Syntheses, Characterizations, and Biochemical Reactivities of 1-*N*-Oxides of 5′-Adenylic and 5′-Inosinic Acids*

Donald B. McCormick

ABSTRACT: The 1-*N*-oxides of 5′-adenylic and 5′-inosinic acids have been synthesized by peroxidation of 5′-adenylic acid with hydrogen peroxide in acetic acid and by deamination of 5′-adenylic acid 1-*N*-oxide with sodium nitrite in acetic acid, respectively. The 1-*N*-oxides were obtained by chromatography on Dowex 1-X8 in formate and chloride forms.

The infrared absorption spectra of the hydrated barium salts of the 1-*N*-oxides and their ultraviolet absorption spectra at different pH values have been characterized. Although the 1-*N*-oxides of both 5′-adenylic and 5′-inosinic acids are readily dephosphorylated by nonspecific phosphomonoesterases, they appear relatively inert as substrates in those systems requiring 5′-adenylic acid. 5′-Adenylic acid 1-*N*-oxide is an effective competitive inhibitor with 5′-adenylate kinase, but does not inhibit 5′-adenylate deaminase from rabbit muscle.

Until fairly recently, direct oxidation of purines and especially their nucleoside and nucleotide forms had not been investigated. Brown (1957) reported the direct oxidation of adenine with hydrogen peroxide in acetic acid, and these investigations were extended to proof of structures for the 1-*N*-oxides of adenine, adenosine, and 2,6-diaminopurine (Stephens *et al.*, 1958; Stephens and Brown, 1958). The direct oxidation of adenine nucleotides to their 1-*N*-oxides by neutral and acid hydrogen peroxide and by perphthalate also was reported (Cramer and Randerath, 1958; Stephens *et al.*, 1959; Cramer *et al.*, 1963). More recently the 1-*N*-oxides of inosine and 5′-inosinic acid have been prepared by deamination of the corresponding adenine-containing compounds (Sigel and Brintzinger, 1965). However, specific details of rate and extent of formation, chromatographic purification on columns, and absorption spectra in infrared and ultraviolet regions do not appear to have been reported in relatively complete form for both 5′-adenylic acid (5′-AMP)[1] 1-*N*-oxide and 5′-inosinic acid (5′-IMP) 1-*N*-oxide.

The photochemical changes induced by ultraviolet radiation of purine *N*-oxides now has been reported (Brown *et al.*, 1964; Levin *et al.*, 1964) as well as certain properties of the chelated structures of 1-*N*-oxides of adenine and adenosine (Perrin, 1960) and 5′-adenylic acid (Sigel and Brintzinger, 1964). Also the incorporation of ADP 1-*N*-oxide into polyadenylic acid (poly-A) has been noted (Cramer *et al.*, 1963). In spite of the interesting influences of the *N*-oxide grouping on the chemical behavior of the molecule, the possibilities that nucleoside 5′-phosphate 1-*N*-oxides may have significant effects in the biochemical roles of natural nucleotides have not been adequately investigated.

The present study was made to describe quantitatively the optimal conditions for syntheses, chromatographic purifications, spectral characteristics, and biochemical reactivities of 1-*N*-oxides of 5′-adenylic and 5′-inosinic acids.

Experimental Section

Materials and Apparatus. Hydrated sodium salts of nucleotides were the purest available, generally 98–100%, from Sigma Chemical Co. 5′-Adenylate kinase (myokinase), which could convert approximately 1 mmole of ADP/mg of protein per min at pH 7.6 and 37°, and 5′-adenylate deaminase, which could deaminate approximately 2 μmoles of AMP/mg of protein per min at pH 6.5 and 25°, were isolated from rabbit muscle as prepared by Sigma. Preparations from livers of rats and rabbits were used as 10% (w/v) homogenates in 0.02 M potassium phosphate buffer, pH 7. All common reagents were of the highest purity from commercial sources. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory.

Static measurements of absorbancy changes during formation of the *N*-oxides and chromatographic resolutions of nucleotides were made with a Beckman DU spectrophotometer. Continual measurements of absorbancy changes during rapid enzymatic deamination were made with attachment of the Gilford Model 2000 multiple-sample absorbance indicator and recorder. Infrared absorption spectra were obtained with the Perkin-Elmer Infracord and ultraviolet absorption spectra with the Cary 14 recording spectrophotometers.

* From the Graduate School of Nutrition and Biochemistry Section of the Division of Biological Sciences, Cornell University, Ithaca, New York. *Received August 13, 1965.* Supported in part by grants from the U. S. Public Health Service and by funds from the State University of New York.

[1] Abbreviations used in this work: 5′-AMP, 5′-adenylic acid; 5′-deoxyAMP, 5′-deoxyadenylic acid; 5′-AMP 1-*N*-oxide, 5′-adenylic acid 1-*N*-oxide; 5′-IMP, 5′-inosinic acid; 5′-IMP 1-*N*-oxide, 5′-inosinic acid 1-*N*-oxide; ADP, adenosine 5′-diphosphate; ADP 1-*N*-oxide, adenosine 5′-diphosphate 1-*N*-oxide; ATP, adenosine 5′-triphosphate; CDP, cytosine diphosphate; ITP, inosine triphosphate.

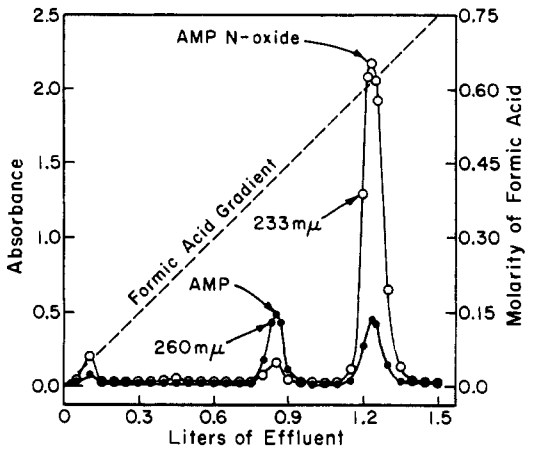

FIGURE 1: Chromatographic purification of 5′-AMP 1-*N*-oxide on Dowex 1-X8 (formate). After 5 days of acid–peroxide treatment, 400 mg of a mixture of 45% 5′-AMP and 55% 5′-AMP 1-*N*-oxide was poured over a 1 × 10 cm column, and 10-ml fractions were collected during linear gradient elution with formic acid. Both absorbancies at 233 (○) and 260 mμ (●) were measured for $^1/_{100}$ dilutions in 0.1 M sodium phosphate, pH 7, against the buffer blank.

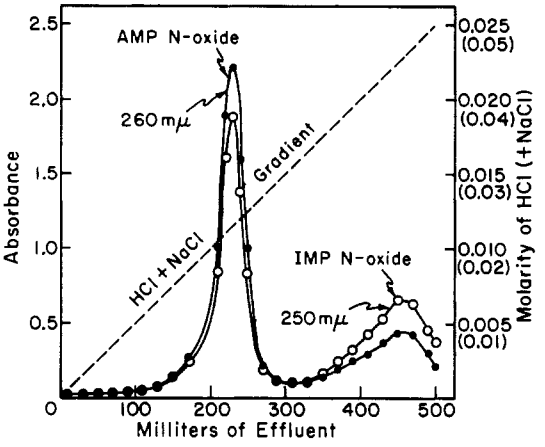

FIGURE 2: Chromatographic purification of 5′-IMP 1-*N*-oxide on Dowex 1-X8 (chloride). After 2.5 hr of nitrous acid treatment, 8 mg of a mixture of 35% 5′-AMP 1-*N*-oxide and 65% 5′-IMP 1-*N*-oxide was poured over a 1 × 10 cm column, and 10-ml fractions were collected during linear gradient elution with HCl containing NaCl. Both absorbancies at 250 (○) and 260 mμ (●) were measured against a water blank.

Syntheses and Isolations. The synthesis of 5′-AMP 1-*N*-oxide was modified from the acid peroxide treatment of Stephens *et al.* (1959). For this, 405 mg (1 mmole) of monosodium 5′-AMP dihydrate was dissolved in 83 ml of glacial acetic acid and 17 ml of 30% H_2O_2 added. The solution was kept in the dark at room temperature for 12–14 days for over 80% conversion to the *N*-oxide. The solution was cooled and 10% palladium on charcoal was stirred in to decompose the excess peroxide. After 1 hr of stirring with gradual warming to room temperature, the mixture was filtered, the catalyst was rinsed on the filter with a little acetic acid, and the combined filtrates were evaporated to near dryness under vacuum while warming below 50°. The residue was taken up in 5 ml of warm ethanol, 5 ml of diethyl ether was added, and the product was allowed to crystallize at refrigerator temperature. After drying *in vacuo* over silica gel, 75% yield of the crude hydrated acid was obtained with mp 180–185° dec. To obtain complete purity, this material was dissolved in 10 ml of water, poured over a 1 × 10 cm column of Dowex 1-X8 (formate), and eluted fractionally during a 12-hr period using a linear gradient established between 1 l. of water and 1 l. of 1 M formic acid. The desired material was eluted within about 300 ml following 1.1–1.2 l. of effluent and the solution was reduced to dryness by lyophilization for 65% over-all yield. The hydrated barium salt was obtained in the usual manner by precipitation with ethanol from a neutral solution to which excess barium acetate was added.

The synthesis of 5′-IMP 1-*N*-oxide was modified from the nitrous acid treatment of Shuster and Kaplan (1953). For this, 40 mg (0.1 mmole) of 5′-AMP 1-*N*-oxide dihydrate was dissolved in 2 ml of 2 *N* acetic acid, and 320 mg of $NaNO_2$ dissolved in 2 ml of water was stirred in over several minutes. The solution was kept in the dark at room temperature for about 3 hr for over 75% conversion to the deaminated product. The solution was adjusted to pH 7 with 1 *N* NaOH and 0.75 ml of 10% barium acetate was added. The product was precipitated with 10 ml of ethanol, collected by centrifugation, and rinsed with ethanol and diethyl ether. After drying *in vacuo* over silica gel, 70% yield of the crude hydrated barium salt was obtained. To obtain complete purity, this material was dissolved in 10 ml of water, poured over a 1 × 10 cm column of Dowex 1-X8 in either formate or chloride form, and eluted fractionally during a 12-hr period. With the formate form, a linear gradient was established between 500 ml of water and 500 ml of 1 M formic acid. The desired material was eluted within 100–150 ml following 800–850 ml of effluent. With the chloride form, a linear gradient was established between 500 ml of water and 500 ml of 0.025 M HCl containing 0.05 M NaCl. The desired material was eluted within 100–150 ml following 750–800 ml of effluent. Reduction of the volume, addition of NaOH to pH 6, and precipitation from acetone–methanol yielded material with the following elemental analysis.

Anal. Calcd for the monosodium dihydrate: C, 28.4; H, 3.8; N, 13.3; P, 7.3. Found: C, 28.6; H, 4.1; N, 13.1; P, 7.2.

Biochemical Assays. With liver preparations, 5 μmoles of 5′-AMP 1-*N*-oxide or 5′-IMP 1-*N*-oxide was incubated with 100 μmoles of Tris–HCl or potassium phosphate buffer, pH 7, and 0.1–0.25 ml of 10% homogenate in 10 ml for 15–30 min at 37°. The contents

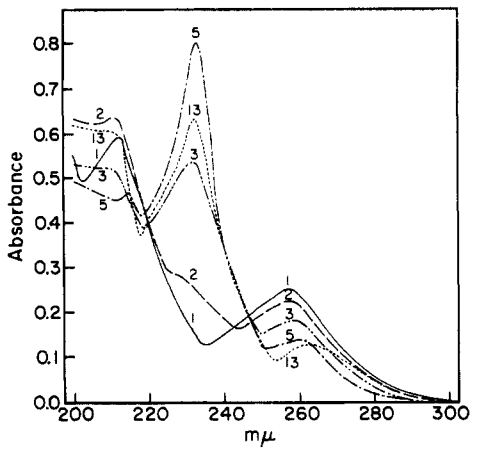

FIGURE 3: Ultraviolet absorption spectra of 5′-AMP 1-*N*-oxide as influenced by pH. Solutions of 2×10^{-5} M 5′-AMP 1-*N*-oxide at pH values indicated by the numbers were made in 0.1 M solutions of: HCl, pH 1; HCl–glycine buffer, pH 2 and 3; sodium acetate buffer, pH 5; NaOH, pH 13.

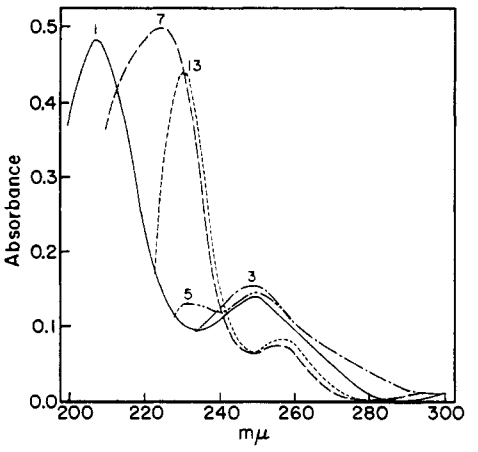

FIGURE 4: Ultraviolet absorption spectra of 5′-IMP 1-*N*-oxide as influenced by pH. Solutions of 6×10^{-6} M 5′-IMP 1-*N*-oxide at pH values indicated by the numbers were made in 0.1 M solutions of: HCl, pH 1; HCl–glycine buffer, pH 3; sodium acetate buffer, pH 5; sodium phosphate buffer, pH 7; NaOH, pH 13.

were acidified with 0.1 ml of 1 N HCl and poured over a 1 × 10 cm column of Dowex 1-X8 (chloride). The column was rinsed with 25 ml of water, and the nucleotides were eluted over 6 hr with a linear gradient established between 500 ml of water and 500 ml of 0.05 M HCl containing 0.1 M $BaCl_2$. Nucleosides and nucleotides were identified by their relative ease of elution and by absorbancy readings at 260, 250, and 233 mμ.

With 5′-adenylate kinase, substrate reactivities were determined by incubating 5 μmoles each of ATP and 5′-AMP or 5′-AMP 1-*N*-oxide, 50 μmoles of $MgSO_4$, 100 μmoles of Tris–HCl buffer, pH 7.5, and 0.5 mg of kinase in 10 ml for 30 min at room temperature. The contents were acidified and chromatographed on Dowex 1-X8 (chloride) as before. Resolution of nucleotides was followed by measuring the effluent absorbance at 260 and 233 mμ. Tests for any inhibition effected by 5′-AMP 1-*N*-oxide were made by incubating 5 μmoles of ATP, 0–10 μmoles of 5′-AMP with and without 5 μmoles of 5′-AMP 1-*N*-oxide, 50 μmoles of $MgSO_4$, 100 μmoles of Tris–HCl buffer, pH 7.5, and 0.5 μg of kinase in 10 ml for 5 min at 37°. The contents were acidified, chromatographed on Dowex 1-X8 (chloride) as before, and ADP was selectively removed and quantitated by absorbance at 260 mμ.

With 5′-adenylate deaminase, the reactions were followed by absorbancy decreases at 265 mμ during 5 min at room temperature as based on the method of Kalchar (1947). The rates of deamination were determined by incubating 0.1 μmole of 5′-AMP, 5′-AMP 1-*N*-oxide, or both, 45 μmoles of sodium citrate buffer, pH 6.5, and 10 μg of deaminase in 3.1 ml. Tests for any inhibition effected by 5′-AMP 1-*N*-oxide were made under these conditions but with 0–0.3 μmole of 5′-AMP with and without 0.3 μmole of 5′-AMP 1-*N*-oxide.

Results

Syntheses and Chromatography. The rate of formation of 5′-AMP 1-*N*-oxide from 5′-AMP in acetic acid containing 5% H_2O_2 at room temperature follows a zero-order course. When aliquots of the reaction mixture are measured near pH 7, the ratios of absorbance at 233–260 mμ and the absolute absorbance at 233 mμ increase in a linear manner for over 1 week. After nearly 2 weeks, the reaction approached completion. The rate of deamination of 5′-AMP 1-*N*-oxide in acetic acid containing a large molar excess of $NaNO_2$ at room temperature follows an apparent first-order course. The decrease in absorbance at 265 mμ measured in neutralized aliquots progressively decreases for 2–3 hr after which time additional nitrite will again produce further deamination which proceeds to near completion.

The chromatographic pattern from Dowex 1-X8 (formate) for separation of a mixture of 5′-AMP 1-*N*-oxide and 5′-AMP which results from a 5-day treatment with acid peroxide is shown in Figure 1. Differentiation of the two nucleoside 5′-monophosphates is readily made on the basis of the much higher absorbance at 233 than 260 mμ of 5′-AMP 1-*N*-oxide near neutrality with the converse situation for 5′-AMP at any pH value. Moreover, the relatively slower elution of 5′-AMP 1-*N*-oxide with increasing formic acid is a reflection of the greater acidity of the *N*-oxide. The chromatographic pattern from Dowex 1-X8 (chloride) for separation of a mixture of 5′-IMP 1-*N*-oxide and 5′-AMP 1-*N*-oxide which results from a 2.5-hr treatment with nitrous acid is shown in Figure 2. Differentiation of these two nucleotide 1-*N*-oxides is made on the basis of greater absorbance at 250 than 260 mμ of the IMP derivative under slightly acid conditions with the converse true of 5′-AMP 1-*N*-oxide. Again as expected,

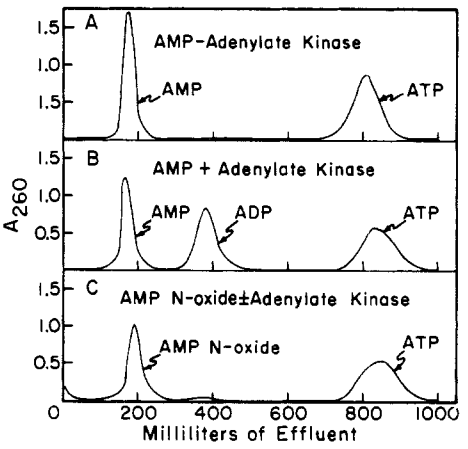

FIGURE 5: Comparison of the substrate reactivities of 5′-AMP and 5′-AMP 1-*N*-oxide with 5′-adenylate kinase. Nucleotide patterns are from chromatographic resolution of 30-min incubations of 5 μmoles each of 5′-AMP and ATP, 50 μmoles of Mg^{2+}, 100 μmoles of Tris–HCl, pH 7.5, without (A) and with 0.5 mg of kinase (B) in 10 ml at room temperature. When 5′-AMP 1-*N*-oxide replaces 5′-AMP (C), the patterns are the same with and without enzyme. Chromatography was accomplished by linear gradient elution from water to 0.05 M HCl plus 0.1 M NaCl on a 1 × 10 cm column of Dowex 1-X8 (chloride).

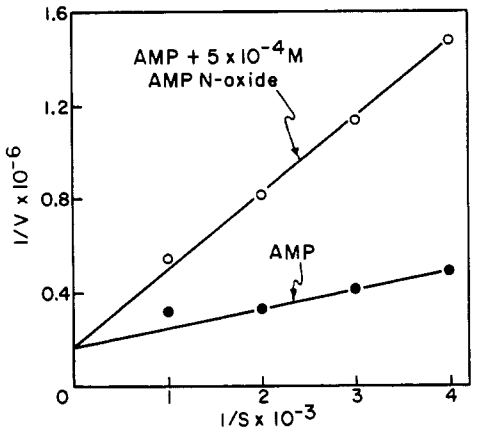

FIGURE 6: Competitive inhibition of 5′-adenylate kinase with 5′-AMP 1-*N*-oxide. Reactions were measured as the amount of ADP formed during 5-min incubations of 0–10 μmoles of 5′-AMP with (○) and without (●) 5 μmoles of 5′-AMP 1-*N*-oxide, 5 μmoles of ATP, 50 μmoles of Mg^{2+}, 100 μmoles of Tris–HCl, pH 7.5, and 0.5 μg of kinase in 10 ml at 37°. Contents were acidified with HCl and poured over a 1 × 10 cm column of Dowex 1-X8 (chloride) from which the ADP was selectively removed during linear gradient elution with increasing concentrations of HCl and NaCl.

5′-IMP 1-*N*-oxide is a stronger acid than 5′-AMP 1-*N*-oxide and is eluted from the anion-exchange column under conditions where more eluting anion has been exchanged.

Spectral Characteristics. Comparison of the infrared absorption spectra of the hydrated sodium salt of 5′-AMP ($NaAMP \cdot 2H_2O$) and the hydrated barium salt of 5′-AMP 1-*N*-oxide revealed that the strong absorption at 1670 cm^{-1} due to the 6-amino group in 5′-AMP is shifted to an increased frequency at 1690 cm^{-1} in the *N*-oxide derivative. Also, absorption near 1100 cm^{-1}, characteristic of the anionic phosphate group of these salts, is intensified with loss of fine structure in the 1-*N*-oxide. This also occurs at 985 cm^{-1}. Comparison of the infrared absorption spectra of the hydrated sodium salt of 5′-IMP ($Na_2IMP \cdot 7H_2O$) and the hydrated barium salt of 5′-IMP 1-*N*-oxide revealed that the strong absorption at 1700 cm^{-1} due to the 6-hydroxyl group in 5′-IMP is shifted in the *N*-oxide to a decreased frequency at 1670 cm^{-1}. Again as with 5′-AMP 1-*N*-oxide, the absorptions characteristic of the anionic phosphate group in the salt of 5′-IMP 1-*N*-oxide are intensified with loss of fine structure.

The ultraviolet absorption spectra of 5′-AMP 1-*N*-oxide at various pH values are shown in Figure 3. Absorption maxima shift from 258 to 263 mμ concomitant with approximately 50% decrease in intensity in going from strong acid to strong alkaline medium. Correspondent with this change, absorption at 233 mμ increases from a minimum in strong acid to a maximum near neutrality and then decreases somewhat in the alkaline range. The maximum (neutral) ratio of absorption at 233:260 mμ is approximately 6, whereas the minimum (acid) ratio is approximately 0.5. The ultraviolet absorption spectra of 5′-IMP 1-*N*-oxide at various pH values are shown in Figure 4. Absorption maxima occur near 250 mμ at acid pH and shift toward 260 mμ with approximately 50% decrease in intensity as the medium becomes more alkaline. As with the 1-*N*-oxide of 5′-AMP, 5′-IMP 1-*N*-oxide shows a large increase in absorption in the lower ultraviolet range, but shows a pronounced bathochromic shift from 205 to 250 mμ with increasing alkalinity.

Biochemical Reactivities. The predominant biochemical change which appears to occur during incubation of 1-*N*-oxides of 5′-AMP and 5′-IMP with liver homogenates is the hydrolytic cleavage of the orthophosphate linkages by nonspecific phosphatases. Considerable amounts of the nucleoside 1-*N*-oxide accumulate, especially using Tris buffer, during prolonged periods of incubation. The nucleosides are readily rinsed through the anion-exchange columns of Dowex which retain the remaining nucleotides.

As shown by the nucleotide patterns in Figure 5, 5′-AMP 1-*N*-oxide appears inactive as substrate for 5′-adenylate kinase under conditions where 5′-AMP with ATP is readily interconverted with ADP. No *N*-oxides of ADP or ATP were detected from the incubation of 5′-AMP 1-*N*-oxide with ATP in this kinase system. However, when 5′-AMP 1-*N*-oxide was tested with the enzyme in the presence of 5′-AMP and ATP, the results shown in Figure 6 were obtained. The 1-*N*-

oxide appears to be an effective competitive inhibitor of adenylate kinase with a calculated K_i of 2×10^{-4} M compared with a K_m value of 5×10^{-4} M for 5′-AMP. Moreover, large concentrations of 5′-AMP can elicit inhibition of the enzyme as seen by the slight deviation from linearity in the Lineweaver–Burk plots with high substrate.

The rate of deamination of 3×10^{-5} M 5′-AMP with 5′-adenylate deaminase in the presence and absence of 5′-AMP 1-*N*-oxide is the same. Over 5 min, a linear decrease of 0.1 in absorbance at 265 mμ was observed with 5′-AMP. No change was observed with 5′-AMP 1-*N*-oxide alone. Thus, the 1-*N*-oxide is inactive both as substrate and as inhibitor for 5′-adenylate deaminase.

Discussion

The peroxidative treatment of 5′-AMP proceeds in a straightforward fashion, but as found before (Stephens *et al.*, 1959), does not quantitatively yield the 1-*N*-oxide. Rather, a mixture which still contains some 5′-AMP is obtained even after many days of reaction. The deamination of 5′-AMP 1-*N*-oxide by nitrous acid proceeds in a manner similar to that for adenylate and cytidylate derivatives (Shuster and Kaplan, 1953). The 1-*N*-oxides of both 5′-AMP and 5′-IMP can be readily purified by chromatography over anion-exchange resins. The decreasing ease of elution of these *N*-oxides indicates increasing over-all acidities which may be compared with similar nucleoside 5′-phosphates in the following order of appearance in the effluent: AMP > AMP 1-*N*-oxide > IMP > IMP 1-*N*-oxide > ADP. Care must be taken to avoid exposure to ultraviolet light which leads to such photodecomposition as found with 1-*N*-oxides of purines (Brown *et al.*, 1964; Levin *et al.*, 1964) and to heating in solution at extremes of pH, especially with 5′-IMP 1-*N*-oxide.

Spectral properties of the 1-*N*-oxides of 5′-AMP and 5′-IMP may be interpreted on the basis of the *N*-oxide function in the purinic structure. In the infrared spectra of 5′-AMP compared with its 1-*N*-oxide, the indication of greater double bond character of the C–N linkage at position 6 in the purine 1-*N*-oxide arises from the shift to higher frequency for the primary absorption attributable to influence of the amino group at this position. This is explainable in terms of the greater tendency of the *N*-oxide toward a 6-imino tautomeric salt form with barium than the corresponding 5′-AMP. This is analogous to the situation indicated by elemental analyses and ultraviolet spectral studies of chelated adenosine 1-*N*-oxide (Perrin, 1960). In the infrared spectra of 5′-IMP compared with its 1-*N*-oxide, the diminished double-bond character of the C–O linkage at position 6 in the purine 1-*N*-oxide is indicated by the shift to lower frequency for the primary absorption due to the hydroxyl group at this position. Again as with 5′-AMP 1-*N*-oxide, this can be explained in terms of a favored enolic tautomeric form for the salt of IMP 1-*N*-oxide. In general, for the 1-*N*-oxides of both 5′-AMP and 5′-IMP, the more positive nature of the 1-nitrogen enhances the loss of a proton at higher pH values. Intensification of absorptions characteristic of the 5′-phosphate groupings in both of the *N*-oxides is similar to the infrared antisymmetric phosphate bond at 1090 cm^{-1} reported in solutions of 5′-AMP 1-*N*-oxide (Sigel and Brintzinger, 1964). The contribution of particular groups in the purine portion of 1-*N*-oxides to absorption in the ultraviolet region of the spectrum has been noted in terms of their discrete pK_a values and agrees well with observations of adenine 1-*N*-oxide and adenosine 1-*N*-oxide made by others (Stephens and Brown, 1958; Perrin, 1960). Decreases in the absorptions of 5′-AMP 1-*N*-oxide and 5′-IMP 1-*N*-oxide around 230 mμ in alkaline solutions are undoubtedly due to loss of a proton from the tautomeric enol forms of these compounds.

Except for the nonspecific enzymatic dephosphorylations of 5′-AMP 1-*N*-oxide and 5′-IMP 1-*N*-oxide, it appears that both compounds are relatively inert metabolically. However, the 1-*N*-oxide of 5′-AMP is an effective competitive inhibitor in one system heretofore presumed relatively specific for the 5′-adenylate structure. With 5′-adenylate kinase, the only previously reported inhibitor which is competitive with 5′-AMP is adenosine 5′-monosulfate (Callaghan and Weber, 1959), although excess substrate has been observed to inhibit (Colowick, 1955), and CDP may have some activity (Noda and Kuby, 1963). The 3-isoadenosine analogs of AMP, ADP, and ATP also have been shown to equilibrate as substrates in this system (Leonard and Laursen, 1965). No other purine or pyrimidine derivative was demonstrated to serve as either substrate or competitive inhibitor for 5′-adenylate kinase. Recently, high concentrations of 5′-AMP 1-*N*-oxide have been found to inhibit liver ATP–AMP transphosphorylase and to have no effect on ITP–AMP transphosphorylase.[2] With 5′-adenylate deaminase, specificity is again quite narrow for 5′-AMP with weak substrate activity exhibited by 5′-deoxyAMP (Lee, 1957). Competitive inhibition of this deaminase has been demonstrated with phosphate, pyrophosphate, and excess substrate (Nikiforuk and Colowick, 1955). It is also interesting, in terms of biological reactivity of 1-*N*-oxides, that ADP 1-*N*-oxide can be incorporated into poly-A by extracts of *Azotobacter vinelandii* which contains polynucleotide phosphorylase (Cramer *et al.*, 1963).

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Effects of Deoxycholate on the Microsome Fraction of Thymus*

E. L. Hess and D. F. Oberhauser

ABSTRACT: Ultracentrifuge patterns obtained with the microsome fraction from calf thymus extracts are clearly distinguished from similar solutions to which deoxycholate (DOC) was added. In solutions which contain DOC both the total area of the pattern and the area under the peak in the region of the meniscus is strikingly larger, whereas the peak which sediments most rapidly, usually ascribed to ribosomes, is reduced in area and in rate of sedimentation. Within the concentration ranges examined, 0.1–0.5% DOC, the above changes are dependent upon the concentration of DOC. In control experiments with the microsome fraction in the absence of DOC, there is a progressive decrease in the total area of the pattern with time of centrifugation and a decrease in the areas of individual peaks, greater than can be attributed to radial dilution effects. Area and weight relationships and ribonucleic acid (RNA) and lipid analyses are consistent with the behavior in transport experiments of two reactants in equilibrium with a product. In sedimentation experiments with the microsome fraction the boundary usually ascribed to ribosomes appears to be a reaction boundary. A destruction of isolated 74S ribosomes as a function of DOC concentration was also observed.

The microsome fraction is defined operationally as subcellular material which, after removal of the mitochondria, sediments at 105,000*g* (Loftfield, 1957; Hess and Lagg, 1963a). In accord with the suggestion of Roberts (1958) and consistent with current usage (Petermann, 1964) the term ribosome will designate ribonucleoprotein particles in the size range 20S–100S. The sedimentation properties of ribosomes observed when the microsome fraction of calf thymus extracts is centrifuged differ from those of isolated ribosomes (Hess and Lagg, 1963a). On the basis of several kinds of evidence we suggested that in the microsome fraction ribosomes, and a fraction called M rich in lipids, exist in equilibrium with an interaction product (Hess and Lagg, 1963a). The behavior of two reactants in equilibrium with a product in transport experiments has been considered by Svedberg and Pedersen (1940), Gilbert and Jenkins (1956), Schachman (1959), and Nichol *et al.* (1964). Direct evidence for an interaction between deoxycholate (DOC)[1] and the lipid-rich microsomal constituent has also been reported (Hess and Lagg, 1963b).

This report will present evidence suggesting that a component seen in ultracentrifuge patterns obtained with the microsome fraction of thymus, which would normally be considered to represent ribosomes, is in reality an interaction product. The addition of deoxycholate to the fraction displaces the equilibrium and alters the quantitative distribution of peaks seen in the pattern.

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[1] Abbreviations used: DOC, deoxycholate; RNA, ribonucleic acid; M, membrane; R, ribosomes.