme is seen directly from the energies in Table 1: In aqueous solution, half of the activation energy comes from the unfavorable formation of the reactant complex. However, in vacuum the formation of this complex is exothermic, and the rate-determining step is the conversion of the reactant complex into product complex. This barrier is 13 kJ/mol with all corrections. Thus, the enzyme may in principle enhance the reaction rate by 10^{14} (–80 kJ/mol) by lowering the effective dielectric constant in the active site. This idea is confirmed by the crystal structure,[2] where hydrophobic residues gather around the β-side of the co-factor. Thus, the present study suggests a dual catalytic strategy of methionine synthase: Deprotonation of the homocysteine substrate and hydrophobic stabilization of the TS. It is likely that other cobalamin-dependent methyl-transferases[19] employ similar mechanisms.[21]

Supporting Information Available: Optimized coordinates of all species are available at http:/pubs.acs.org.

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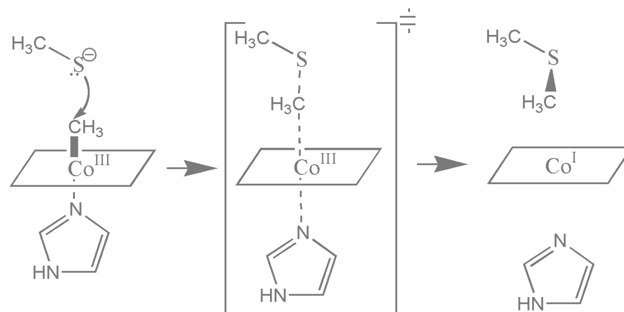
We present here a theoretical study of the conversion of homocysteine to methionine by methionine synthase. The reaction pathway is based on density functional calculations with large basis sets, including thermodynamic, relativistic, and solvent effects. We find that the suggested S_N2 mechanism explains well the experimentally observed reaction rate. The results show that the reaction is highly polar, as reflected in the change of charge density along the reaction coordinate. It is enhanced in the protein by two effects: Deprotonation of the bound substrate and desolvation of substrate and cofactor in the rate-determining step.



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