Purification and Characterization of Guanosine 3′:5′-Monophosphate-specific Phosphodiesterase from Guinea Pig Lung*

(Received for publication, December 14, 1976)

CRAIG W. DAVIS† AND J. F. KUO§

From the Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia 30322

A low \( K_m \) guanosine 3′:5′-monophosphate (cyclic GMP)-specific phosphodiesterase was purified about 250-fold from crude extracts of the guinea pig lung by steps of DEAE-cellulose chromatography, hydroxylapatite treatment, and preparatory polyacrylamide gel electrophoresis. The relative rate of hydrolysis of cyclic GMP and adenosine 3′:5′-monophosphate (cyclic AMP), using 1 \( \mu \)M substrate concentrations, was at least 1,000:1. The apparent \( K_m \) for cyclic GMP (0.8 \( \mu \)M) was about 200 times lower than the apparent \( K_m \) for cyclic AMP (150 \( \mu \)M). No significant hydrolysis of inosine 3′:5′-monophosphate (cyclic IMP) and the 8-bromo and 8-benzylamino derivatives of cyclic GMP, cyclic AMP, or cyclic IMP by the purified enzyme was noted. The specificity of the enzyme was unaltered by pH, most metal ions, temperature, or the endogenous protein activator and Ca\(^{2+}\).

The purified cyclic GMP-specific enzyme was extremely labile and required bovine serum albumin for stabilization. The molecular weight of the enzyme was estimated by linear sucrose density gradient ultracentrifugation, hydroxylapatite treatment, and Sephadex G-200 gel filtration to be 137,000 and 168,000, respectively. The enzyme required Mg\(^{2+}\), Mn\(^{2+}\), or Co\(^{2+}\) for its activity and was not stimulated by protein activator. In addition, maximal enzyme activity was observed at a pH range of 7.5 to 8.0 and at a temperature range of 37–42°C, with an activation energy of 11.9 kcal/mol.

Cyclic AMP and cyclic IMP were found to inhibit the cyclic GMP-specific enzyme in a competitive manner with apparent \( K_i \) values of 8.1 \( \mu \)M and 1.1 \( \mu \)M, respectively. Inhibition by cyclic IMP was highly specific for the cyclic GMP-specific enzyme when compared to a cyclic AMP phosphodiesterase purified from the same tissue.

The role of cyclic nucleotides as intracellular mediators of the actions of numerous physiologic and pharmacologic agents is well recognized. Recent evidence suggests the biological actions of cyclic AMP and cyclic GMP are probably independent of each other, and in some instances, mutually opposing (1, 2). The hydrolytic cleavage of the cyclic nucleotides to their corresponding 5′-nucleotides by phosphodiesterases may be the most important physiological mechanism for terminating their actions. Since the intracellular levels of cyclic nucleotides are, in part, determined by the relative activities of the phosphodiesterases, a better understanding of these enzymes is in order.

Multiple forms of phosphodiesterase have been shown to occur in a wide variety of tissues (3–8). Early investigations described enzymes having a high specificity for cyclic AMP (4, 9–11). Only recently have phosphodiesterases with a higher affinity for cyclic GMP been reported (10, 12–17); those enzyme preparations, however, lacked a rigid specificity for cyclic GMP, probably due to a low degree of purity and a possible contamination of enzyme specific for cyclic AMP.

We have previously shown (18) that an enzyme activity specific for cyclic GMP (Peak 1) and an enzyme activity specific for cyclic AMP (Peak 2) were obtained by DEAE-cellulose chromatography of lung extracts from guinea pigs. Furthermore, we noted that the ratio of the pulmonary content of the two enzyme activities was the highest in the fetus, whereas it was the lowest in the adult (18). It is conceivable that cyclic GMP and cyclic AMP are hydrolyzed by separate enzymes, perhaps in addition to a class of enzymes that hydrolyzes both cyclic GMP and cyclic AMP. The present investigation describes an extensive purification and characterization of a cyclic GMP-specific phosphodiesterase from guinea pig lungs, and its properties are compared with those of the cyclic AMP-specific enzyme purified from the same tissue.

**EXPERIMENTAL PROCEDURES**

Materials — [γ-\(^{32}\)P]ATP (10 Ci/mmole), cyclic [\(^{32}\)H]GMP (9.8 Ci/mmole), and cyclic [\(^{32}\)H]AMP (27.9 Ci/mmole) were purchased from New England Nuclear; hydroxylapatite (Bio-Gel HTP), AG 1-X8 (Cl− form, 100 to 200 mesh), and 2-mercaptoethanol, were from Bio-Rad; DEAE-cellulose and snake venom (Crotalus adamanteus) were obtained from Sigma; and Sephadex G-200, from Pharmacia. Various cyclic nucleotides and their analogs were obtained from either Sigma, Boehringer Mannheim, or Plenum Scientific Research. Acrylamide, \(N,N'\)-methylenebisacrylamide, ammonium peroxydisulfate, and \(N,N,N',N'\)-tetramethylmethylenediamine (TEMED) were from Eastman Organic Chemicals.
Purification of Cyclic GMP-specific Phosphodiesterase from Guinea Pig Lung—Mature, female guinea pigs (600 to 800 g) were killed by decapitation and the lungs were immediately removed and rinsed in ice-cold 50 mM Tris/Cl buffer (pH 7.5) containing 3.75 mM 2-mercaptoethanol (extraction buffer). Tissues with any evidence of hemorrhage were discarded. The tissues were homogenized in 2 volumes of the extraction solution for 20 s using a Sorvall Omni-Mixer. The homogenate was centrifuged at 105,000 × g for 45 min and the supernatant was filtered through glass wool to remove the fat. The supernatant fluid (crude extract) so obtained was used for subsequent purification procedures.

An aliquot (25 to 35 ml) of the crude extract was applied to a DEAE-cellulose column (2.5 x 40 cm) previously equilibrated with extraction buffer. The column was then washed with 300 ml of the extraction solution followed by a second wash (400 ml) with the same buffer containing 0.1 M potassium chloride. The wash fluid contained 60 to 70% of the protein originally present in the crude extract without detectable phosphodiesterase activity. The enzyme was eluted from the column using a linear gradient of potassium chloride (0.1 to 0.6 M; total volume, 800 ml) added to the extraction buffer. The fractions containing peak enzyme activity were pooled (40 to 50 ml), and 5 ml of hydroxyapatite gel suspension (350 mg of dry powder/ml) was then added. Quantitative absorption of the enzyme occurred and the gel was collected by a brief centrifugation. The gel was washed twice with 25-ml volumes of 25 mM potassium phosphate buffer (pH 7.0) by suspending and stirring the gel in the buffer; no appreciable enzyme activity was detected in the washing solution. The enzyme was finally eluted twice from the gel using 25-ml volumes of 100 mM potassium phosphate buffer (pH 7.0). The two eluates were pooled and concentrated by vacuum dialysis using a collodion bag apparatus to a final volume of 3 ml. Preparatory polyacrylamide gel electrophoresis (Ortec System, model 4010) was carried out using a discontinuous buffer system of Tris/borate (pH 9.0) and Tris/sulfate (pH 9.0), with acrylamide gel slabs (72 x 95 x 3 mm) cast in a pore-size gradient consisting (from bottom to top of gel) of 8% (59 mm), 6% (9 mm), and 4.5% (4 mm) gel. The enzyme (0.5 ml, 1 mg of protein) was mixed with a buffered sucrose solution to a final sucrose concentration of 15 to 25% and applied to the gel. The sample was electrophoresed by applying a constant 350 V with a variable pulse rate. The initial rate of 75 pulses/s was maintained for 15 to 20 min and was increased every 10 min by 75 until a pulse rate of 300 pulses/min was reached. The enzyme activity and appropriate incubation times were used so that 10 to 30% of the cyclic nucleotides were hydrolyzed under the assay conditions. All assays were performed in duplicate and the values were corrected for blank values obtained in the absence of added phosphodiesterase. One unit of enzyme activity is defined as that amount of activity that hydrolyzes 1 pmol of cyclic nucleotide/min under the assay conditions.

Other Methods—Cyclic AMP-specific phosphodiesterase was purified from guinea pig fetal lung using DEAE-cellulose chromatography (Peak 2, Fig. 1). Phosphodiesterase activator was prepared from the rabbit brain and the guinea pig lung by the method of Cheung (20). Cyclic GMP-dependent protein kinase was purified from the fetal guinea pig lung through the Sephadex G-200 step (21). The assay conditions for cyclic GMP-dependent protein kinases were as reported elsewhere (21, 22). For the estimation of hydrolysis of cyclic AMP as well as the 8-bromo and 8-phenylthio derivatives of cyclic GMP, cyclic AMP, or cyclic IMP, 1 or 10 µM concentrations of these compounds were incubated with cyclic GMP-specific phosphodiesterase (0.12 µg; Fig. 2 and Table I) at 37° as described above. The remaining (unhydrolyzed) amounts of the compounds were then estimated by the method (23) based upon their ability to activate guinea pig lung cyclic GMP-dependent protein kinase (21). Isoelectric focusing was performed using an 110-mV isoelectric focusing column (LKB Instruments) and ampholytes (Bio-Lyte) with a pH range of 3 to 10. Linear sucrose density gradient ultracentrifugation was car-

![Fig. 1. DEAE-cellulose chromatography of phosphodiesterases in extracts of guinea pig lung. The linear gradient of KCl (100 to 600 mM) was from Fractions 114 to 230. The flow rate was 0.80 ml/min and fraction 0.5 ml. An aliquot (0.01 ml) from each fraction was assayed for phosphodiesterase activity as described in "Experimental Procedures." Using 1 µM cyclic GMP or cyclic AMP as substrate.](http://www.jbc.org/)

![Fig. 2. Preparative polyacrylamide gel electrophoresis of cyclic GMP phosphodiesterase from the guinea pig lung. The enzyme (0.9 mg) from the hydroxylapatite step was used for the electrophoresis which was carried out as described in "Experimental Procedures." The gel slab was cut into 2-mm sections and the enzyme was eluted using 0.3 ml of 50 mM Tris/Cl (pH 7.5) containing 3.75 mM mercaptoethanol. An aliquot (0.03 ml) of the eluates were assayed for phosphodiesterase activity using 1 µM cyclic GMP or cyclic AMP under the standard assay conditions. The incubation time was 25 min.](http://www.jbc.org/)
**RESULTS**

**Purification of Cyclic GMP-specific Phosphodiesterase**—The majority (about 94%) of phosphodiesterase activity for either cyclic GMP or cyclic AMP in the guinea pig lung was found in the 105,000 × g supernatant fluids of the tissue homogenate, whereas only 6% of the enzyme activities were found in the 105,000 × g pellets (data not shown). The elution pattern of phosphodiesterases from crude extracts of guinea pig lung on DEAE-cellulose chromatography is illustrated in Fig. 1. The cyclic GMP-specific phosphodiesterases was eluted as a sharp peak at 250 mM KCl (Peak 1), which represented about 85 to 90% of the recovered enzyme activity for the hydrolysis of cyclic GMP. A second enzyme activity (Peak 2), eluted at 350 mM KCl, was specific for cyclic AMP hydrolysis and accounted for 90% of the recovered phosphodiesterase activity for cyclic AMP hydrolysis. Combined fractions from Peak 2 (Fractions 168 to 176), representing an 8-fold purification of cyclic AMP enzyme activity over the crude extract, were used without further purification as the source of the cyclic AMP-specific phosphodiesterase.

The electrophoretic pattern of the cyclic GMP-specific phosphodiesterase from the hydroxylapatite step on preparatory polyacrylamide gel slab is depicted in Fig. 2. A small activity peak, migrating closer to the anode, preceded the major activity peak, which was followed by a third, slower migrating activity. None of the three activity peaks demonstrated any significant hydrolysis of cyclic AMP. The enzyme eluted from the preparatory gel electrophoresis step revealed two protein bands (Fig. 3), indicating the enzyme preparation was about 50% homogeneous. It is worth noting that the position of the heavier band corresponded to the most active enzyme band observed following preparative gel electrophoresis (Fig. 2).

The results of the enzyme purification are summarized in Table I. The specific activity (units per mg of protein) of the resulting enzyme was 27,000, which represents a 250-fold purification over that observed in the crude extract. The recovery of enzyme activity was estimated to be 4%. The relative rate of hydrolysis of cyclic GMP compared to hydrolysis of cyclic AMP was at least 1,000:1.

**Enzyme Stabilization**—The enzyme stored at −100°C was found to lose 96% of its original activity in 24 h. Sucrose (20%, v/v) or glyceraldehyde (20%, v/v) were only slightly effective in preventing the loss in enzyme activity. In contrast, the enzyme stored in the presence of bovine serum albumin (final concentration, 2 mg/ml) showed no appreciable loss (less than 5%) in activity over 5 weeks (data not shown). Because of the extreme instability of the enzyme in the absence of added albumin, it is felt that a 250-fold purification of the enzyme (Table I) may be much lower than that which has actually been achieved.

**Apparent Molecular Weight**—Linear sucrose density gradient ultracentrifugation (Fig. 4) of cyclic GMP-specific phosphodiesterase from the DEAE-cellulose step (Peak I, Fig. 1) revealed two active molecular species. The major activity peak with a sedimentation coefficient of 7.5 S (apparent $M_r = 137,000$) was preceded by a minor peak of enzyme activity having a sedimentation coefficient of 11.2 S (apparent $M_r =$...
Cyclic GMP-specific Phosphodiesterase 4081

220,000). The existence of a low and a high molecular weight component of the enzyme from the DEAE-cellulose step was confirmed by Sephadex G-200 gel filtration (Fig. 5). Only one molecular species (M₀ = 168,000) was noted, however, if the enzyme from the gel electrophoresis was used (Fig. 5). It was noted that the high molecular weight enzyme species was extremely unstable even in the presence of added albumin. It is not clear whether the two molecular species of the enzyme are inter-convertible.

Kinetics—The reaction kinetics of the cyclic GMP-specific phosphodiesterase as a function of incubation time and enzyme protein were performed. The reaction was linear for incubation times at least up to 20 min and for protein at least up to 0.16 µg (figure not shown). It should be noted that no appreciable hydrolysis of cyclic AMP could be detected in all cases. The cyclic AMP-specific phosphodiesterase similarly displayed linearity for incubation time up to 25 min and for enzyme protein up to 18 µg (data not shown). The hydrolysis of cyclic AMP was about 20 times greater than the hydrolysis of cyclic GMP by the cyclic AMP enzyme under these assay conditions.

The cyclic GMP enzyme displayed linear substrate kinetics using concentrations of cyclic GMP from 0.7 to 3.3 µM (Fig. 6). At cyclic GMP concentrations above 5 µM, the kinetic plot became hyperbolic indicative of substrate inhibition. The apparent Kₘ of the enzyme for cyclic GMP was 0.83 µM. Kinetic analysis revealed that the apparent Kₘ for cyclic AMP of the cyclic GMP enzyme was greater than 150 µM (data not shown). The cyclic AMP phosphodiesterase exhibited linear substrate kinetics over the entire range (1.0 to 6.7 µM) of cyclic AMP concentrations examined, with an apparent Kₘ for cyclic AMP of 1.6 µM (Fig. 6).

Substrate Specificity and Effect of Cyclic Nucleotides on Enzyme Activity—The hydrolytic rate of 10 µM cyclic IMP by cyclic GMP-specific phosphodiesterase was 10% of that of 1 µM cyclic GMP. The 8-bromo and 8-benzylamino derivatives of either cyclic GMP, cyclic AMP, or cyclic IMP at concentrations of 1, 5, and 10 µM were not hydrolyzed by the enzyme.

The hydrolysis of 1 µM cyclic GMP by the cyclic AMP enzyme was slightly inhibited by cyclic AMP (Fig. 7); a 35% inhibition by 1 mM cyclic AMP was observed. Similarly, cyclic GMP was found to be a weak inhibitor of hydrolysis of 1 µM cyclic AMP by the cyclic AMP enzyme (Fig. 7); a 50% inhibition was noted in the presence of 1 mM cyclic GMP. Kinetic analysis indicated the cyclic AMP inhibition of cyclic GMP hydrolysis by phosphodiesterases purified from guinea pig lung. Cyclic AMP phosphodiesterase (0.04 µg) was from the gel electrophoresis step, and was assayed as described under "Experimental Procedures," using cyclic GMP (0.67 to 74.0 µM) as substrate. Cyclic AMP phosphodiesterase (4 µg) was from the DEAE-cellulose step and was assayed using cyclic AMP (0.3 to 6.3 µM) as substrate. Kinetic plots were determined by linear least square analysis. PDE, phosphodiesterase.

![Fig. 4 (left). Sucrose density gradient ultracentrifugation of cyclic GMP phosphodiesterase. The enzyme (1.6 mg) was from the DEAE-cellulose step. Aliquots (0.01 ml) of each fraction were assayed in the presence of 1 µM cyclic GMP under the conditions described in "Experimental Procedures." C, catalase; GADPH, glyceraldehyde-3-phosphate dehydrogenase; ADH, alcohol dehydrogenase (horse liver).](http://www.jbc.org/)

![Fig. 7. Inhibition by cyclic nucleotides of cyclic GMP and cyclic AMP hydrolysis by phosphodiesterases purified from guinea pig lung. The hydrolysis of 1 µM radioactive cyclic GMP by the cyclic GMP enzyme (gel electrophoresis step, 0.04 µg) and the hydrolysis of 1 µM radioactive cyclic AMP by the cyclic AMP enzyme (DEAE-cellulose step, 4 µg), was measured in the absence and presence of nonradioactive cyclic AMP and cyclic GMP (10⁻⁴ to 10⁻³ M), respectively. The activities of the respective enzymes for hydrolysis of cyclic GMP or cyclic AMP were 1.09 and 3.42 units, respectively, under the assay conditions described under "Experimental Procedures." PDE, phosphodiesterase.](http://www.jbc.org/)
hydrolysis to be of the competitive type, with an apparent $K_i$ of 8.1 mM (Fig. 8).

Cyclic IMP, which was hydrolyzed slightly by the cyclic GMP enzyme as shown above, was a very potent inhibitor of cyclic GMP hydrolysis by the enzyme. Kinetic analysis revealed that the inhibition of cyclic IMP by cyclic IMP was competitive with an apparent $K_i$ value of 1.1 $\mu$M (Fig. 9). Interestingly, the cyclic AMP enzyme was inhibited by only 24% in the presence of cyclic IMP at a concentration as high as 1 mM (figure not shown).

**Effect of Temperature, Divalent Metal Ions, Activator Protein, and pH on Enzyme Activity**—When the cyclic GMP-specific enzyme was preincubated at 25–42°C, a 25 to 35% loss in activity was observed within 5 min, after which the activity was relatively stable for up to 25 min (figure not shown). At temperatures above 50°C, loss in enzyme activity was directly related to length of incubation time, with the half-life of the cyclic GMP enzyme at 50°C being 3 min, whereas that at 60°C being about 1 min. The effect of temperature on the rate of hydrolysis of cyclic GMP by the cyclic GMP-specific phosphodiesterase was also investigated. The rate of hydrolysis increased between 10 and 42°C, with a calculated energy of activation ($E_a$) of 11.9 kcal/mol (figure not shown). The temperature coefficient ($Q_10$) calculated over this temperature range was 1.44. The calculated energy of inactivation ($-E_a$) was found to be 227 kcal/mol.

The dose-dependent activation of the cyclic GMP enzyme by divalent metal ions is illustrated in Fig. 10. Activity of the enzyme in the absence of added metal ions was about 6% of the maximal activity stimulated by Mg$^{2+}$. The strict requirement for divalent cations for activity was found to be more apparent with increasing purity of the enzyme. In addition to Mg$^{2+}$, Mn$^{2+}$ or Co$^{2+}$ was found to stimulate enzyme activity, while Zn$^{2+}$ or Cu$^{2+}$ was inactive. The apparent $K_i$ for Mn$^{2+}$ was 0.5 mM which was 3.5 times lower than that of Mg$^{2+}$. The maximal activity stimulated by Mn$^{2+}$ or Co$^{2+}$ was about 45% of that by Mg$^{2+}$. Unexpectedly, Co$^{2+}$, in contrast to Mg$^{2+}$ or Mn$^{2+}$, disproportionately stimulated cyclic AMP hydrolysis by the cyclic GMP enzyme. The effect of combinations of the cations were also studied (data not shown). Stimulation of enzyme activity caused by Mg$^{2+}$ and Mn$^{2+}$ was almost additive, while Co$^{2+}$, in combination with either Mg$^{2+}$ or Mn$^{2+}$, appeared to inhibit the stimulatory effect of either metal ion.

The phosphodiesterases from extracts of rabbit brain were stimulated by added activator (15 $\mu$g, prepared from rabbit brain) in the presence of Ca$^{2+}$ (40 $\mu$M), as previously demonstrated by others (20, 27). No significant stimulation of the purified pulmonary cyclic GMP-specific enzyme by the activator, either present alone or in combination with varying concentrations (4 to 400 $\mu$M) of Ca$^{2+}$, was observed. In addition, the specificity of the enzyme for cyclic GMP was unaltered (data not shown). In contrast, the purified cyclic AMP enzyme from the lung was stimulated by the activator in a dose-dependent manner, with activity increased 150% in the presence of 0.0 $\mu$g of the activator, Ca$^{2+}$ (4 to 400 $\mu$M), when present alone, was also found to stimulate the cyclic AMP enzyme activity, but a synergism with the activator was not observed. Qualitatively similar results were obtained with respect to both the purified pulmonary enzymes when an activator prepared from guinea pig lung, instead of rabbit brain, was used (data not shown). The stimulation of cyclic GMP hydrolysis by the activator using lung extracts was found to be slight, and this effect could be reproduced by bovine serum albumin, indicating the stimulation observed may be due to stabilization of the enzyme by added protein.

The pH profile for the cyclic GMP enzyme is shown in Fig. 11. Maximal activity was found between pH 7.5 and 8.0. No significant hydrolysis of cyclic AMP by the enzyme was observed for the entire pH ranges tested, using three different buffer systems. Isoelectric focusing of the cyclic GMP enzyme from the DEAE-cellulose step revealed a minor enzyme activity peak with a pl of pH 7.4, and a major enzyme activity peak with a pl of pH 5.3 (data not shown). This is in line with our observation that the phosphodiesterase activity for cyclic GMP in extracts of the lung could be quantitatively precipitated at pH 5.1.
than cyclic AMP, employing 1 PM substrate concentration. In this respect, the enzyme from the guinea pig lung in the present study represents the most highly purified low $K_m$, inhibiting cyclic AMP phosphodiesterase since in most instances employing cyclic IMP phosphodiesterase in crude extracts of the guinea pig lung (29) and the rabbit skeletal muscle (35). It is conceivable, therefore, that binding of Co$^{2+}$ may, in a manner not entirely clear at this time, alter the characteristics of the enzyme in such a way as to somewhat diminish its rigid specificity for the two cyclic nucleotides. It is also possible that our enzyme preparation may still be contaminated with the cyclic AMP enzyme whose activity was not detected in the absence of Co$^{2+}$.

Phosphodiesterase activators from the rabbit brain or the guinea pig lung neither stimulated cyclic GMP hydrolysis by the enzyme purified from the guinea pig lung, nor altered its substrate specificity. This is in marked contrast to the stimulation by the activator of cyclic GMP enzyme preparations from the brain (13), the heart (12, 16), and the coronary artery (15) reported by others. Furthermore, their brain (13) and heart (16) enzyme preparations, upon stimulation by the activator, began to hydrolyze cyclic AMP at a rate comparable to that of cyclic GMP hydrolysis. It is not clear whether the alteration in the specificity of the enzymes in the latter instances by the activator was due to a unique property of the enzymes, or to activation of contaminating cyclic AMP phosphodiesterase in their preparations.

The competitive inhibition of cyclic GMP hydrolysis by cyclic IMP was intriguing. Previously cyclic IMP has also been shown to effectively inhibit a purified silkmoth enzyme having a higher specificity for cyclic GMP than for cyclic AMP (14). The present study indicates that inhibition by cyclic IMP was highly specific for the cyclic GMP enzyme when compared to the cyclic AMP enzyme, suggesting a possibility that cyclic IMP may have a potential therapeutic value in the regulation of the cyclic nucleotide levels in the lung.

**Acknowledgments**—The authors thank Ms. Janice G. Patrick and Ms. Nancy L. Brackett for their excellent technical assistance and Dr. Donald P. Groth for performing isoelectrofocusing of the enzyme.

**REFERENCES**

Cyclic GMP-specific Phosphodiesterase

Purification and characterization of guanosine 3':5'-monophosphate-specific phosphodiesterase from guinea pig lung.
C W Davis and J F Kuo


Access the most updated version of this article at http://www.jbc.org/content/252/12/4078.citation

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/252/12/4078.citation.full.html#ref-list-1