Journal of the Arkansas Academy of Science

Volume 39 Article 42

1985

Demonstration of a Heat-Stable Cyclic GMP Phosphodiesterase in the Medium of Physarum flavicomum

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Recommended Citation

Lynch, Thomas J. and Ross, Mark S. (1985) "Demonstration of a Heat-Stable Cyclic GMP Phosphodiesterase in the Medium of Physarum flavicomum," *Journal of the Arkansas Academy of Science*: Vol. 39, Article 42.
Available at: https://scholarworks.uark.edu/jaas/vol39/iss1/42

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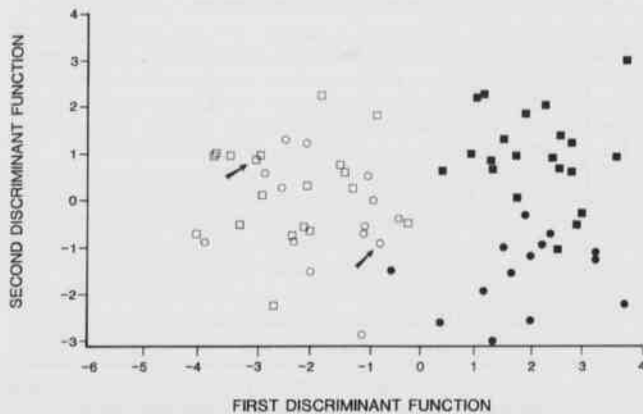

Figure. Ordination with respect to the first two discriminant functions of 68 skeletal specimens of Black-capped and Carolina Chickadees. (Darkened symbols represent Black-capped Chickadees, open ones Carolina Chickadees. Squares are male birds, circles, females. Arrows indicate the specimens in question.)

Table 2. Skeletal characteristics and standardized discriminant function coefficients that maximize the discrimination among 68 male and female Black-capped and Carolina Chickadees from the Central Plains and Ozark Plateaus regions.

Skeletal characteristic	Discriminant function	
	First	Second
Femur length	0.75	-0.25
Humerus length	0.42	-0.36
Ulna length	-1.02	0.81
Carpometacarpus length	0.25	0.46
Keel length	0.15	0.68
Synsacrum width	0.47	-0.40

in the smallest group. Because we had only 14 female Black-capped and Carolina Chickadees, it was necessary to *a priori* reduce the number of variables used to 13. Only the 15 variables that showed univariate significance were considered (Table 1), and from these culmen depth and scapula length were omitted (the former because it is difficult to measure accurately, the latter because of great variation within groups). The 13 variables were used in a step-wise DFA, using the criterion of maximizing the Mahalonobis distance among the 4 groups (males and females of both Black-capped and Carolina Chickadees). The infrequent missing values were estimated using the mean values for the appropriate group (e.g., Carolina female). The whole analysis was repeated using 75 chickadees by adding specimens from a wider geographical range. The results of the two analyses were essentially similar to those described below, which are based on the one using the 68 specimens.

The step-wise DFA identified 6 variables that maximized the separation among the 4 groups of chickadees. These variables, and their standardized discriminant functions coefficients are listed in Table 2. As can be seen from the Figure, the two species are separated along the first discriminant function axis (DF-1), accounting for 82.4% of the variance among the four groups.

Ulna length is the variable that contributes most to the separation between species with femur length and synsacrum width being of secondary and tertiary importance (Table 2). In total, Black-capped Chickadees, which have relatively large positive values on DF-1, have a combination of a relatively short ulna, long femur and humerus, and wide synsacrum. The measures of wing length, other than ulna length, interestingly are positively correlated to DF-1. The second DF (accounting for an additional 17.4% of the variation among the groups) separates male and female Black-capped Chickadees but not the sexes of Carolina Chickadees (Figure). Ulna length, again, is the most important variable on the axis, with male *atricapillus* having longer ulnae and keels, but relatively narrower synsacra and shorter humerae than females (Table 2).

In evaluating the precision of the discriminat function analysis, 82% of the specimens (56 out of 68) were correctly categorized as to group affinity. Eleven of the misidentifications were with regard to sex within species, and only one specimen was grouped with the wrong species. This was the female Black-capped Chickadee from Phillips Co., Kansas (KU 61722 ♀), that is clustered with the Carolina Chickadees in the Figure. The analysis identified it as a female Carolina Chickadee. Thus 99% of the specimens were correctly positioned with respect to species.

The two specimens in the Kansas collection that were suspect as to identification were both identified by the analysis as being Carolina Chickadees (marked by the two arrows in the Figure). As is shown in the Figure, the male specimen clearly is a Carolina Chickadee whereas the female specimen is close to the misclassified Black-capped Chickadee. In fact this female bird was identified with a rather high probability of being a Carolina Chickadee. It had a 69% probability of being a female Carolina (which it is), and 26% probability of being a male Carolina, which leaves only a 5% probability of being a Black-capped Chickadee.

In summary, Black-capped and Carolina Chickadees are readily identified from skeletal material and two suspect skeletal specimens labeled Black-capped Chickadees from Arkansas were indeed Carolina Chickadees. Thus, there are still no records of the Black-capped Chickadee in Arkansas.

(In completing this study, Rising's contribution was supported by the Natural Sciences and Engineering Research Council of Canada. Jim Dick of the Royal Ontario Museum, and Marion Mengel and Steven Johnson, of the University of Kansas Museum of Natural History, were very helpful in making specimens available. Thanks are due Janet Mannone for managing the data. J. D. Black was especially helpful for his comments.)

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DEMONSTRATION OF A HEAT-STABLE CYCLIC GMP PHOSPHODIESTERASE IN THE MEDIUM OF *PHYSARUM FLAVICOMUM*

Although the role of cyclic AMP in cellular regulation has been well characterized during the last two decades, the function of cyclic guanosine monophosphate (cyclic GMP) remains vague. Cyclic GMP phosphodiesterase is the enzyme responsible for degrading cyclic GMP to 5′GMP and thus is of major importance in maintaining cellular levels of cyclic GMP. We have identified an extracellular cyclic GMP phosphodiesterase in

Table 1. Effect of Heat on Cyclic GMP Phosphodiesterase Activity.

	nMole/min/ml Unheated	nMole/min/ml Heated	% Activity Remaining
Starting Medium	146	86	60%
30-80% Pellet	160	134	84%

Samples of starting medium (sm) and ammonium sulfate 30-80% pellet were assayed for cGMP phosphodiesterase activity without heating (unheated) and after a 5 min exposure to a boiling water bath (heated). The sm is an average of four experiments and the 30-80% pellet is an average of three experiments.

Table 2. Effect of Various Compounds on Cyclic GMP Phosphodiesterase Activity.

Additive	Concentration	nMole/min/ml	% Activity
Control	0	162	100%
MIX	7 mM	50	31%
Theophylline	10 mM	63	39%
Caffeine	10 mM	94	58%
Chlorpromazine	500 µM	207	128%
Chlorpromazine	1 mM	207	128%
Compound 48/80	10 µg	253	156%
Compound 48/80	100 µg	225	139%

Dialysed samples of the ammonium sulfate 30-80% pellet were assayed in the presence of the above indicated reagents. MIX is 3-isobutyl-l-methylxanthine.

Table 3. Effect of pH on cGMP Phosphodiesterase Activity.

pH	Starting Medium nMole/min/ml	Starting Medium % Activity	30-80% Pellet nMole/min/ml	30-80% Pellet % Activity
4	24	11%	56	7%
5	7	6%	67	8%
6	125	56%	659	79%
7	225	100%	830	100%
8	93	41%	167	20%

Cyclic GMP phosphodiesterase was determined at the pH values indicated. A citrate buffer was used for pH 4 and 5, Pipes buffer for pH 6 and 7, and Tris for pH 8.0.

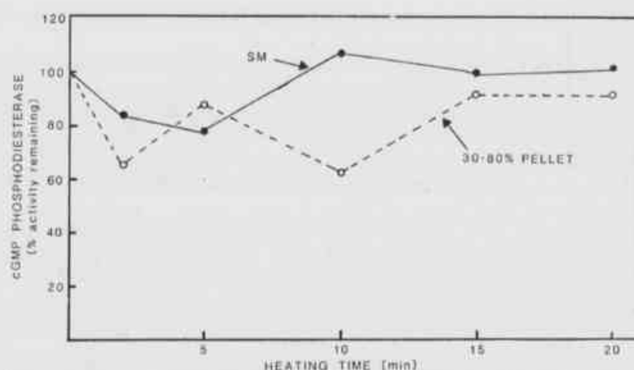

Figure 1. Samples of starting medium (SM) and 30-80% ammonium sulfate pellets were assayed for cyclic GMP phosphodiesterase activity after being heated in a boiling water bath for the times indicated on the abscissa.

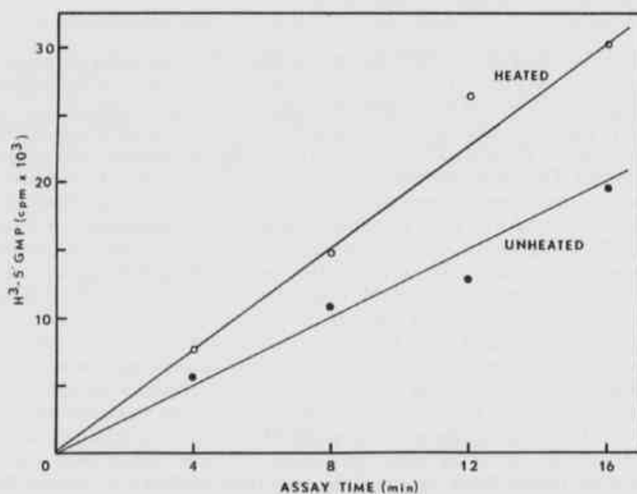

Figure 2. Cyclic GMP phosphodiesterase activity in heated and unheated samples was measured by paper chromatography. The 30-80% ammonium sulfate pellet for heat treatment was placed in a boiling water bath for 10 min and then returned to 30°C. Reaction mixtures of 300 µl each were prepared for heat-treated and non-heat treated samples. Duplicate 0.025 ml samples were withdrawn at the indicated times and added to tubes containing 0.005 ml of 2 M HCl to stop the reaction. Cold 5' GMP (0.005 ml of 20 mM solution) was added and aliquots were spotted on Whatman filter paper. Descending paper chromatography was done overnight and the ³H-5' GMP spots cut out and counted.

the medium from the plasmodium of the myxomycete *Physarum flavicomum*. This cyclic GMP phosphodiesterase appears to be very similar to the previously reported extracellular cyclic AMP phosphodiesterase in that both are extremely heat stable proteins (Lynch and Farrell, 1985).

Plasmodia of *P. flavicomum* were grown as previously described by Lynch and Farrell (1984). After reaching stationary phase (about 7 days) the microplasmodia were separated from the medium by centrifugation. The medium was either used immediately or stored frozen at −20°C. Cyclic GMP phosphodiesterase activity was determined by an anion exchange resin procedure using ³H-cyclic GMP as the substrate (Lynch and Cheung, 1975). All values were corrected for binding of ³H-guanosine to the AGI-X2 resin. Cyclic GMP phosphodiesterase activity was also determined by descending paper chromatography using a solvent system of 1M ammonium acetate and 95% ethanol (15/35, v/v). Spots corresponding to (³H)-5'GMP were visualized under ultraviolet light, cut out and the radioactivity determined.

Extracellular cyclic GMP phosphodiesterase activity was determined in crude medium and also in partially purified fractions. The enzyme was purified from spent medium by ammonium sulfate fractionation. Medium (100 ml) was adjusted to 30% ammonium sulfate, centrifuged at 48,000 xg for 30 min and the pellet discarded. The supernatant fraction was raised to 80% ammonium sulfate and centrifuged at 48,000 xg for 30 min. This 30-80% pellet was collected and resuspended in a small volume of 20 mM Tris HCl, pH 7.6 and dialyzed overnight against the same buffer.

The activity of phosphodiesterase in the starting medium and in a partially purified ammonium sulfate pellet is shown in Table 1. The same table also shows that this enzyme is heat stable, retaining up to 84% of the unheated activity after exposure to a boiling water bath for 5 min. Extracellular cyclic AMP phosphodiesterase from the same organism was previously shown to also be heat stable (Lynch and Farrell, 1985). Table 1 shows heat resistance at 5 min but gives no indication of how long the enzyme can withstand exposure to 100°C. Figure 1 shows cyclic GMP phosphodiesterase activity remaining after heating for up to twenty minutes in a boiling water bath. Both the starting medium and the ammonium sulfate pellet retained almost full phosphodiesterase activity even after twenty minutes of exposure to heat.

The effect of various compounds on partially purified cyclic GMP phosphodiesterase activity is shown in Table 2. MIX, theophylline and caffeine are all competitive inhibitors of phosphodiesterase. All three compounds inhibited enzyme activity with MIX being the most potent. Chlorpromazine (a phenothiazine) and Compound 48/80 (a condensation product of N-methyl-p-methoxy-phenethylamine with formaldehyde) are thought to inhibit calmodulin dependent phosphodiesterase by binding to calmodulin (Levin and Weiss, 1976; Gietzen et al., 1983). Both of these compounds stimulated plasmodial cyclic GMP phosphodiesterase. The mode of action of these two compounds on this enzyme is unknown.

Table 3 shows cyclic GMP phosphodiesterase activity at various pH values. a pH of 7.0 showed maximum enzyme activity with markedly reduced activity at pH 4.0 and 5.0. This is similar to cyclic AMP phosphodiesterase from the same organism in that the optimum pH for enzyme activity does not coincide with the optimum pH for growth. Uninoculated growth medium had a pH of 4.3 and after inoculation and growth, the pH of the medium rose to between 5 and 6. Under the growth conditions, cyclic GMP phosphodiesterase should be relatively inactive during the early stages of growth (the first few days after inoculation). Then, as the pH increases and approaches 5.0, the enzyme should become increasingly active.

All the previous enzyme assays were done using the resin assay which measures ^{3}H-guanosine as the end product. To insure the validity of our heat stability data, an alternate procedure for measuring phosphodiesterase activity was utilized. This procedure involved separating the product of phosphodiesterase (^{3}H-5′-GMP) by paper chromatography. Figure 2 shows a continuous assay of cyclic GMP phosphodiesterase for both heated and unheated samples as measured by paper chromatography. As can be seen, the heated sample retained enzymatic activity and product accumulation was linear with time.

As mentioned previously, extracellular cyclic AMP phosphodiesterase from the same organism is also heat stable. Work is presently underway in our laboratory to determine if the cyclic AMP enzyme is a di tinct protein from the cyclic GMP phosphodiesterase reported here and whether the two enzymes are indeed one and the same protein.

ACKNOWLEDGMENTS

We would like to thank Mrs. Ann Durgun for excellent clerical assistance. This research was supported by NSF EPSCOR Grant #ISP-8011447, by an Altheimer Foundation Grant and by the College of Sciences ORST (Office of Research in Science and Technology).

LITERATURE CITED

GIETZEN, K., E. SANCHEZ-DELGADO, and H. BADER. 1983. Compound 48/80: A powerful and specific inhibitor of calmodulin-dependent ca^{2+}-transport ATPase. IRCS Medical Sci. 11:12-13.

LEVIN, R. M., and B. WEISS. 1976. Mechanism by which psychotropic drugs inhibit adenosine cyclic 3′ 5′-monophosphate phosphodiesterase of brain. Mol. Pharmacol. 12:581-589.

LYNCH, T. J., and W. Y. CHEUNG. 1975. Underestimation of cyclic 3′,5′-nucleotide phosphodiesterase by a radioisotopic assay using an anion-exchange resin. Anal. Biochem. 67:130-138.

LYNCH, T. J., and M. E. FARRELL. 1984. Comparison of cAMP phosphodiesterase from the haploid and diploid stages of the myxomycete *Physarum flavicomum*. Cytobios. 39:71-79.

LYNCH, T. J., and M. E. FARRELL. 1984. Extracellular phosphodiesterase from the growth medium of the myxomycete *Physarum flavicomum*. Proc. Ark. Acad. Sci. 38 p. 60-63.

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NOTES ON THREE PALUSTRINE NATURAL COMMUNITY TYPES IN THE ARKANSAS OZARKS

Within the last five years three distinct palustrine (*sensu* Cowardin et al., 1979) community types not previously described in the literature as occurring in Arkansas have been recognized by the authors. The two fen community types have been reported previously in the Ozark Plateaus of southeastern Missouri (Orzell, 1983). Each of the recognized community types occur within the Ozark Natural Division (Pell, 1983) and is largely restricted to that part of the state. This paper represents an attempt to describe and classify these little-known natural communities.

The basis for our classification is the repeated occurrence on the landscape of particular combinations of vegetation structure and physiognomy, soil moisture regime, soil reaction, geologic substrate, topographic position, and species composition. Species composition, though obviously important, is not an overriding concern; in fact, examples of a given "natural community type" may vary considerably in this respect. A natural community classification emphasizes consistent ecological differences, not those due to the vagaries of dispersal or disturbance history. In many cases, more traditionally defined "plant community types" may be recognized within each natural community type.

The natural community descriptions presented here include information on distribution, topography, soils, community size and structure, natural processes, status and threats, and dominant and characteristic plant species. Dominant plants are species consistently occurring as major vegetation components (relative cover > 25 percent, based on visual estimates) of the type, though they need not dominant every example of that type. Characteristic plants are those typical or representative species that provide a reliable indicator of a given type. Dominant and characteristic species are not necessarily restricted to the types but, when considered together with other natural features, are diagnostic. Characteristic species were identified by constructing synthesis tables from species checklists compiled during field surveys (Mueller-Dombois and Ellenberg 1974). Four-