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(11) Abbreviations: ATP, adenosine 5′-triphosphate; bpy, 2,2′-bipyridyl; ITP, inosine 5′-triphosphate; M^{2+} = Co^{2+}, Ni^{2+}, Cu^{2+}, or Zn^{2+}; NTP = nucleoside 5′-triphosphate = ATP or ITP; NS = nucleoside = adenosine or inosine; TP, tripolyphosphate. The phosphate groups of the triphosphate chains are labeled in the usual way as α, β, and γ, where the latter refers to the terminal phosphate group.
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Model Compounds for Protein–Nucleic Acid Interactions. 5.[1a] 5-*S*-Cysteinyluracil Monohydrate, a Photoaddition Product between an Amino Acid and Pyrimidine Base

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Abstract: The molecular and crystal structure of a pyrimidine–amino acid irradiation product, 5-*S*-cysteinyluridine, has been determined by x-ray crystallography. The cell dimensions are $a = 14.59$ (3) Å, $b = 6.82$ (1) Å, $c = 5.05$ (1) Å, $\beta = 95.1$ (1)°, space group $P2_1$ with two formula units of $C_7H_9N_3O_4S \cdot H_2O$ per cell. The pyrimidine rings are linked together in ribbons by four hydrogen bonds. Seven other hydrogen bonds interconnect the other polar functions and indicate how the unusual conformation of the cysteinyl group is stabilized. The irradiation product in its observed conformation can be fit into a RNA helix and proposed structures of intermediates in the in vivo covalent coupling of cysteine to uracil are described.

The cross-linking of nucleic acids to protein is one of the consequences of radiation in biological systems.[2] The importance of these cross-links in aging, carcinogenesis, and radiation biology has been recently reviewed.[3] Results of studies with model systems suggest that addition of cysteine residues to pyrimidine bases is one mechanism for the formation of cross-links in biological systems. One model reaction is the addition of cysteine to uracil under the influence of radiation.[4,5]

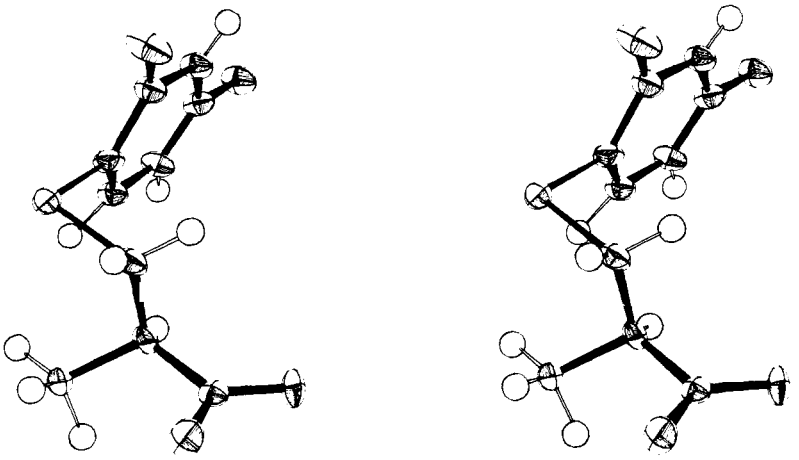


Figure 1. ORTEP drawing showing molecular conformation.

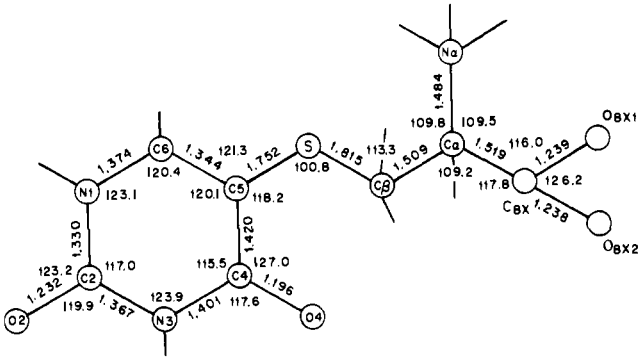
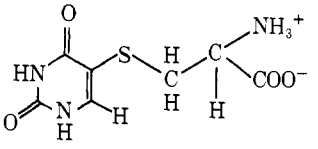


Figure 2. Distances and angles. The average standard deviations of the distances are 0.009 Å for the C-C, C-N, and C-O bonds and 0.006 for the C-S bond lengths.

Smith and Aplin[6] have identified a uracil-cysteine adduct (I) as a product of uracil irradiated with ultraviolet light in the presence of cysteine. Subsequent studies[9] have shown that uracil forms about six photoaddition products with cysteine, and the relative amounts of the various products depend on the environment and conditions of irradiation.

Nucleic acid-protein cross-linking has been used to study[7,8] the interaction between aminoacyl-tRNA synthetases and tRNA's. Knowledge on the spatial structure of these addition products could give valuable information on the stereochemistry of the residues at the contact sites. With this objective we have been studying the crystal structures of amino acid-pyrimidine addition products. In this paper, we describe the crystal structure of 5-*S*-cysteinyluracil (I).



I

Experimental Section

Small colorless tabular crystals were obtained by evaporation of a solution of I in 0.2 M acetic acid. Preliminary oscillation and precession photography revealed $2/m$ diffraction symmetry and the systematic absences $0k0$ for $k = 2n + 1$. The presence in the molecule of at least one asymmetric center (C_α of the cysteine) indicated the space group $P2_1$. Least-squares analysis of the setting angles for 25 reflections on a Picker four-circle diffractometer yielded the unit cell constants of Table I. No experimental value for the density of these crystals was obtained because of their very small size and number.

Table I. Crystallographic Data for 5-*S*-Cysteinyluracil Monohydrate

Formula	$C_7H_9N_3O_4S \cdot H_2O$
a	14.59(3) Å
b	6.82 (1) Å
c	5.05 (1) Å
β	95.10 (1)°
ρ_{calcd}	1.626 g/cm^3
Space group	$P2_1$
$Z = 2$	

Intensity data were collected using graphite-monochromated Mo K radiation ($\lambda = 0.7107$ Å) in a θ–2θ step scan procedure. Lorentz and polarization corrections, including polarization due to reflection from the assumed ideally mosaic monochromator, were applied. Because of the small crystal size (0.08 × 0.14 × 0.24 mm) absorption effects were considered to be negligible [μ(Mo Kα) = 3.14 cm^{-1}; μr_{max} = 0.038]. A discrepancy index of 0.08 over the remeasured and equivalent reflections was obtained from the sorting and averaging process which reduced the 3115 reflections, obtained by sampling all quadrants of reciprocal space within a d^* limit of 1.65 Å^{-1}, to 1326 unique data. Error estimates were derived from the variance of the counting statistics. An E map which revealed 16 plausible locations for the 15 nonhydrogen atoms in the molecule was obtained straightforwardly with use of the MULTAN program set.[10] The extra atomic site implied that both carbon atoms ortho to the sulfur atom, i.e., C(4) and C(6), might bear exocyclic nonhydrogen substituents. Both of these sites were included as oxygen atoms in the initial Fourier expansion and least-squares refinement of the model but it soon became apparent that the data contained little contribution from an oxygen atom substituent of C(6) and this oxygen was accordingly replaced by a hydrogen atom. Continued full-matrix least-squares refinement of the model against the full set of F_o^2 using the scattering factors of Cromer and Mann[11] for the nonhydrogen atoms and Stewart et al.[12] for the hydrogen atoms has resulted in the final residuals.

$$R = \sum|F_o^2 - k^2F_c^2|/\sum F_o^2 = 0.101$$

$$R_w = [\sum w|F_o^2 - k^2F_c^2|^2/\sum wF_o^4]^{1/2} = 0.107$$

$$S = [[\sum w|F_o^2 - k^2F_c^2|^2]/(N_o - N_v)]^{1/2} = 1.7$$

The structure factors have been deposited on microfilm (see paragraph at end of paper regarding supplementary material). Table II gives the position and temperature parameters.

Discussion

Description of the Molecular Structure. The photoproduct is confirmed as being composed of a covalently linked amino acid, cysteine, and the nucleic acid base, uracil. The amino acid is in the usual amphionic form (Figure 1) and, as shown in Figure 2, there are no unusual distances or angles in any part

Table II. Positional and Thermal Parameters[a]

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
N1	9160 (1)	−7577 (0)	7155 (2)	3.00 (5)	2.02 (7)	3.78 (5)	0.32 (7)	−0.43 (3)	−0.01 (7)
C2	9519 (4)	−5941 (10)	8239 (12)	2.34 (24)	3.02 (27)	3.35 (27)	−0.21 (21)	0.33 (19)	0.34 (24)
O2	10044 (4)	−5922 (11)	10292 (12)	3.44 (21)	3.73 (32)	2.96 (21)	−0.12 (24)	−1.24 (18)	0.39 (26)
N3	9266 (4)	−4219 (10)	7002 (10)	2.91 (24)	3.03 (30)	2.43 (23)	−0.36 (21)	−0.74 (19)	0.39 (23)
C4	8656 (2)	−4093 (9)	4701 (7)	2.88 (15)	2.60 (18)	2.84 (15)	−0.08 (24)	−0.27 (12)	0.03 (26)
O4	8518 (4)	−2504 (11)	3751 (14)	4.99 (27)	3.03 (34)	5.08 (30)	−0.16 (25)	−1.76 (23)	−0.27 (30)
C5	8271 (3)	−5897 (13)	3747 (10)	1.91 (19)	3.08 (25)	2.77 (22)	−0.37 (31)	0.00 (16)	0.11 (35)
C6	8544 (3)	−7588 (13)	4935 (10)	2.21 (18)	2.93 (24)	3.42 (20)	−0.22 (31)	0.30 (15)	−0.46 (33)
S	7481 (3)	−5872 (9)	922 (10)	2.46 (23)	4.01 (25)	2.00 (22)	−0.47 (19)	0.05 (18)	−0.07 (20)
CB	6408 (4)	−5587 (12)	2440 (13)	2.56 (30)	2.65 (34)	2.49 (30)	−0.13 (26)	−0.49 (23)	−0.32 (29)
CA	6034 (3)	−7492 (7)	3408 (7)	2.43 (19)	2.76 (20)	1.44 (13)	0.04 (17)	−0.50 (13)	−0.27 (14)
NA	5932 (3)	−8927 (8)	1193 (12)	2.50 (25)	2.72 (24)	1.74 (24)	0.06 (21)	0.09 (19)	−0.03 (22)
CBX	5102 (3)	−7124 (8)	4435 (10)	2.49 (21)	1.84 (24)	2.17 (20)	0.18 (19)	0.16 (16)	0.47 (18)
OBX1	5105 (2)	−6849 (8)	6863 (7)	4.16 (16)	3.86 (28)	1.06 (15)	0.91 (18)	0.32 (12)	−0.03 (18)
OBX2	4422 (4)	−7048 (10)	2785 (10)	2.36 (23)	6.58 (28)	2.12 (20)	0.46 (22)	−0.33 (17)	0.23 (20)
W	7408 (3)	−911 (12)	8899 (12)	4.77 (21)	5.05 (25)	5.41 (27)	0.88 (29)	0.76 (19)	−0.67 (36)
	x	y	z	B					
HN1	925 (3)	−877 (9)	788 (1)	4.0 (11)					
HN3	949 (3)	−311 (8)	782 (10)	5.5 (10)					
HC6	840 (5)	−902 (11)	395 (12)	0.2 (16)					
H1CB	647 (4)	−460 (10)	394 (14)	2.3 (16)					
H2CB	606 (3)	−486 (8)	122 (11)	2.2 (10)					
HCA	646 (5)	−811 (13)	450 (17)	2.7 (25)					
H1NA	661 (5)	−940 (11)	63 (17)	3.1 (20)					
H2NA	559 (6)	−1023 (14)	220 (22)	8.7 (32)					
H3NA	558 (4)	−845 (9)	−23 (12)	6.5 (13)					
H1W	744 (4)	−149 (11)	1078 (12)	6.7 (17)					
H2W	728 (5)	−101 (19)	743 (16)	10.8 (23)					

[a] The x, y, and z coordinates are multiplied by 10^4 and those of the hydrogen atoms by 10^3. Values in parentheses are estimated standard deviations given with respect to the last digit reported. The anisotropic temperature factor expression is $T = \exp[0.25(-h^2a^{*2}B_{11} - k^2b^{*2}B_{22} - l^2c^{*2}B_{33} - 2hka^*b^*B_{12} - 2hla^*c^*B_{13} - 2klb^*c^*B_{23})]$. The isotropic temperature factor expression is $T = \exp(-B \sin^2\theta/\lambda^2)$.

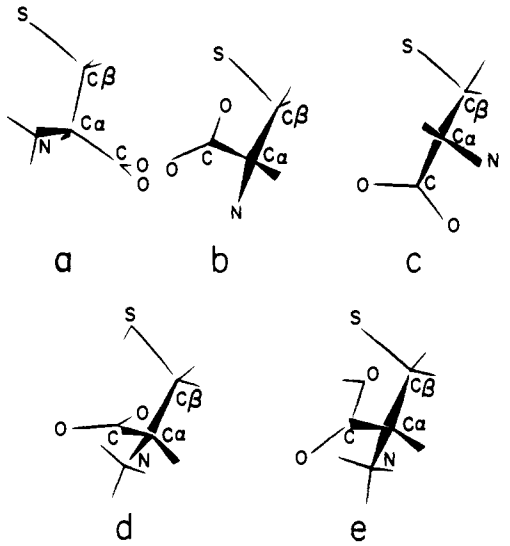


Figure 3. Comparison of conformations: (a) this structure, (b) monoclinic cysteine molecule I,[16] (c) monoclinic cysteine molecule II,[16] (d) cysteine,[15] (e) ½ cystine.[14]

Table III. Torsion[a] Angles

Atoms	Value, deg
C^β-S-C(5)-C(4)	−90.3
C(5)-S-C^β-C^α	−82.6
S-C^β-C^α-N^α	−55.3
S-C^β-C^α-C(BX)	−175.5
N-C^α-C(BX)-O(BX1)	144.5
C^β-C^α-C(BX)-O(BX1)	−95.1
N-C^α-C(BX)-O(BX2)	−38.3
C^β-C^α-C(BX)-O(BX2)	82.0

[a] The torsion angle defined by the positions of the four atoms A-B-C-D is the dihedral angle between the plane containing ABC and that containing BCD. A positive angle is one that would require a clockwise rotation of atom A to achieve superposition of the AB and CD bond vectors when viewed in the B-C direction.

of the structure. The sulfur and exocyclic carbon atoms are approximately coplanar and this plane, which passes through the S atom, is inclined 100° to the C(5)-S bond and 100° to the plane of the pyrimidine ring. A more precise description of the molecular conformation is embodied in the torsion angles of Table III. A comparison of the conformation of this structure with those found for other cysteinyl derivatives (Figure 3) indicates that the present molecule exists in an unusual conformation in the crystal. In order to judge the relative probabilities of the available conformational states, a potential energy surface for rotations about the bonds S-C^β and C^β-C^α was calculated. This map was computed with the program WMIN of Busing employing interatomic interaction terms from Hopfinger[13] in a Lennard-Jones 6-12 nonbonded potential function

$$P_{\tau_1\tau_2} = \left[\sum_{i=1}^{m}\sum_{j=1}^{n}\frac{B_{ij}}{R_{ij}^{12}} - \frac{A_{ij}}{R_{ij}^{6}}\right]_{\tau_1,\tau_2}$$

The summation at each point (τ_1,τ_2) of the surface was performed over each intergroup pair of atoms. The "m" group of atoms were those comprising the pyrimidine base and the "n" group were those of the amino acid excluding the sulfur atom. The potential energy surface contained a broad minima which extended over 75% of the possible conformations. The experimentally observed conformation is within this allowed domain and thus the pervasive hydrogen bonding must provide the stabilization for the experimentally determined conformation.

Hydrogen Bonding. Although each aromatic base participates in four hydrogen bonds, the α-aminocarboxylate in eight, and the water molecule in a total of three such interactions, there are no direct hydrogen bonds between the pyrimidine

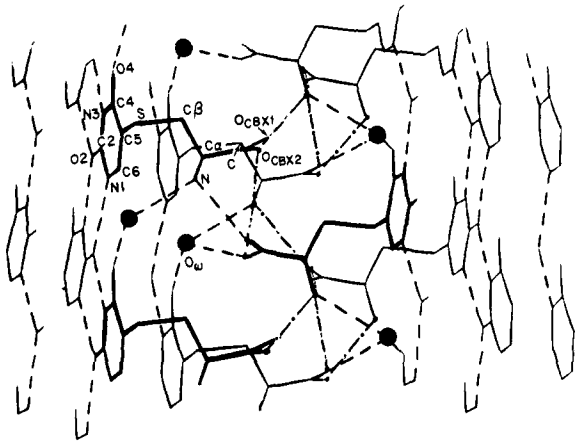


Figure 4. Hydrogen bonding.

Table IV. Hydrogen Bonding Distances

Donor atom	Acceptor atom	Distance, Å	Symmetry
N(1)	O(2)	2.820	$2-x,-\frac{1}{2}+y,2-z$
N(3)	O(2)	2.773	$2-x,\frac{1}{2}+y,2-z$
N^{α}	O(BX1)	2.789	$x,y,-1+z$
	O(BX1)	2.737	$1-x,-\frac{1}{2}+y,1-z$
	O(BX2)[a]	2.941	$1-x,-\frac{1}{2}+y,-z$
	O(W)	2.872	$x,-1+y,-1+z$
O(W)	O(4)	3.017	$x,y,1+z$
	O(BX2)	2.837	$1-x,\frac{1}{2}+y,1-z$

[a] This is a close contact.

rings and the aminoacyl groups.

Infinite planar ribbons of hydrogen-bonded bases form an important feature of this structure. These chains are illustrated in Figure 4 which also indicates that sulfur atoms are positioned over the center of each pyrimidine ring. The 3.4-Å distance between the sulfur atom and the center of the aryl system is typical of this kind of interaction involving polar exocyclic groups and polarizable π-electron clouds.[17]

The water molecule is a donor in the hydrogen bond to the ring substituent O(4) and an acceptor in its linkage to the α-amino group. By examining a model of this structure it was possible to discern that if the aromatic ring were rotated 180° about the S-C(5) bond, the new O(4) would be in a favorable position to accept a hydrogen bond from the water molecule. If this occurred both ring atoms ortho to the cysteinyl substituent would appear to bear oxygen atoms. The behavior observed during the early stages of this analysis is consistent with a limited occurrence of this static disorder. The other intermolecular hydrogen bonds tabulated in Table IV surround the screw axes and so link the molecules in helices.

Models for Cysteine–RNA Complexes. Photocross-linking has emerged as a powerful tool for examining protein–nucleic acid complexes. Nothing is known, at present, about the conformational constraints on the reacting species and resultant products. The simplest interactions to consider at this time are the adducts which may be formed between fragments of helical RNA and cysteine upon irradiation. Examination of a model of RNA[18] shows that a cysteine molecule could form a covalent bond with the C(5) atom of a uracil residue and that the resultant product could have the conformation found in this study without disturbing the ribose phosphate backbone (Figure 5, a). It is also possible to construct models for likely ways in which the cysteine might be aligned with respect to an RNA chain *prior* to the photochemical event that produces a cysteine–uracil adduct whose conformation is the same as the one

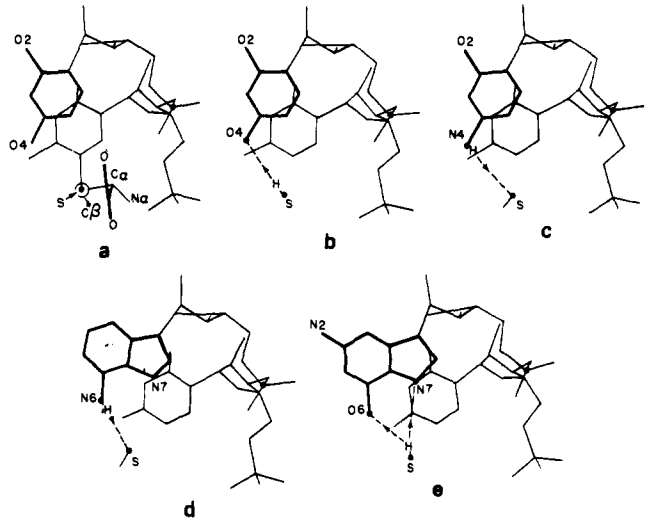


Figure 5. (a) The cysteine–uracil adduct in an RNA helix. (b) A model for the proposed SH- - -O(4) hydrogen bond between UpU and cysteine. (c) A model for the proposed N(4) H- - -S hydrogen bond between UpC and cysteine. (d) A model for the proposed N(6)–H- - -S hydrogen bond between UpA and cysteine. (e) A model for the proposed bifurcated N(7)- - -SH- - -O(6) hydrogen bond between UpG and cysteine.

found here. To do that we consider the four possible dinucleotide fragments of RNA chains with uridine as the 3′ member, i.e., uridylyl-3′,5′-adenosine phosphate (UpA), uridylyl-3′,5′-guanosine phosphate (UpG), uridylyl-3′,5′-uridine phosphate (UpU), and uridylyl-3′,5′-cytosine phosphate (UpC). First, we shall assume that in the photoreaction the thiol of the cysteine attacks trans to the uracil rings. We can then propose four possible prereaction hydrogen-bonded complexes. The lengths of hydrogen bonds are assumed to be 3.5 Å. For UpU (Figure 5, b) the thiol can donate its hydrogen to the O(4) of the uracil which is in the plane above the reacting uracil. For UpC (Figure 5, c) the N(4) H donates to the sulfur. One can postulate an N(6)–H- - -S hydrogen bond anchoring the UpA–cysteine complex (Figure 5, d) and a bifurcated N(7)- - -SH- - -O(6) stabilizing the UpG–cysteine complex (Figure 5, e). In all cases the cysteine needs to shift a relatively short distance to bond with the C(5) of the uracil in the plane below. It is also possible that before the reaction the sulfur is in the same plane as the uracil ring and that a SH- - -O(4) hydrogen bond to the reacting uracil is formed.

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Supplementary Material Available: a listing of observed and calculated structure factors (7 pages). Ordering information is given on any current masthead page.

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A Surface-Modified Gold Minigrid Electrode Which Heterogeneously Reduces Spinach Ferredoxin

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Abstract: The preparation and characteristics of gold minigrid electrode with its surface modified by a polymeric form of 1,1′-dimethyl-4,4′-dipyridyl dichloride are described. The prepared electrode is stable to a wide range of applied potentials and to chemical reaction with molecular oxygen and Fe(III). The electrode has the ability to heterogeneously reduce spinach ferredoxin at a rate which is considerably faster than that of a nonmodified gold minigrid electrode.

The direct electrochemical reduction or oxidation of components of the plant photosynthetic apparatus using a solid electrode is usually an irreversible electrode reaction which prohibits the use of a variety of voltammetric techniques[1,2] for its characterization. The use of mediated, or indirect, electrochemical methods has permitted the application of a growing number of instrumental techniques to the study of this problem.[1-5] This present study was undertaken to determine the electron-transfer features of spinach ferredoxin in an optically transparent thin-layer electrochemical cell (OTTLE cell)[6] based on the successful application of this method to components of the mammalian oxidative phosphorylation apparatus by Heineman et al.[7,8] and in the study of vitamin B_{12}.[9] During the course of this investigation an aspect of 1,1′-dimethyl-4,4′-dipyridyl dichloride (common name methyl viologen, MV) chemistry which has not been previously reported to the authors' knowledge was discovered.

The electrochemistry of MV has been widely studied[3,10-15] and its electrochemical reactions are

$$MV^{2+} + e^- \rightarrow MV^{+}\cdot \quad (1)$$

$$MV^{+}\cdot + e^- \rightarrow MV^0 \quad (2)$$

Prominent among the chemical reactions which complicate the above electrochemical reactions are the dimerization of the cation radical[3,16] and the disproportionation of the cation radical to the dicationic and the neutral species.[17] We wish to report results which indicate that under certain experimental conditions another species, proposed to be polymeric, is formed which is analogous to the polyviologen product of the chemical reactions of a series of viologens described by Simon and Moore.[18]

The significance of these results is that the polymeric form of MV is readily prepared by electrochemical means on a gold minigrid surface. Once formed it is stable to the application of between +0.50 and −0.95 V vs. Ag|AgCl and to chemical degradation with added MV^{2+}, molecular oxygen, and ferric iron. This polymeric film of MV formed on the gold surface is also stable to extended exposure to air. The utility of this surface-modified electrode is that it renders the gold surface electroactive in the reduction and oxidation of spinach ferredoxin. In the absence of the polymeric film and solution, MV^{2+} gold electrodes reduce ferredoxin only at a very slow rate.[19] The results of this investigation show that these electrodes may be prepared simply and that the surface-modified electrode has the capability of heterogeneous, or direct, electron transfer with at least one reasonably large biological molecule from the plant photosynthetic apparatus. The kinetic and analytical utility of this electrode is currently being investigated in our laboratory.

There are important consequences to being able to eliminate the need for mediators in the study of photosynthetic components. Optical studies of the chromophores of the plant photosynthetic apparatus are hampered due to the large absorbances of the mediators (cation radicals of the viologens) in the 390 and 600-nm range.[20] This requires that the mediators be present in low concentration in electrochemical titrations leading to slow rates of reduction. Secondly, the cation radical forms of the viologen mediators produce a large electron paramagnetic resonance (EPR) signal which must be accounted for in studies of the difference spectra often obtained in such studies (i.e., light minus dark EPR spectra). The electrode described in this paper may allow ready potentiometric poising of photosynthetic samples without the need for viologen mediators in solution, thereby permitting much more sensitive optical and EPR measurements.

Experimental Section

The electrochemical instrumentation consisted of a Wenking Model 61RS potentiostat with controlled voltages obtained from a Wavetek Model 112 signal generator and a battery. These voltages were added with a conventional operational amplifier adder circuit before being applied to the potentiostat.

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