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Isolation and Characterization of a Methionine Adduct of DOPA *o*-Quinone

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The *o*-quinone of DOPA, an important intermediate implicated in many biological processes, has been found to react with methionine. The product has been isolated and studied, and tentative structure has been assigned.

INTRODUCTION

The *o*-quinone of DOPA[1] has been implicated in many biological processes such as melanine biosynthesis (*1*), treatment of schizophrenia (*2*), and mechanism of polyphenol oxidase (*3*). The substance, however, has never been isolated. Perhaps the only evidence for its existence comes from transient kinetic studies (*4*). Furthermore, the well-known reaction of *o*-quinone chemistry, i.e., the reaction with —SH group of cysteine, has not been observed in case of the *o*-quinone of DOPA (*5*). In this context, mention should be made of the work on the interaction of other related quinones and thiol compounds because of its possible significance in melanin formation and other biological processes (*6, 7*).

As the reaction with side chains of amino acids is an underlying feature of biological importance of *o*-quinones (*1, 8–10*), we have examined the reaction between methionine and the *o*-quinones of DOPA, homoprotocatechuic acid, adrenalin, and noradrenalin (*11*), since such a reaction has been reported in the case of *o*-benzoquinone (*12–14*). In the present paper, we wish to report our results on the nature of the reaction product between the *o*-quinone of DOPA and methionine. These results indicate that the product is a sulfonium compound which is not stable because of an abnormally low pK_a value for its phenolic ionization.

MATERIALS AND METHODS

N-Acetyl methionine, methionine, and DOPA were purchased from Sigma Chemical Company. All other chemicals were of analytical grade.

[1] Abbreviations used: DOPA, 3,4-Dihydroxyphenylalanine; NAM, *N*-acetyl methionine.

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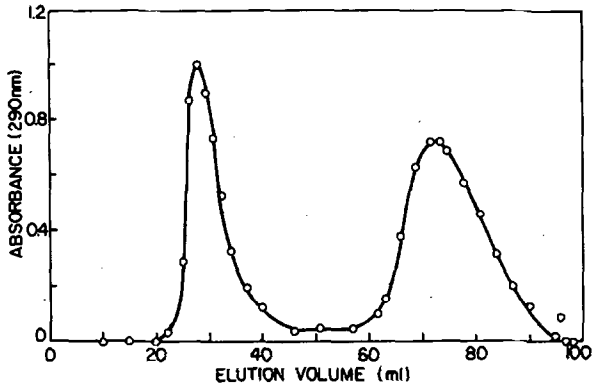


FIG. 1. Gel filtration of DOPA and NAM derivative on Sephadex G-10. The column size was 1.5 × 40 cm. The filtration was carried at 20°C. The column was eluted with 0.1 *N* HCl.

Isolation of DOPA Quinone Derivative of Methionine

DOPA (100 mg) and methionine (1.4 g) were dissolved together (12 ml, 1 *N* sulfuric acid). Ceric sulfate solution (50 mg/ml, 1 *N* sulfuric acid) was slowly added to above solution. Excess oxalic acid was added to this reaction mixture to precipitate all cerium as cerrous oxalate. The latter was filtered off, and excess lead acetate was added to the clear supernatant so that all the remaining oxalate precipitated out as lead oxalate. The mixture was again filtered, and H_2S was passed through the supernatant. Excess lead which precipitated out as lead sulfide was filtered off. The clear filtrate was kept under vacuum to remove dissolved H_2S and was concentrated to about 10 ml.

An aliquot (2 ml) from the above solution was loaded on a Sephadex G-10 column (Fig. 1), and ultraviolet spectra of all the fractions were recorded. The

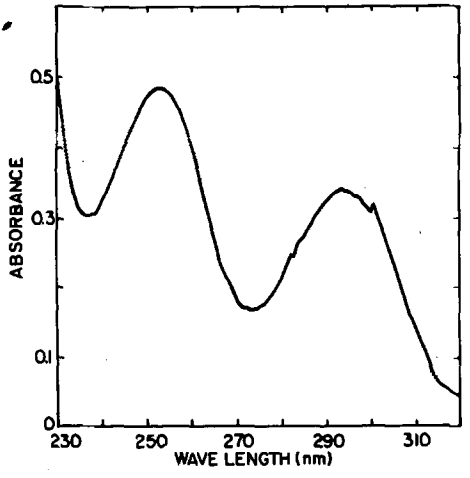


FIG. 2. Spectra of NAM derivatives of *o*-quinone of DOPA. The spectra of the lyophilized sample was recorded in 0.1 *N* HCl.

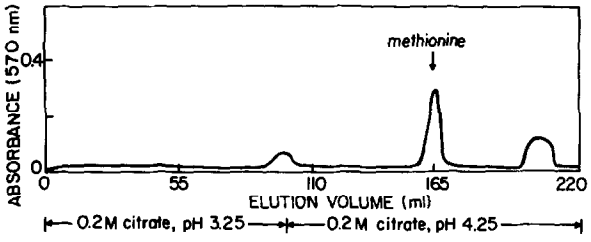


FIG. 3. Amino acid analysis of the acid hydrolysate of methionine derivative of *o*-quinone of DOPA. The column length was 80 cm. It was operated at 50°C with the buffer flow rate of 90 ml/hr.

material eluting between 60 and 90 ml was found to have the same ultraviolet spectra as that of authentic DOPA. The material eluting between 25 and 40 ml, however, gave rise to spectra which were different from that of DOPA. This fraction was concentrated by lyophilization, and its spectra is shown in Fig. 2. The reaction product with NAM was also isolated similarly and had similar ultraviolet spectra.

Acid Hydrolysis of the Methionine Derivative

The sample (5 mg; amount measured by measuring the absorbance at 290 nm and assuming the same extinction coefficient for the compound as known for the *o*-benzoquinone derivative (*12*)) was hydrolyzed in 5.8 *N* HCl (6 ml) under vacuum for 22 hr at 110°C. The hydrolysate was dried under KOH in vacuum. The residue was dissolved in citrate buffer (0.2 *M*, pH 3.25) and analyzed for amino acids on an EEL 193 automatic amino acid analyzer (Fig. 3) (*15*).

Hydrolysis of the Methionine Derivative by Alkali

The methionine derivative (5 μmol, 1 ml aqueous solution) was kept at pH 12.0 for 10 min, after which the pH was brought to 3.0. This sample was analyzed on the amino acid analyzer (Fig. 4) (*15*).

All ultraviolet spectra were recorded on Cary-14 spectrophotometer.

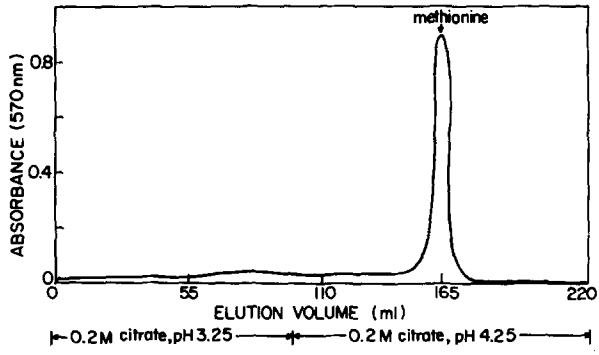


FIG. 4. Amino acid analysis of the alkali hydrolysate of methionine derivative of *o*-quinone of DOPA. The column length was 80 cm. It was operated at 50°C with the buffer flow rate kept at 90 ml/hr.

RESULTS

A major difficulty in studying DOPA quinone and its chemistry has been the fast cyclization of the side chain to form hetrocyclic compounds (*1*). The DOPA quinone derivative of NAM forms colored product with ninhydrin solution, showing that the side chain of DOPA quinone has not cyclized. The elution position on the Sephadex G-10 column indicates that it has a higher molecular weight than DOPA.

Acid hydrolysis of the derivative releases methionine (Fig. 3). If the molar extinction coefficients of the derivative are assumed to be of the same order as that of the *o*-benzoquinone derivative of methionine, the amount of derivative hydrolyzed and loaded for analysis can be calculated. Such calculations show that 1 mol of the compound contains 1 mol of methionine. Other minor ninhydrin positive decomposition products arise from the remaining part of the molecule.

Hydrolysis with alkali also releases methionine (Fig. 4). Here also the data indicate that 1 mol of the compound contains 1 mol of methionine.

Vithayathil and Murthy (*12*) have reported the ultraviolet spectra of the methionine derivative of *o*-benzoquinone. This compound has two absorption maxima at 287 and 250 nm. The DOPA quinone derivative has absorption maxima at 293 and 253 nm, respectively. As in the case of the *o*-benzoquinone derivative, the absorption around the 250-nm region is higher than the absorption around the 290-nm region.

The spectral titration of the NAM derivative of *o*-benzoquinone derivative (Fig. 5) reveals that with an increase in pH from 2 to 3 (i) the absorption maximum at 293 nm underwent a red shift with a concomitant increase in the absorption, and

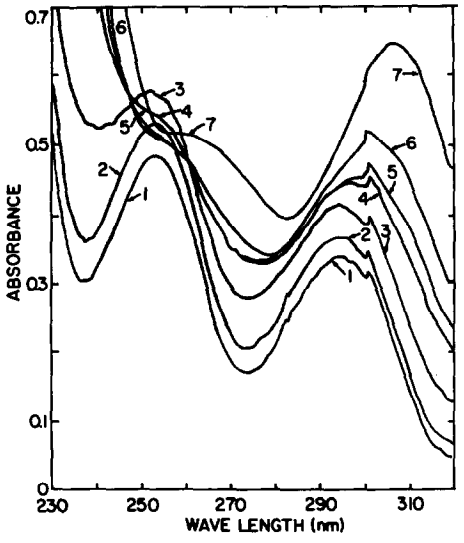


FIG. 5. Spectra of the NAM derivative of *o*-quinone of DOPA at different pH. The concentration of the derivative was same at all pH. 1 : 1.8 pH; 2 : 2.0 pH; 3 : 2.5 pH; 4 : 2.9 pH; 5 : 3.1 pH; 6 : 3.5 pH; 7 : 4.8 pH.

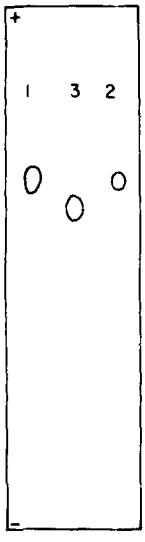


FIG. 6. Paper electrophoresis of DOPA, NAM, and methionine derivatives of *o*-quinone of DOPA. Electrophoresis was carried out in 5% formic acid at a voltage gradient of 20 V/cm for 2 hr. 1, DOPA; 2, NAM derivative; 3, methionine derivative.

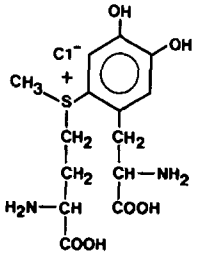
(ii) the absorbance at 250 nm was significantly decreased. Addition of acid to the solution at pH 3 resulted in the reversal of the spectrum to one identical with spectrum at pH 2. The spectral changes indicate a phenolic group ionizing with pK_a around 3. The pK_a value of a phenolic group (9.8) is known to decrease when a sulfonium group is directly attached to the aromatic ring (*12*).

Paper electrophoresis of the NAM and methionine derivatives at pH 2.0 show that these compounds are homogenous (Fig. 6). Again, because of the abnormally low pK_a value for phenolic ionization, it is difficult to make comments about the charges on the molecules, as the pK_a for the carboxyl group ionization also falls in the same pH range. It is seen that the mobility of the DOPA quinone derivative of NAM is not different from that of DOPA. One explanation could be that carboxyl groups in the derivative ionize at slightly lower pH than the carboxyl group of DOPA. In that case the negative charges on these groups neutralize the expected additional positive charge corresponding to the sulfonium group of the NAM derivative.

DISCUSSION

Two general reactions for *o*-quinones with side chains of amino acids are the reactions with the —SH group and the —NH_2 group. It is believed that the —SH group does not react with DOPA quinone *in vitro* (*5*), even though such a reaction has been observed *in vivo* (*2*). Vithayathil and Murthy have described the reaction of *o*-benzoquinone with methionine and showed that the product is a sulfonium compound (*12*). We have now shown that DOPA quinone also reacts with

methionine (*11*). The evidence presented in this paper fits best with a structure which is analogous to the *o*-benzoquinone derivative (*12*). Hence the DOPA quinone derivative of methionine can be tentatively assigned the structure



It has been difficult to gather further evidence in support of this structure because of the ionization of phenolic group in the compound at an abnormally low pH. Any attempt to obtain the compound in a solid state from its acidic solutions results in isolation of a dark red product, typical of polymerized products normally obtained from oxidation of polyphenolic substances. Thus, the unstable nature of the compound has imposed limitations on the methods which can be used for structural characterization of this compound.

It is possible that this compound is involved in the many biological processes where DOPA quinone is implicated. Also, the reaction described here has proved to be useful in the chemical modification of enzymes (*12–14*). Thus, data on the derivative described here may be useful when using *o*-quinones as chemical modification reagents.

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