3,4-Dihydroxy-L-phenylalanine as the Tyrosinase Cofactor

OCCURRENCE IN MELANOMA AND BINDING CONSTANT*

(Received for publication, June 6, 1967)

SEYMOUR H. POMERANTZ AND MARGARET C. WARNER
From the Department of Biological Chemistry, University of Maryland School of Medicine, Baltimore, Maryland 21201

SUMMARY

3,4-Dihydroxy-L-phenylalanine (dopa) has been shown to account for the cofactor activity for tyrosinase contained in extracts of hamster melanoma. Dopa has been identified by several different comparisons with an authentic sample and by conversion to two derivatives. The amount of dopa in the tumor (4 to 8 µg per g) has been estimated by cofactor assay and fluorometry. A $K_m$ value for dopa as a cofactor was estimated to be about $2 \times 10^{-4} \text{m}$, much lower than its $K_m$ as a sole substrate ($5 \times 10^{-4} \text{m}$).

The hydroxylation of tyrosine catalyzed by tyrosinase requires a reduced cosubstrate. For tyrosinase from hamster melanoma and, by extension, for other mammalian tyrosinases, the most efficient cosubstrate in vitro is 3,4-dihydroxy-L-phenylalanine (1). Only a catalytic amount of dopa need be added because dopa is a product of the hydroxylation reaction (Equation), and some steady state level is present even as dopa is converted to melanin (2).

In contrast to the requirement of dopa by tyrosinase, a reaction:

\[
\text{L-Tyrosine-3,5-}^3\text{H} + \text{L-dopa} + \text{O}_2 \rightarrow \text{L-dopa-5-}^3\text{H} + \text{L-dopa quinone} + 3\text{H}_2\text{O} \tag{1}
\]

reduced pteridine is required for the tyrosine hydroxylase of adrenal medulla (3, 4).

This paper reports the concentration from hamster melanoma of a substance with cofactor activity for tyrosine hydroxylation by tyrosinase, the identification of this substance as L-dopa, the measurement of the concentration of dopa in melanoma, and an approximate binding constant for this substance as a cofactor. A preliminary account of part of this work has appeared (5).

* This research was supported in part by Research Grant CA-07993 from the National Cancer Institute, National Institutes of Health.

1 The abbreviation used is: dopa, 3,4-dihydroxyphenylalanine; dopamine, 3,4-dihydroxyphenylethylamine.

EXPERIMENTAL PROCEDURE

Materials—Melanomas were propagated in hamsters, and tyrosinase was partially purified from these tumors and assayed spectrophotometrically as reported previously (6). The tyrosinase used was the DEAE-cellulose fraction purified 200 to 300-fold. L-Tyrosine decarboxylase from Streptococcus faecalis was purchased from Sigma; N-methyl-N-nitroso-p-toluensulfonamide from Aldrich and the N-14C-methyl compound from New England Nuclear; and L-dopa-2,5,6-3H from Nuclear Chicago. The origins of other materials were given previously (1).

Chromatography Solvents—The solvents used in paper chromatography were n-butyl alcohol-acetic acid-water (80:20:20) (Solvent A); tert-butyl alcohol-formic acid-water (70:15:15) (Solvent B); and methyl ethyl ketone-propionic acid-water (60:20:24) (Solvent C).

Radioassay—All radioassays were done in polyethylene vials on a Packard model 3315 spectrometer with a dioxane scintillator solution (7). Automatic external standardization was employed to determine efficiencies.

Electrophoresis—High voltage paper electrophoresis was performed on a 110 cm flat plate (8) with Whatman No. 3MM paper. At pH 1.9 a potential of 35 volts per cm and a current of 25 to 35 ma was employed for 2 hours, and the buffer was composed of 0.36 M formic acid and 1.83 M acetic acid. The pH 4 buffer was 0.2 M pyridine-acetic acid and it was used at 35 volts per cm at a current of 35 to 45 ma for 1 hour.

Hydroxylation of Tyrosine—This reaction was estimated by determining the radioactivity of an aliquot of the $3\text{H}_2\text{O}$ produced from incubations of L-tyrosine-3,5-3H and tyrosinase (1). The dopa-catalyzed reaction was run for 20 min with the addition of $1.2 \times 10^{-4} \text{M}$ dopa, an amount which gives a constant rate of hydroxylation (1). In order to correct for the inhibitory effects of some tissue fractions, aliquots of these fractions were added to standard dopa incubations.

Cofactor Assay—The assay for cofactor activity in melanoma extracts was based on the reduction of the lag preceding tyrosine hydroxylation in the absence of dopa. This type of incubation was performed for 60 min. A typical cofactor reaction mixture contained an aliquot of the fraction to be tested (adjusted to about pH 6.5 if necessary), 1 µmole of L-tyrosine 3,5-3H (5 to
and another faster moving ninhydrin-positive area just behind and showed one ninhydrin-positive spot corresponding to dopa. The reaction mixture was examined by electrophoresis at pH 1.9 generated from N-methyl-N-nitroso-p-toluenesulfonamide (9).

A pound was prepared by treatment of dopa with diazomethane. This was done with the above reaction mixture omitting 3H-dopa. The appearance within 1 hour of the pink color of dopachrome indicated the presence of cofactor.

The reaction mixture, incubated at 37°, contained L-tyrosine-3,5-3H (1.0 μmole, 6.34 X 10^6 dpm); sodium phosphate buffer (35 μmoles, pH 6.8); purified tyrosinase (1.2 units); and additional components as indicated in a volume of 1.25 ml. 

### TABLE I

**Quantitation of cofactor in melanoma fraction**

The reaction mixture, incubated at 37°, contained L-tyrosine-3,5-3H (1.0 μmole, 6.34 X 10^6 dpm); sodium phosphate buffer (35 μmoles, pH 6.8); purified tyrosinase (1.2 units); and additional components as indicated in a volume of 1.25 ml. 

The concentration of cofactor activity and the extent of hydroxylation were always performed in both quantitative and qualitative assays.

**Preparation of O,O'-dimethyl-L-dopa Methyl Ester—**This compound was prepared by treatment of dopa with diazomethane generated from N-methyl-N-nitroso-p-toluenesulfonamide (9). The reaction mixture was examined by electrophoresis at pH 1.9 and showed one ninhydrin-positive spot corresponding to dopa and another faster moving ninhydrin-positive area just behind dopamine. This compound was established as O,O'-dimethyl-L-dopa methyl ester by treatment of L-dopa-2,5,6-3H with 14CH2N2. As shown by the data in Table II, three methyl groups were introduced into dopa by this treatment.

Two types of observations, illustrated in Fig. 2, showed that a factor was present in melanoma which shortened the lag in tyrosine hydroxylation. From Fig. 2a it is seen that increased amounts of crude enzyme not only increase the rate of oxygen uptake but also reduce the lag period. Fig. 2b shows that addition of boiled purified enzyme to a tyrosinase reaction will also reduce the lag.

The method summarized is the procedure finally adopted after numerous trials. Hamster melanoma (400 g) was homogenized in the cold with 1.2 liters of 0.01 M sodium phosphate, pH 6.5, made 5% in HClO4 by the addition of concentrated acid, and then centrifuged. The supernatant solution was titrated to pH 7.9 with 5 N KOH, allowed to stand in an ice bath for 30 min, and centrifuged to remove KClO4. The filtrate (Fraction A) was made 0.1 N in HCl and applied at room temperature to a column (1.9 X 20 cm) of Dowex 50(H+)-X8, 100 to 200 mesh. The column was eluted successively with 100 ml of 0.1 N HCl, 100 ml of 1.0 N HCl, 200 ml of 2.0 N HCl, and 600 ml of 4 N HCl. The last fraction, which contained the cofactor activity, was concentrated under reduced pressure below 0°. This fraction (B)
was then mixed with 40 ml of 0.1 m sodium borate, pH 8.6, brought to pH 8.5 to 8.6 by the addition of 1 N NaOH, and then applied at room temperature to a column (1.9 x 30 cm) of Dowex 2 (borate)-X8, 100 to 200 mesh (10). The column was eluted successively with 300 ml of 0.1 m sodium borate, pH 8.6; 600 ml of 2.5% boric acid; and 600 ml of 0.2 m HCl divided into two fractions. The second 0.2 N HCl fraction (350 ml), containing cofactor activity, was applied to a column (0.9 x 50 cm) of Dowex 50 (H+)-X8, 200 to 400 mesh. The column was washed with 1 N HCl and then eluted with a linear gradient of HCl (1 N → 4 N). Aliquots were removed from alternate tubes and concentrated to dryness below 0°; the presence of cofactor in the residue was determined qualitatively. The activity appeared first on elution with 2 N HCl. The active fractions were pooled and evaporated to dryness with a vacuum pump. The residue was diluted to 2 to 5 ml (Fraction C), and the entire fraction was streaked on large sheets of Whatman No. 3MM paper for descending chromatography in Solvent A. It was found that the streak could contain as much as 1 μmole of amino acid per cm without disturbing the movement of bands. The developed paper was cut into 1-cm strips, and about 1% of each area was cut from the centers, placed in tubes, and assayed qualitatively. The appropriate strips were eluted with 0.01 N HCl, concentrated by lyophilization (Fraction D), and subjected to electrophoresis at pH 1.9. Strips were cut out, assayed, and eluted as before (Fraction E).

An amino acid analysis by the ninhydrin procedure (11) was obtained on each fraction in order to gauge the effectiveness of the procedure. Table III shows the typical purification obtained by the above method. The activity shown for Fraction A is probably lower than the activity actually present in the original perchlorate extract; however, activity was usually not measured until after neutralization and removal of KCIO₄. The activity in Fraction A in seven preparations on this scale ranged from 363 to 840 units.

Fraction E was purified from several other ninhydrin-positive bands which were present in Fraction D; however, it is unlikely that it was pure, as will be shown later by direct analysis for dopa.

**Identification of Dopa in Active Fractions**

Comparisons of crude Fractions A and B with dopa, with respect to stability to heat, acid, and alkali, gave equivocal results. When the cofactor had been partially purified, several lines of evidence were gathered which prove that L-dopa is responsible for the activity.

**Co-electrophoresis and Co-chromatography with Dopa-2-¹⁴C**—Control experiments showed that the cofactor activity of 0.001 μmole of L-dopa (0.05 unit) was barely detectable after electrophoresis and extraction of the dopa area. Therefore co-electrophoresis and co-chromatography were performed on samples of cofactor mixed with 0.001 μmole of m-dopa-2-¹⁴C, with the results shown in Figs. 3 and 4. There was a close coincidence between the counts due to dopa and the cofactor activity extracted from the paper.

**Treatment with Tyrosinase**—Incubation of Fraction D with tyrosinase gave a pink reaction mixture that had an absorption

---

**Table III**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Total units</th>
<th>Total amino acid</th>
<th>Specific activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>μmoles</td>
<td>μmoles</td>
<td>μmoles/μmole amino acid</td>
<td>%</td>
</tr>
<tr>
<td>A</td>
<td>1445</td>
<td>780</td>
<td>8600</td>
<td>0.091</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>10.5</td>
<td>438</td>
<td>1610</td>
<td>0.290</td>
<td>50</td>
</tr>
<tr>
<td>C</td>
<td>5.0</td>
<td>198</td>
<td>202</td>
<td>9.8</td>
<td>25</td>
</tr>
<tr>
<td>D</td>
<td>2.0</td>
<td>123</td>
<td>5.48</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>E</td>
<td>2.0</td>
<td>31</td>
<td>1.08</td>
<td>29</td>
<td>4</td>
</tr>
</tbody>
</table>

**Figure 2** Demonstration of a heat-stable factor in melanoma which shortens the lag in tyrosine hydroxylation. a (left), oxygen uptake in air in a Warburg respirometer with tyrosine (1.88 μmoles); sodium phosphate buffer, pH 6.8 (300 μmoles); and crude enzyme (1.2 μmoles per mg) at the protein levels shown on graph in a volume of 3.0 ml at 37°. b (right), time course of tyrosine hydroxylation (measured in the absence of dopa) with tyrosinase (1 μmole); sodium phosphate buffer, pH 6.8 (35 μmoles); and purified enzyme (84 μg, 31 units per mg) in a volume of 1.25 ml at 37°. Boiled enzyme of this same specific activity was added to II, III, and IV as shown on the graph. Reactions were stopped at appropriate times by the addition of 0.10 ml of 20% HPO₄.
spectrum similar to that of dopachrome with a broad maximum between 460 and 500 μm.

**Methylation of Cofactor with $^{14}$CH$_2$N$_2$**—The cofactor (Fraction D) was methylated with $^{14}$CH$_2$N$_2$, and this product was compared with the O',O'-dimethyldopa methyl ester prepared from L-$^3$H-dopa and $^{14}$CH$_2$N$_2$. Fig. 5 compares the electrophoretogram of the purified trimethyl dopa compound with the crude material obtained by methylation of cofactor. The faster moving of the two radioactive bands from the cofactor reaction corresponds to the trimethyl dopa compound. The band near the origin is a contaminant which was also observed in the crude dopa reaction mixture. Elution of the fast moving band with 95% ethyl alcohol and chromatographic comparison with the trimethyl dopa compound in Solvent C again showed correspondence between the cofactor and dopa derivatives.

**Decarboxylation with Tyrosine Decarboxylase**—L-Dopa and cofactor (Fraction D) were each incubated with L-tyrosine decarboxylase from *S. faecalis* (which is also active against L-dopa) in the presence of $^{3}$HOH. This procedure introduces $^3$H into the amino carbon of dopamine, the expected product (12). During electrophoresis both cofactor and dopa gave radioactive bands that coincided with dopamine, as shown in Fig. 6. The contaminating $^3$H material in both preparations that migrated just behind dopamine was not further investigated.
Comparison of Cofactor and Dopa at Levels Beyond Range of Linear Response—At a concentration of $1.2 \times 10^{-4} \text{ M}$, dopa eliminates the lag in tyrosine hydroxylation (1). Near this concentration the degree of hydroxylation is no longer a linear function of dopa or natural cofactor. The data in Table IV compare the extent of hydroxylation achieved by natural cofactor and by dopa. It is seen that 0.10 μmole of dopa (5.0 units) leads to the same amount of hydroxylation as 6.2 units of natural cofactor.

Direct Dopa Assays on Fractions D and E—A colorimetric assay for dopa (13) was performed on the two most highly purified fractions. Table V shows that there is satisfactory agreement between the data from this colorimetric measurement and the dopa levels calculated from estimates of cofactor activity. Furthermore, the spectrum of the colored derivative derived from Fraction D was the same as that obtained from authentic dopa.

The data summarized above established that purified Fractions D and E contain dopa, and that the dopa in these fractions accounted for the cofactor activity contained in them. Because of the substantial losses incurred during the purification procedure, it was still necessary to determine whether all or most of the cofactor activity in the homogenate was due to dopa.

To obtain this information DL-dopa-2,14C was added to a homogenate of hamster melanoma, and the material was put through the customary purification procedure. The results, shown in Table VI, show that the radioactivity from added dopa is closely associated with the cofactor activity. A more nearly constant ratio of 14C to cofactor would have been desirable, but it is likely that this was prevented by the inherent errors in the colorimetric assay and by the fact that radioactivity from dopa may remain in a fraction even though the compound was degraded to an inactive substance.

Supporting evidence was obtained by comparing the amount of dopa estimated by cofactor assay with the quantity determined by a fluorometric procedure (14) and by an amino acid analyzer. The data presented in Table VII indicate a rough agreement between these independent methods. While it would not be possible to identify dopa in a crude homogenate with certainty by the fluorescence or amino acid analyzer methods, the results obtained by these methods are clearly consistent with the proposal that all or most of the cofactor activity in the homogenate is due to dopa.

### Table IV

<table>
<thead>
<tr>
<th>Cofactor</th>
<th>Units</th>
<th>Equivalent amount of dopa</th>
<th>3HOH in 1-ml aliquot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmole</td>
<td>dpm</td>
<td></td>
</tr>
<tr>
<td>Fraction D</td>
<td>6.2</td>
<td>0.20*</td>
<td>18,700</td>
</tr>
<tr>
<td>Dopa, 0.05 μmole</td>
<td>2.5*</td>
<td>13,700</td>
<td></td>
</tr>
<tr>
<td>0.10 μmole</td>
<td>5.0*</td>
<td>18,800</td>
<td></td>
</tr>
<tr>
<td>0.15 μmole*</td>
<td>7.5*</td>
<td>22,200</td>
<td></td>
</tr>
</tbody>
</table>

* Determined from the approximate relation that 1 cofactor unit is equivalent to 0.02 μmole of dopa.

* This amount eliminates the lag completely.

### Table V

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total amino acids</th>
<th>Dopa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmoles</td>
<td>μmoles</td>
</tr>
<tr>
<td>D</td>
<td>5.48</td>
<td>2.46</td>
</tr>
<tr>
<td>E</td>
<td>1.08</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Comparison of amounts of dopa in melanoma homogenate determined by cofactor assay, fluorometric analysis, and amino acid analyzer

Hamster melanoma (5 g) was homogenized in 20 ml of 0.4 N HClO4. After centrifugation at 30,000 X g for 10 min, the supernatant volume was brought to 25 ml. One-half the volume was used for cofactor assay after neutralization to pH 7.0 with KOH, centrifugation, and then back titration to pH 6.5 with 0.1 N HCl. The remainder was treated with alumina according to the method of Anton and Sayre (15), and aliquots were assayed for dopa fluorometrically (14) with a Turner filter instrument. Internal controls were used to correct for losses. The fluorescence spectrum of the derivative of the tissue compound was obtained on an Amino-Bowman spectrophotofluorometer and was found to be very similar to the fluorescence spectrum of the derivative of authentic dopa. The bulk of the alumina eluate was submitted to analysis on a Technicron amino acid analyzer. There were peaks due to tyrosine, phenylalanine, and ammonia in addition to the one corresponding to dopa. The position assigned to dopa was supported by comparison with a known quantity of dopa.
Binding Constant for L-Dopa When It Serves as Cofactor

The $K_m$ for L-dopa as a substrate is about $5 \times 10^{-4}$ M (6). However, the lag in tyrosine hydroxylation can be eliminated with dopa at $1.2 \times 10^{-4}$ M. It thus appeared that dopa might bind the enzyme at a second site when it serves as a cosubstrate with tyrosine. This was investigated further by performing hydroxylation experiments with either no added dopa or with suboptimal concentrations. The data from one experiment are plotted in Fig. 7. The lag times ($t$) for the various dopa concentrations are estimated by extrapolation of the linear portions of the curves to the abscissa. The lag probably represents the time required for the accumulation of some steady state level of dopa which is independent of its initial concentration. This is supported by the fact (Fig. 7) that the linear rate of hydroxylation is independent of the initial dopa concentration. Dopa may accumulate through the operation of the following reactions.

\[
\text{Tyrosine} + \text{dopa} \rightarrow \text{dopaquinone}
\]

\[
\text{dopaquinone} \rightarrow 2 \text{(2,3-dihydro-5,6-dihydroxyindole-2-carboxylic acid)}
\]

\[
2 \text{(2,3-dihydro-5,6-dihydroxyindole-2-carboxylic acid)} + \text{dopaquinone} \rightarrow 2 \text{dopachrome} + \text{dopa}
\]

\[
\text{Sum: Tyrosine} \rightarrow 2 \text{dopa} + 2 \text{dopachrome}
\]

The operation of this scheme would lead to the observation (6, 16) that dopaquinone is formed at twice the rate of dopachrome.

If the lag time ($T$) in the absence of dopa is known, a plot of $(T - t)$ versus initial dopa gives a hyperbolic curve which approaches $T$ as an asymptote as dopa increases and $t$ becomes small. This is analogous to a plot of velocity versus substrate concentration. Since it is difficult to estimate $T$ reliably, another way of treating the data is to plot $(1/t)$ versus initial dopa. Following the analogy to Michaelis-Menten kinetics,

\[
(T - t) = \frac{T}{1 + \frac{K_m}{[\text{dopa}]}}
\]

\[
\frac{1}{t} = \frac{1}{T K_m [\text{dopa}]} + \frac{1}{T}
\]

The data in Fig. 7 were used to obtain the least squares plot shown in Fig. 8, which yielded a $K_m$ of approximately $2 \times 10^{-4}$ M for dopa as a cofactor and 200 min for $T$.

**DISCUSSION**

The evidence clearly establishes L-dopa as responsible for all or nearly all of the cofactor activity in hamster melanoma. The concentration of dopa on the basis of cofactor and fluorometric assays is about 4 to 8 µg per g of tumor or 2 to 4 µmoles/100 g. Both the presence of dopa in a tissue which contains tyrosinase and the low binding constant of dopa as a cofactor suggest that dopa is the natural cofactor for the enzyme. The bulk of the dopa in this tumor probably accumulates during melanogenesis, since dopa was shown to be present in mealworm tyrosinase mixtures in vitro even after the utilization of all tyrosine (2). On the assumption that dopa is the specific physiological cofactor for tyrosine, there is still a question as to the origin of the initial dopa in the melanoma. It is possible that tyrosine hydroxylase is present in the tumor and that this enzyme, with a tetryhydropteridine as cofactor, triggers melanogenesis by the synthesis of a small amount of dopa. Attempts have been made to detect tyrosine hydroxylase in both melanotic and amelanotic hamster melanoma but so far without success.

Anton and Sayre (18), by means of a fluorometric method, could not detect dopa (<0.05 µg per g) in adrenal or other tissues from several animals. They did find large quantities of dopa in the urine of neuroblastoma patients. The absence of dopa in adrenal is probably the result of the high activity of dopa decarboxylase. Dopa has been reported in the medium of pheochromocytoma tumors grown in tissue culture (17), and 3-methoxy-4-hydroxyphenylalanine was found in the urine of a patient with a sympathogonioma (18). There are some reports.

*S. H. Pomerantz, unpublished experiments.
that dopa was detected in the urine of melanoma-bearing mice (10) and humans (19), but there are also negative reports for humans (20). From these results it is clear that dopa is probably not present in measurable levels in normal tissues.

Takahashi and Fitzpatrick (21) reported very large quantities of dopa (230 μg per g) in acid hydrolysates of the trichloracetic acid precipitate of Harding-Passey mouse melanoma, but only 1 μg per g in the trichloracetic acid supernatant fraction. With a similar hydrolysate of hamster melanoma, we found 64 cofactor units/100 g or about 2.6 pg of dopa per g, somewhat less than we would have arisen from dopa absorbed on the large amount of melanin contained in the tumor, since it has been shown (22) that radioactivity can be removed from 14C-melanin by refluxing for 24 hours in 2 N HCl. The difference between the two observations cannot be adequately explained, although it could be the result of differences between the mouse and hamster melanomas. However, it is still surprising that such a large quantity of dopa would survive 12 to 18 hours of reflux in air in 6 N HCl since dopa is severely degraded by repeated exposure to flash evaporation at 50° from dilute HCl solutions (1). In any event their conclusion that dopa is bound in peptide linkage in this melanoma would appear to be highly speculative at present.

The observation that dopa has two binding values supports the hypothesis that dopa binds at different sites on the enzyme when functioning as a cosubstrate with tyrosine or as the sole substrate (aside from oxygen). This idea receives additional support from two inhibitor studies. Both diethyldithiocarbamate (1, 6) and β-n-propylgallate2 are much better inhibitors of dopa oxidation than of tyrosine hydroxylation by tyrosinase.

The calculation of a binding constant for dopa as a cofactor must be viewed as a gross approximation because the lag time is variable from one enzyme preparation to another and also varies somewhat between experiments with the same preparation. Since dopa is present in the original homogenate and since the lag time increases as the enzyme becomes more highly purified,2 it is tempting to suggest that this is related to the removal of dopa during purification. At some state of purity an indefinite lag might be observed in the absence of added dopa. If so, the present calculation of cofactor $K_m$ of $2 \times 10^{-4}$ M would be higher than the true value.

Acknowledgment—We wish to thank Mrs. Jean Li for technical assistance.

REFERENCES

3,4-Dihydroxy-l-phenylalanine as the Tyrosinase Cofactor: OCCURRENCE IN MELANOMA AND BINDING CONSTANT
Seymour H. Pomerantz and Margaret C. Warner


Access the most updated version of this article at http://www.jbc.org/content/242/22/5308

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/242/22/5308.full.html#ref-list-1