No Analytical crib available

March 31, 2007

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Acceptable answers to the Biochemistry Cume, DNA replication, March 31, 2007

Question 1. (20 points)

DNA replication starts at specific sequences known as replication origins. Initiation is achieved by several proteins and leads to the opening of the double helix by spending energy (i.e. ATP hydrolysis). An enzyme (known as helicase) separates the DNA duplex. The helicase in bacterial cells is known as DnaB. The energy for opening the double-helix comes from hydrolysis of ATP by helicase. Single-stranded DNA binding proteins (SSDBP) bind selectively and cooperatively to single-stranded DNA. Therefore, SSDBP would bind to and stabilize the single-stranded DNA generated at the replication forks by the helicase. After replication of the single-stranded DNA, SSDBP dissociates from DNA because it has a very low affinity for double-stranded DNA.

Question 2 (40 points)

A. Synthesis of nucleic acids by RNA and DNA polymerases always move in one direction. Therefore, during DNA replication, one the DNA strands can be replicated continuously. The other strand is replicated discontinuously, creating short pieces of DNA (known as Okazaki fragments).

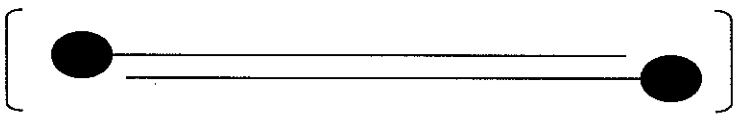
B. For DNA synthesis, DNA polymerases require a primer. In vivo, the primers consist of relatively short RNA segments synthesized by a specific RNA polymerase known as primase. The primase synthesizes the RNA on the single-stranded DNA produced by the action of DNA helicase. DNA polymerases can synthesize DNA by adding deoxy-nucleotides to the 3' end (3' hydroxyl group of the last ribose) of the primer RNA.

C. DNA polymerase I has a 5' exonuclease activity, which can hydrolyze and thus remove the RNA primers from the DNA.

D. In the course of removing the RNA primers, DNA polymerase I also synthesizes DNA to fill in the gaps generated from the degradation of the primers. DNA polymerase I uses the 3' end of a newly synthesize DNA as a primer for replicating the DNA. This process fills in the gaps between the newly synthesized DNA to produce the larger pieces of Okazaki fragments. The whole process would generate double-stranded DNA that contains "nicks", breaks in the phosphordiester backbone of double-stranded DNA. These nicks are closed by DNA ligase to produce contiguous double-stranded DNA.

Question 3 (10 points)

After the completion of DNA replication, the two daughter sequences are entangled. Therefore, they must be separated. This is done by a type II DNA topoisomerase. In bacteria, the enzyme is known as DNA gyrase. Type II DNA topoisomerase introduce double-stranded cuts in DNA. During this process, two subunits of the enzyme become covalently attached to DNA (shown in black circles) to produce the following intermediate.



Because of the linear structure of the intermediate, the replicated DNA can become untangled and thus can be segregated.

Question 4 (30 points)

A. Tumor cells proliferate rapidly. This would require continuous rounds of DNA replication.

B. Anti-cancer drugs are designed to inhibit DNA replication and thus cell proliferation.

C. Thymine is the only base that selectively appears in DNA. Thymidylate Synthase synthesizes dTMP from dUTP. During this reaction, Thymidylate Synthase adds a methyl group to the 5 position in U. In subsequent reactions, dTTP (the precursor needed for DNA replication) is synthesized from dTMP. Therefore, inhibition of Thymidylate Synthase would inhibit DNA replication and thus proliferation of cancerous cells.

D. 5-Fluorodeoxyuridylate is an irreversible inhibitor of Thymidylate Synthase. For the mechanism, see the book entitled Biochemistry, by Vogt and Vogt. The strategic position of Thymidylate synthase in DNA biosynthesis has led to the clinical use of F-dUMP as an antitumor agent.

Answers

INORGANIC Chemistry Cumulative Exam
March 31, 2007

Given

(40) 1. Proposed mechanism for the oxidation of cysteine (CSH) under pseudo-first-order conditions (excess CSH) in buffered solutions pH 2.7 - 9.5.

Proposed mechanism

$$CSH \underset{}{\overset{K_{a2}}{\rightleftharpoons}} CS^- + H^+ \quad K_{a2} = 10^{-8.18} \quad \text{rapid pre-equil}$$

$$CS^- + ClO_2 \xrightarrow{k_1} CS\cdot + ClO_2^- \quad \text{rate detn.}$$

$$CS\cdot + ClO_2 \xrightarrow{\text{fast}} \text{products}$$

(a) Derive the rate expression given $[CSH]_T = [CSH] + [CS^-]$ for the loss of ClO_2

$$\frac{d[\text{products}]}{dt} = k_1[CS^-][ClO_2], \quad K_{a2} = \frac{[CS^-][H^+]}{[CSH]}$$

$$[CSH] = \frac{[CS^-][H^+]}{K_{a2}}, \quad [CSH]_T = \frac{[CS^-][H^+]}{K_{a2}} + [CS^-] = [CS^-]\left(\frac{[H^+]}{K_{a2}} + 1\right)$$

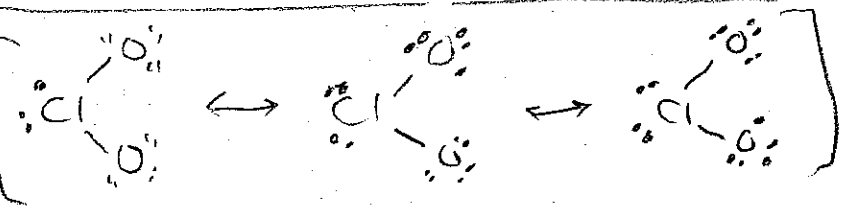
$$[CSH]_T = [CS^-]\left(\frac{[H^+] + K_{a2}}{K_{a2}}\right), \quad [CS^-] = \left(\frac{K_{a2}}{[H^+] + K_{a2}}\right)[CSH]_T$$

$$\frac{d[\text{products}]}{dt} = \frac{k_1 K_{a2}[CSH]_T[ClO_2]}{[H^+] + K_{a2}}$$

$$\boxed{-\frac{d[ClO_2]}{dt} = \frac{2k_1 K_{a2}[CSH]_T[ClO_2]}{[H^+] + K_{a2}}}$$

in terms of $[CSH]_T$, $[ClO_2]$ and $[H^+]$

(b) 2 oxygens = 12e
1 chlorine = 7e
19e total



One unpaired electron shared between the 3 atoms, with slightly greater density on Cl than on the oxygens.

1. (b) <u>continued</u> ClO_2 is a strong oxidizing agent that easily accepts an electron to <u>give ClO_2^- (chlorite ion) as a product.</u> Or ClO_2 with an unpaired e can also react with the CS• radical to give an adduct <u>CS-O-Cl-O</u> where CS = the cysteine radical. This can react further to generate oxidized CS-O and give HOCl.

(60) 2. <u>Given</u> the mechanism <u>where $BrOOO^-$ is a steady-state intermediate</u>,

$$O_3 + Br^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (BrOOO^-)_{ss} \qquad (1)$$

$$(BrOOO^-)_{ss} + H^+ \xrightarrow{k_2} HOBr + O_2 \qquad (2)$$

$$(BrOOO^-)_{ss} + H_2O \xrightarrow{k_3} HOBr + O_2 + OH^- \qquad (3)$$

$$HOBr + Br^- + H^+ \xrightarrow{fast} Br_2 + H_2O \qquad (4)$$

$$H^+ + OH^- \xrightarrow{fast} H_2O \qquad (5)$$

(a) The overall reaction is:

$$O_3 + 2Br^- + 2H^+ \longrightarrow Br_2 + H_2O + O_2$$

The reaction is acid assisted because the rate increases with acid, but acid is also consumed (so it is not just a catalyst).

7. (b) Derivation of the rate expression,

$$\frac{d[BrOOO^-]_{ss}}{dt} = k_1[O_3][Br^-] - k_{-1}[BrOOO^-]_{ss} - k_2[BrOOO^-]_{ss}[H^+] - k_3[BrOOO^-]_{ss} = 0$$

$$\therefore (k_{-1} + k_2[H^+] + k_3)[BrOOO^-]_{ss} = k_1[O_3][Br^-]$$

and $$[BrOOO^-]_{ss} = \frac{k_1[O_3][Br^-]}{k_{-1} + k_2[H^+] + k_3}$$

$$\frac{d[HOBr]}{dt} = (k_2[H^+] + k_3)[BrOOO^-]_{ss}$$

$$\frac{d[HOBr]}{dt} = \frac{k_1[O_3][Br^-](k_2[H^+] + k_3)}{k_{-1} + k_2[H^+] + k_3}$$

which from eq 4 is also equal $\frac{d[Br_2]}{dt}$ and $= -\frac{d[O_3]}{dt}$ from the stoichiometry

(c) A steady-state intermediate is present in sufficiently low concentration that its rate of loss or formation approaches zero. above $\frac{d[BrOOO^-]}{dt} \cong 0$.

A transition state is a highly transitory energy maximum, while a steady state species is not an energy maximum, but is an unstable intermediate present at low concn.

<u>transition state compositions</u>

are $[BrOOOH(H_2O)_n]^{\ddagger}$ and $[BrOOO^-(H_2O)_n]^{\ddagger}$

Organic Cumulative Exam Crib

03/31/2007

See David Liu website

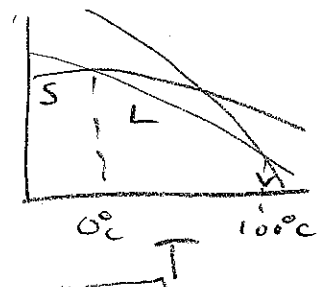
P. Chem March 31, 2007

1) $\boxed{\mu_i = \left(\frac{\partial A}{\partial N_i}\right)_{T,V,N_j}}$

2) at 0°c $\mu_S = \mu_L < \mu_V$

at 50°c $\mu_L < \mu_V$ & $\mu_L < \mu_S$

at 100°c $\mu_V = \mu_L < \mu_S$



3) Gibbs-Duhem: $\boxed{\sum_i N_i d\mu_i} = -S\cancel{dT}^{0} + V\cancel{dP}^{0} \boxed{= 0}$ at const. T and P

4) $\boxed{\Psi} = G - \left(\frac{\partial G}{\partial N_i}\right)_{T,P,N_{j\neq i}} N_i \boxed{= G - \mu_i N_i}$