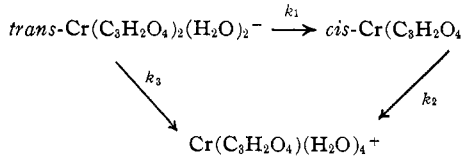
moting the exchange of water between hexaaquochromium(III) and solvent. It is highly unlikely that the modes of action of the anions are the same in both cases since one involves a positively charged complex and the other a negatively charged species. The present study clearly indicates that nitrate only affects the entropy of activation for the isomerization reaction and the positive trend in this parameter is in complete agreement with the rationale presented above for the mode of action of the anions. Further investigations on similar systems are currently being studied.[17]

Since the observed product of the trans–cis isomerization of *trans*-$Cr(C_3H_2O_4)_2(H_2O)_2^-$ is the monomalonate complex of chromium(III), it was of interest to learn whether this is due to direct aquation of the trans isomer or results from the trans–cis isomerization of the trans isomer followed by aquation of the cis isomer which is formed.



The pathway (k_1, k_2) in which the trans isomer proceeds to the mono complex exclusively through the cis isomer is treated mathematically by Frost and Pearson.[18] For our system the rate constants were taken to be $k_1 = 0.4 \times 10^{-4} + 1.6 \times 10^{-4}[H^+]$ and $k_2 = 7.22 \times 10^{-4}[H^+]$, both for a temperature of 45°. The series-first-order equations were then solved at each of six acid concentrations for the maximum per cent cis isomer ($\beta_{max} = [cis]_{max}/[trans]_0$) as well as the per cent trans isomer (α) and per cent mono complex (γ) corresponding to β_{max}. Having determined these values at τ_{max} (related to the time required for β to reach its maximum), we can calculate the apparent molar extinction coefficient of the solution by using the equation $\epsilon_{app} = \alpha(\epsilon_{trans}) + \beta(\epsilon_{cis}) + \gamma(\epsilon_{mono})$, where ϵ_{app} should have its maximum value at $[cis]_{max}$. Table V presents the results of such a calculation. The results clearly indicate that at $[H^+] = 0.05$ and $0.10\ M$ the apparent molar extinction coefficients should exceed that for the mono complex alone. The absorbance data at these acid concentrations were used to determine the experimental apparent molar extinction coefficients. At $[H^+] = 0.10\ M$, ϵ_{app}(exptl) = 31.6 M^{-1} cm^{-1}, and at $[H^+] = 0.05\ M$, ϵ_{app}(exptl) = 36.0 M^{-1} cm^{-1}, both in excellent agreement with the calculated values. This strongly argues that the trans isomer proceeds to the monomalonate complex exclusively through the cis isomer.

TABLE V

CALCULATED APPARENT MOLAR EXTINCTION COEFFICIENTS FOR THE ACID-CATALYZED ISOMERIZATION OF *trans*-$Cr(C_3H_2O_4)_2(H_2O)_2^-$ AS A FUNCTION OF ACID CONCENTRATION[a]

$[H^+]$, M	10^4k_1,[b] sec^{-1}	10^4k_2,[c] sec^{-1}	α	β_{max}	γ	ϵ_{app} (calcd)[d]	ϵ_{app} (exptl)
1.00	2.00	7.22	0.610	0.170	0.220	26.2	...
0.80	1.68	5.76	0.602	0.176	0.212	26.1	26.2
0.50	1.20	3.61	0.577	0.193	0.240	27.4	27.1
0.20	0.72	1.44	0.500	0.250	0.250	29.3	29.5
0.10	0.56	0.72	0.414	0.325	0.261	31.9	31.6
0.05	0.48	0.36	0.371	0.421	0.262	35.4	36.0

[a] Temperature 45°; λ 560 nm. [b] $k_1 = 0.4 \times 10^{-4} + 1.6 \times 10^{-4}[H^+]$. [c] $k_2 = 7.22 \times 10^{-4}[H^+]$. [d] At 560 nm, $\epsilon_{trans} = 17.5\ M^{-1}$ cm^{-1}, $\epsilon_{cis} = 50.7\ M^{-1}$ cm^{-1}, and $\epsilon_{mono} = 31.2\ M^{-1}$ cm^{-1}.

No definitive statement can be made presently with regard to the origin of the catalytic effect of acid on the trans–cis isomerization; however, the presence of acid is expected to facilitate ring opening which is postulated as the rate-determining step for the isomerization reaction. The lower entropy of activation is surprising but is consistent with the participation of a cation in the rate-determining step.[10]

(17) Preliminary results indicate that free oxalate anion increases the rate of isomerization of *trans*-$Cr(C_2O_4)_2(H_2O)_2^-$: K. R. Dekker, F. C. Maenpa, and D. H. Huchital, work in progress.

(18) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, Wiley, New York, N. Y., 1961, pp 166–169.

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Electrochemical Studies of the Interactions of Riboflavin and of Its Reduction Products with Metal Ions in Dimethyl Sulfoxide

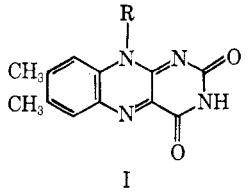
BY DONALD T. SAWYER* AND RICHARD L. McCREERY

Received July 7, 1971

The nonaqueous electrochemistry of riboflavin and its metal ion interactions has been studied at a platinum electrode in dimethyl sulfoxide. Cyclic voltammetry, chronopotentiometry, and controlled-potential electrolysis have been used to establish that riboflavin is reduced in neutral solutions by two one-electron steps with a stable anion radical produced by the first of these; under acidic conditions it is reduced by a single two-electron process. The interactions of the riboflavin radical anion with iron(II), nickel(II), yttrium(III), lanthanum(III), thorium(IV), calcium(II), and sodium(I) ions have been investigated and the relative stabilities of the resulting complexes have been determined. Disproportionation of the radical anion appears to be promoted by the higher valent metal ions.

Introduction

The flavoproteins, which represent an important class of enzymes for biological oxidations, contain flavin mononucleotide (FMN) and flavin–adenine dinucleotide (FAD) as prosthetic groups with the isoalloxazine ring system (I) acting as the electron-transfer (or dehydrogenation) catalyst. In addition to FMN and FAD a number of flavoproteins also contain metal



I

FMN, R = $CH_2(CHOH)_3CH_2OPO_3H_2$
FAD, R = $CH_2(CHOH)_3CH_2O(PO_3H)_2$-adenosine
riboflavin, R = $CH_2(CHOH)_3CH_2OH$
lumiflavin, R = CH_3

ions as prosthetic groups that participate in electron-transfer reactions. Because the flavin coenzymes can undergo reduction either by a one-electron step to a radical intermediate (semiquinone form) or by a two-electron step to fully reduced flavin (dihydroquinone form), they provide versatile catalysts for a wide variety of oxidation–reduction cycles.[1]

The many complications with direct electrochemical investigations of enzymes, or even of FMN and FAD, make model compounds attractive as a starting point for meaningful studies. Recognition of this has prompted the use of riboflavin, lumiflavin, 3-methyllumiflavin, and 8-quinolinol as model compounds, primarily in aqueous solution.[2-5] The structure of the semiquinone form of lumiflavin, FMN, and several of its derivatives in aqueous solutions has been studied by esr spectroscopy.[6] Analysis of the spectra permitted calculation of the spin densities in the isoalloxazine ring; the effects of pH and of metal ion complexation on the esr spectra also were studied. A related study of the interaction of molybdenum(VI,V) and iron(III,II) with the semiquinone form of flavin in aqueous solutions has been made using spectrophotometry and polarography.[5] The results indicate significant metal chelate formation but are complicated by the aqueous solvent.

Although an aqueous system superficially appears to be representative of a biological medium, spectroscopic evidence indicates that the protein fragment of flavoenzymes provides a nonaqueous environment with a much more aprotic solvent condition.[7,8] As a result, a nonaqueous electrochemical study of several model compounds for flavin enzymes has been made to obtain thermodynamic and kinetic data that may be more relevant to biological systems.[9] The model compounds, which included phenazine, 3-methyllumiflavin, and riboflavin, have been studied in dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and acetonitrile (CH_3CN).

Knowledge of the effect of metal ions (particularly in terms of complexation) on the electrochemistry of flavin model compounds is a necessary part of a better understanding of the biochemistry of the metalloflavoenzymes. The goal has been to determine the formulas in nonaqueous solvents of the metal complexes formed between the metal ions and the various oxidized states of the model compounds. Previous investigations have included a study of the molybdenum(VI) and molybdenum(V) complexes with 8-quinolinol in DMSO.[10] The present study has been concerned with the interactions of the radical anion of riboflavin with iron(II), nickel(II), yttrium(III), lanthanum(III), thorium(IV), calcium(II), and sodium(I) ions and the relative stabilities of the resulting complexes.

Experimental Section

The electrochemical measurements were performed with a versatile instrument constructed from Philbrick operational amplifiers following the design of DeFord.[11] A Sargent Model SR strip-chart recorder was used in conjunction with the DeFord instrument. Cyclic voltammograms were recorded with a Moseley X-Y recorder or a Tektronix Model 564 oscilloscope. A Hewlett-Packard Model 202A low-frequency function generator was employed to generate a triangular wave for fast-scan cyclic voltammetry. A three-electrode assembly was used for all electrochemical measurements. For cyclic voltammetric and chronopotentiometric experiments a Beckman platinum-inlay electrode was employed; a platinum gauze electrode was used for the coulometric and electrolysis experiments. The area of the inlay electrode was determined by chronopotentiometric reduction of ferricyanide ion. The reference electrode consisted of an aqueous Ag–AgCl electrode in 0.4 *F* tetramethylammonium chloride solution.

For cyclic voltammetry and chronopotentiometry the electrochemical cell (Leeds and Northrup No. 7961 coulometric cell) consisted of a tall-form beaker fitted to a polyethylene top by means of a polyethylene snap ring. The top was provided with holes for the gas-inlet tubes, the working electrode, the platinum counter electrode (which was located in a glass tube with a fine-porosity fritted-glass disk), and a cracked glass-bead salt bridge containing the Ag–AgCl reference electrode. A Luggin capillary was used in conjunction with the reference electrode in all cases except for the sealed-cell coulometric experiments. The shield tubes were filled with the supporting electrolyte used in the sample solution. The solution was stirred with a magnetic stirrer and a Teflon-covered stirring bar. For the coulometric experiments a glass gastight cell was employed.

Dimethyl sulfoxide (DMSO) (J. T. Baker Analyzed reagent grade) was obtained in pint bottles to minimize water contamination; the water content varied between 0.02 and 0.05%. Tetraethylammonium perchlorate (TEAP) was prepared by stoichiometric combination of reagent grade perchloric acid and reagent grade tetraethylammonium bromide. The product was allowed to crystallize from the cooled solution and was recrystallized twice from cold water. Riboflavin was obtained from the Aldrich Chemical Co.

The metal salts used in the electrochemical studies were $Fe(ClO_4)_2 \cdot 6H_2O$, $Fe(ClO_4)_3 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, YCl_3, $LaCl_3$, $Th(ClO_4)_4 \cdot xH_2O$, $CaCl_2 \cdot 2H_2O$, and $NaClO_4 \cdot H_2O$. The perchlorates were obtained from the G. Frederick Smith Chemical Co., Columbus, Ohio, and the salts of yttrium and lanthanum were purchased from Alfa Inorganics, Beverly, Mass.

Results

A neutral solution of riboflavin containing 0.1 *F* TEAP in dimethyl sulfoxide yields a cyclic voltammogram which corresponds to a quasireversible one-electron couple followed by an irreversible one-electron reduction. The first couple has cathodic and anodic peak potentials of −0.82 and −0.55 V *vs.* sce, respectively, for a scan rate of 0.1 V/sec. The second reduction is a broad peak with a potential of −1.3 V *vs.* sce. Controlled-potential electrolysis of riboflavin at −0.9 *vs.* sce causes it to be reduced by one electron to the riboflavin radical anion. The latter species is stable in the absence of oxygen for at least several hours.

The interaction of neutral, unreduced riboflavin with

(1) H. R. Mahler and E. H. Cordes, "Biological Chemistry," Harper and Row, New York, N. Y., 1966, pp 572–580.
(2) B. Janik and P. J. Elving, *Chem. Rev.*, **68**, 295 (1968).
(3) S. V. Tatwawadi and A. J. Bard, *Anal. Chem.*, **36**, 2 (1964).
(4) S. V. Tatwawadi, J. S. V. Santhananm, and A. J. Bard, *J. Electroanal. Chem.*, **17**, 411 (1968).
(5) P. Hemmerich and J. Spence, "Flavins and Flavoproteins," E. C. Slater, Ed., Elsevier, New York, N. Y., 1966, p 82.
(6) A. Ehrenberg, L. E. G. Eriksson, and F. Muller, ref 5, p 37.
(7) V. Massey and H. Ganther, *Biochemistry*, **4**, 1161 (1965).
(8) A. Kotaki, M. Naok, and K. Tagi, *J. Biochem. (Tokyo)*, **59**, 625 (1966).
(9) D. T. Sawyer and R. Y. Komai, unpublished results, 1971.
(10) A. F. Isbell, Jr., and D. T. Sawyer, *Inorg. Chem.*, **10**, 2449 (1971).
(11) D. D. DeFord, private communication; presented at the 133rd National Meeting of the American Chemical Society, San Francisco, Calif., 1958.

metal ions has been studied in DMSO by measuring the peak potentials for the $Fe^{3+} \rightleftharpoons Fe^{2+}$ couple in the absence and in the presence of excess riboflavin. The $Fe^{3+} \rightarrow Fe^{2+}$ and $Fe^{2+} \rightarrow Fe^{3+}$ peaks occur at +0.20 and +0.27 V *vs.* sce, respectively. After the addition of a fivefold excess of neutral riboflavin, the cathodic and anodic peaks shift to +0.21 and +0.28 V *vs.* sce, respectively. The absence of significant potential shifts with the addition of riboflavin indicates a weak or nonexistent interaction between iron ions and neutral riboflavin in DMSO.

To investigate the effects of Fe^{2+} on the radical anion form of riboflavin, a solution of singly reduced riboflavin has been prepared by electrolyzing a 1 m*M* solution of riboflavin at −0.9 V. The singly reduced riboflavin exhibits a cyclic voltammogram with an anodic peak at −0.64 V *vs.* sce and a cathodic peak at −0.87 V *vs.* sce (Figure 1a). The addition of 0.36 mol

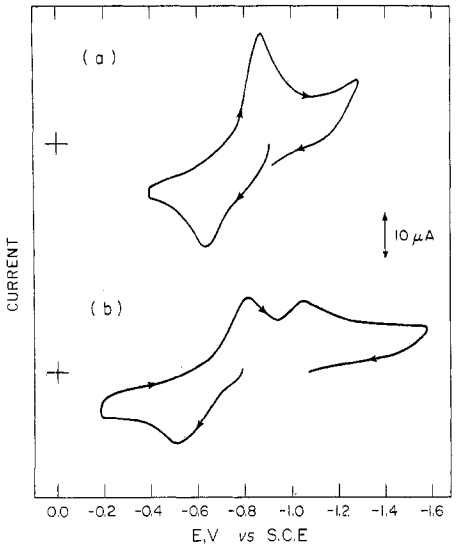


Figure 1.—(a) Cyclic voltammogram of a 1.0 m*M* solution of riboflavin after electrolytic reduction at −0.9 V *vs.* sce at a platinum electrode in DMSO. (b) Cyclic voltammogram of a solution that is 0.83 m*M* in riboflavin anion and 0.30 m*M* in $Fe(ClO_4)_3 \cdot 6H_2O$ in DMSO. Scan rate, 0.1 V/sec.

of Fe^{2+} per mole of reduced riboflavin causes the anodic peak to shift to −0.50 V *vs.* sce and the cathodic peak to shift to −0.81 V *vs.* sce (Figure 1b). The Fe^{2+} reduction occurs at −1.01 V *vs.* sce in DMSO and is shifted to −1.06 V *vs.* sce in the presence of reduced riboflavin (Figure 1b). These shifts indicate that Fe^{2+} forms a complex with the riboflavin radical anion.

The interactions of the riboflavin radical anion with other metal ions have been studied by reducing a 0.5 m*M* degassed riboflavin solution at −0.9 V *vs.* sce prior to adding a tenfold excess of the metal ion. The metal ions selected for study are poor oxidizing agents and are not electroactive in the region of interest. To ensure accurate values for the shifts in peak potentials, cyclic voltammograms have been recorded before and after metal ion addition. The results are summarized in Table I.

The presence of Ca^{2+} and Ni^{2+} ions produce the same qualitative effects on the electrochemistry of the riboflavin anion as Fe^{2+}. In contrast, addition of Y^{3+}, La^{3+}, and Th^{4+} ions appears to cause a chemical reaction which produces neutral riboflavin. After the

TABLE I

VOLTAMMETRIC DATA FOR THE RIBOFLAVIN RADICAL ANION IN THE PRESENCE OF VARIOUS METAL IONS IN 0.1 *F* TEAP IN DMSO[a]

Cation	−ΔEp, mV Anodic	−ΔEp, mV Cathodic	Cation	−ΔEp, mV Anodic	−ΔEp, mV Cathodic
Fe^{2+}	177	−83	Th^{4+} [b]	306	−88
Ni^{2+}	209	−52	Ca^{2+}	17	−15
Y^{3+} [b]	(370)[c]	−80	Na^{+}	−5	0
La^{3+} [b]	(400)	−84			

[a] Riboflavin anion concentration, 0.42 m*M*; metal ion concentration, 4.2 m*M*; scan rate, 0.1 V/sec. [b] Evidence of disproportionation. [c] Parentheses indicate approximate values due to broad, ill-defined peaks, ±50 mV.

addition of Th^{4+}, for example, the rest potential (potential at zero current) shifts in a positive direction and the cathodic peak current indicates the presence of neutral riboflavin (Figure 2a). When the scan is started anodically, the anodic peak current indicates the loss of some riboflavin anion (Figure 2b).

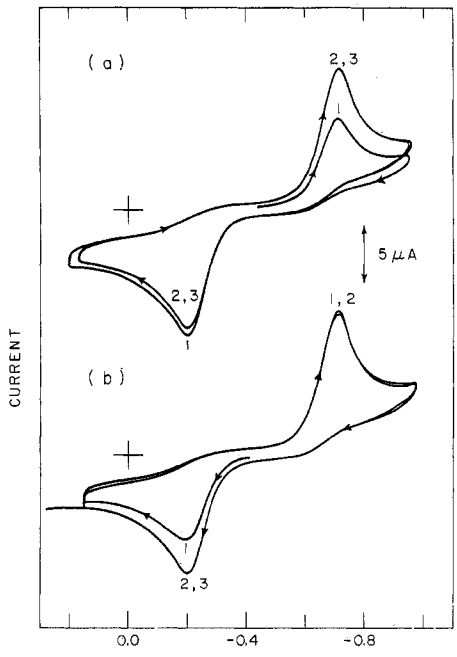


Figure 2.—Cyclic voltammogram of a 0.42 m*M* solution of riboflavin anion after the addition of enough $Th(ClO_4)_4 \cdot xH_2O$ to make the solution 4.1 m*M* in Th^{4+}; the numbers indicate the order of the scans: (a) initial cathodic scan; (b) initial anodic scan. Scan rate, 0.1 V/sec.

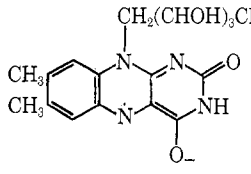
To eliminate the possibility that acid might be the cause of this apparent chemical reduction, $Th(ClO_4)_4 \cdot xH_2O$ has been added to DMSO and the pH noted. Addition of enough damp $Th(ClO_4)_4 \cdot xH_2O$ to make the solution 4 m*M* in Th^{4+} produces a pH change of 0.9 unit. In contrast, addition of enough 2 *F* $HClO_4$ to make a DMSO solution 1 m*M* in H^+ causes a pH change of 4.4 pH units.

Discussion and Conclusions

The present results for a nonaqueous medium indicate that unreduced riboflavin does not interact significantly with iron(II). This is in contrast with the reported stable complex ($\log K_s = 8.0$) for aqueous conditions.[12] However, the latter result has been questioned on the

(12) A. Albert, *Biochem. J.*, **54**, 646 (1953).

basis that riboflavin does not affect the acid–base titration curve of iron(II).[13] Current evidence[6] indicates that the stable tautomer of unreduced riboflavin is that illustrated in the Introduction (I). Such a species would have limited effectiveness as a coordinating agent. However, the radical anion of riboflavin has a tautomer form which is structurally similar (II) to the



II

anion of 8-quinolinol, an extremely effective complexing agent.

The data in Table I indicate that this radical anion forms stable complexes with several metal cations in dimethyl sulfoxide. The highly charged metal ions cause greater peak potential shifts, implying that they form stronger complexes. If the formation of such complexes is assumed, the shift of the anodic peak potential should be more directly related to their stability. The shift in the cathodic peak also is dependent upon the rate of complex formation as well as the reversibility of the reduction reaction. On this basis the apparent order of stability is $La^{3+} = Y^{3+} > Th^{4+} > Ni^{2+} > Fe^{2+} > Ca^{2+} > Na^{+}$. The interaction between neutral riboflavin and metal ions appears to be insignificant, especially in view of its minimal effect on the reduction potential of iron(III).

The riboflavin anion is known to disproportionate in aqueous solution by the reaction[14]

$$2Rib^{-} \rightleftharpoons Rib + Rib^{2-} \quad (1)$$

In nonaqueous systems, the equilibrium for this reaction lies far to the left.[9] However, in the presence of the Y^{3+}, La^{3+}, and Th^{4+} ions the equilibrium appears to be shifted to the right in DMSO as the result of the formation of a more stable Rib^{2-} complex of the metal ions. This causes a significant decrease in the concentration of Rib^{-} accompanied by the formation of neutral riboflavin. The enhancement of the disproportionation of Rib^{-} by multivalent metal ions may occur through initial formation of bis complexes, $M(Rib^{-})_2$, which would then provide a convenient pathway for electron redistribution.

The interactions of metal ions with the various forms of riboflavin may depend in part upon the magnitudes of the charge on the riboflavin species and the metal ion. On this basis, neutral riboflavin would form weak complexes, while singly reduced riboflavin would interact strongly with highly charged metal cations. Highly charged metal ions also promote the disproportionation of the riboflavin anion to form a stronger complex with the doubly reduced Rib^{2-} species.

Acknowledgments.—This work was supported by the National Science Foundation under Grant No. GP-16114.

(13) T. R. Harkins and H. Freiser, *J. Phys. Chem.*, **63**, 309 (1959).

(14) A. Ehrenberg, F. Muller, and P. Hemmerich, *Eur. J. Biochem.*, **2**, 286 (1967).

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The Stoichiometry and Kinetics of the Thermal Decomposition of Molten Anhydrous Lithium Nitrite[1]

BY A. K. K. LEE AND E. F. JOHNSON*[2]

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A semiflow batch reactor has been used to study the thermal decomposition of molten anhydrous lithium nitrite at 250, 300, and 350°. The gaseous products of decomposition from a thin quiescent layer of molten nitrite were swept out of the reactor by a steady flow of argon gas. Kinetic curves of both the gas phase and the molten phase were obtained. The observed rate of overall nitrite decomposition can be represented by a first-order rate equation with the following rate constants: $k = (1.10 \pm 0.05) \times 10^{-6}$ sec^{-1} at 250°, $k = (1.30 \pm 0.07) \times 10^{-6}$ sec^{-1} at 300°, and $k = (1.25 \pm 0.07) \times 10^{-6}$ sec^{-1} at 350°. Individual reaction steps are proposed, which lead to the key reactions

$$3LiNO_2 \rightleftharpoons LiNO_3 + Li_2O + 2NO$$
$$2LiNO_2 \rightleftharpoons Li_2O + N_2O + O_2$$
$$2LiNO_2 + 2NO \longrightarrow 2LiNO_3 + N_2$$
$$2LiNO_3 \rightleftharpoons 2LiNO_3 + O_2$$

The observed overall stoichiometries of decomposition are consistent with combinations of these reactions.

Introduction

Considerable efforts have been made to study the thermal decomposition of molten nitrates and nitrites because the thermal stability and the rate of decomposition of these salts are important factors in molten salt technologies. Surveys[3,4] of the thermal decomposition of nitrates and nitrites indicate that the earlier studies of the decomposition of nitrites are limited to the iden-

(1) This work was supported in part by U. S. Atomic Energy Commission Contract AT(30-1)-1238.

(2) To whom correspondence should be addressed at the Plasma Physics Laboratory, Princeton University.

(3) L. E. Gastwirt and E. F. Johnson, "The Thermal Decomposition of Lithium Nitrate," Report MATT-98, Princeton Plasma Physics Laboratory, Princeton, N. J., 1961.

(4) A. K. K. Lee, Ph.D. Thesis, Princeton University, 1968.