tem. The rate of dissociation of alkylisocyanides from the proteins is faster than CO dissociation (Table I).

It should be noted that the marked difference in binding of alkylisocyanides to hemoglobin and myoglobin with increasing steric bulk of the alkyl group, R,[13] is due almost entirely to a difference in "on" rate, not to differences in "off" rates.[14] We have made preliminary measurements on the rate of isocyanide dissociation from the free heme complexes by displacement with carbon monoxide according to the reaction

$$\mathrm{LFeP(RNC)} + \mathrm{CO} = \mathrm{LFeP(CO)} + \mathrm{RNC}$$

where L = piperidine and P = protoporphyrin IX. We find the rate of displacement of benzyl isocyanide to be comparable to rates observed for other isocyanides in the protein systems. This result is consistent with our previous report that the protein has little effect on the rate of CO dissociation.[7] It should be noted that in the case of polar molecules bound to the protein such as NO^- or O_2^-, one observes a substantially different rate of dissociation outside of the protein environment[15,16] consistent with the idea that the protein serves to insulate the heme group from solvation effects.[16,17] This effect of solvent on the "off" rates explains the much poorer oxygen binding observed in cobalt[18] and iron model[19] systems compared to the binding in the proteins.

While the dissociation of benzyl isocyanide from ferrous phthalocyanine is slow in the dark, the reaction is rapid in the presence of normal fluorescent room lighting. Over a thousandfold increase in rate is observed between dark and full illumination. The observed increase in the rate of dissociation in the presence of light results in a shift of the equilibrium

$$\mathrm{L_2FePc} + \mathrm{RNC} = \mathrm{LFePc(RNC)} + \mathrm{L} \qquad (3)$$

The effect is quite dramatic. Solutions containing concentrations of L and RNC to shift the equilibrium to the right appear blue. In the presence of light the color rapidly changes to the green color of L_2FePc. On placing the solution in the dark, the color changes back to blue. The change in color from blue to green and back again has been repeated several hundred times without any loss in reversibility. The relative rate of return to "true equilibrium" in the dark may be varied by changing the ligand L.

Photodissociation of CO and RNC from hemoglobin and myoglobin is well known.[1] A similar effect of light on the equilibrium for CO binding to hemoglobin and myoglobin has recently been reported.[20,21]

We know of no previous suggestion that solar energy storage in biological systems may occur by a process similar to that observed in the iron phthalocyanine model system. The remarkable versatility of such a process and the similarity between the iron phthalocyanine and biological metal complexes make such a proposal very attractive. We are continuing our investigations of the kinetics and equilibria of substitution reactions of iron phthalocyanine and porphyrin complexes with the goal of optimizing energy storage and determining how this stored energy may be used to drive unfavorable reactions in much the same way ATP is used to drive biological reactions.[22]

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Dennis V. Stynes
Department of Chemistry, York University
Downsview, Ontario, Canada
Received May 7, 1974

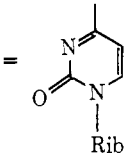
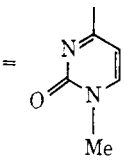
Mechanism of Reduction of 1-Methyl-4-thiocyanatouracil by Bisulfide. A Route to *in Vitro* Labeling of tRNAs with Sulfur-35[1,2]

Sir:

It has been recently demonstrated[3-5] that 4-thiocyanatouridine and its methyl analog undergo nucleophilic C–S bond scission by OH^-, with the formation of the thio and oxo compounds in the ratios 2:7 and 1:1, respectively, according to 1 and 2. The stoichiom-

$$9\mathrm{RSCN} + 18\mathrm{OH^-} = 7\mathrm{RO^-} + 2\mathrm{RS^-} + 7\mathrm{SCN^-} + 2\mathrm{OCN^-} + 9\mathrm{H_2O} \qquad (1)$$

$$2\mathrm{R_mSCN} + 4\mathrm{OH^-} = \mathrm{R_mO^-} + \mathrm{R_mS^-} + \mathrm{SCN^-} + \mathrm{OCN^-} + 2\mathrm{H_2O} \qquad (2)$$

R =  R_m = 

etry of the reactions has been established by estimating spectrophotometrically the amounts of RS^-, RO^-, R_mS^-, R_mO^-, and SCN^- formed in these reactions. In the case of reaction 2, the quantitative uptake of alkali has been determined by titration. These results indicate that the pyrimidine ring C–S bond is more vulnerable to nucleophilic attack than the exocyclic C–S bond in RSCN. In a parallel reaction, both RSCN and R_mSCN are reduced quantitatively by the nucleophile SH^-. If this reaction occurs mostly by the pyrimidine ring C–S fission, one should be able to incorporate ^{35}S label in RSH by using $^{35}SH^-$ as a reagent. We have investigated the mechanism of the

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reaction of R_mSCN with SH^-. For this purpose, $R_m{}^{35}SCN$ was prepared by the following reaction sequence

$$R_mSH \xrightarrow{CNBr} R_mSCN \xrightarrow{{}^{35}SH^-} R_m{}^{35}SH \xrightarrow{CNBr} R_m{}^{35}SCN$$

The separation of $R_m{}^{35}SH$ and $^{35}SH^-$ was achieved by chromatography on DEAE-Sephadex, taking advantage of the difference in the pK_a's of the two compounds (R_mSH, pK 8.2; H_2S, pK 7). The $R_m{}^{35}SCN$ thus prepared showed a specific activity of 160 cpm/nmol. It was treated with SH^- and the specific activity of the product R_mSH was found to be 49 cpm/nmol. This indicates that 70% of the reaction proceeds with ring C–S bond fission as depicted below. There was no exchange reaction between R_mSH and $^{35}SH^-$ under the experimental conditions used.

$$R_mSH \xleftarrow[\text{path 1, 70\%}]{SH^-} R_m\overset{1}{-}{}^{35}S\overset{2}{-}CN \xrightarrow[\text{path 2, 30\%}]{SH^-} R_m{}^{35}SH$$

These results encouraged us to try to label mixed *E. coli* tRNAs *in vitro* with $^{35}SH^-$ for biological studies. 4-Thiouridine present in mixed *E. coli* tRNAs was converted to 4-thiocyanatouridine by CNBr following a modified method.[6] One milliliter of aqueous tRNA (A_{260} = 61, A_{340} = 1.15) was treated with 25 μl of 0.5 *M* phosphate buffer, pH 8, followed by 10 μl of 1 *M* ethanolic CNBr. The reaction mixture was allowed to stand at 27° for 15 min and then evaporated at 27° under high vacuum. The residue was made up to 1 ml with oxygen-free water and treated with 25 μl of 0.4 *M* $NaH^{35}S$ (Amersham, 5.4 Ci/mol) in nitrogen atmosphere and allowed to stand 1 hr at 27° and overnight at 4°. It was then freed from excess reagent by chromatography on a 1-ml column of hydroxylapatite. The unreacted $NaH^{35}S$ was eluted with 0.05 *M* phosphate buffer, pH 6.8. The tRNA was eluted with 0.2 *M* phosphate buffer, pH 6.8, and it was dialyzed against distilled water at 4° to remove the phosphate. The radioactivity incorporated in the tRNA was found to be 9550 cpm/A_{260}. By comparison, a very small amount of radioactivity, 290 cpm/A_{260}, was incorporated by direct exchange between tRNA and $NaH^{35}S$.

In order to demonstrate that the radioactivity was located in the 4-thiouridine moiety of the tRNA, we hydrolyzed the tRNA to nucleoside level in two steps, by incubating the tRNA with RNase T_1 at 37° at pH 7.2 for 30 min followed by incubation at 45° for 3 hr with phosphatase and venom phosphodiesterase at pH 8.8. The hydrolyzed tRNA was then analyzed on a Bio-Rad A6 column (20 × 0.63 cm) by both the anion-exclusion method of Singhal[7] using ammonium carbonate, pH 9.8, and the cation-exchange method using 0.05 *M* NH_4Cl, pH 5.3. In the former system, the radioactivity was found to be associated with the 4-thiouridine peak and no other nucleoside was found to be associated with radioactivity. Unfortunately, this system does not separate SH^- from 4-thiouridine; in addition, there is an overlapping of the enzyme peak with the 4-thiouridine peak. In order to rule out the possibility of the presence of $^{35}SH^-$ as a contaminant, we used the second system, which does separate 4-thiouridine, $^{35}SH^-$, and the enzyme mixture. In this system, the radioactivity was found to be associated with the enzyme peak (breakthrough) and the 4-thiouridine peak. No radioactivity was found in the fractions eluting at the SH^- peak position. About 17% of the total radioactivity was associated with the enzyme peak and 83% with the 4-thiouridine peak. There could be several explanations for the radioactivity in the enzyme peak: (1) some minor nucleoside may incorporate ^{35}S by this process and elute at this position; (2) oligonucleotides containing labeled 4-thiouridine due to incomplete hydrolysis may elute at this position; (3) enzymes may incorporate radioactivity from labeled 4-thiouridine. We have checked the last possibility by incubating a fresh enzyme mixture with radioactive 4-thiouridine and found that the enzyme did incorporate radioactivity. This activity could not be dialyzed against distilled water but could be dialyzed against β-mercaptoethanol. Although the first possibility cannot be ruled out, the second one is remote since the reaction conditions were carefully selected to ensure complete hydrolysis of the tRNA. We have also subjected brewer's yeast tRNA (Schwartz) to this labeling procedure. It was found to incorporate a small amount of radioactivity, 180 cpm/A_{260}, which was associated with the enzyme peak in the chromatogram of the tRNA hydrolyzate (Bio-Rad A6, 0.05 *M* NH_4Cl, pH 5.3).

The exchange method of labeling compounds by heating with ^{35}S in benzene–pyridine has been used successfully;[8] obviously such harsh treatment cannot be used with sensitive macromolecules like tRNA. The present method accomplishes the labeling of 4-thiouridine containing tRNA's *in vitro* with ^{35}S in a simple and mild way.

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Bimal C. Pal,* Diane Grob Schmidt
Biology Division, Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830
Received June 8, 1974

A Method for Direct Hydroxylation of Enolates. Transition Metal Peroxide Reactions

Sir:

Base catalyzed oxygenation can be used to prepare α-hydroxy derivatives from occasional ketones and esters having a tertiary α-carbon.[1] The most practical procedure employs triethyl phosphite for *in situ* reduction of labile hydroperoxide intermediates,[2] a modification

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