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Shift of Oxidases with Morphogenesis in the Slime Mold, Physarum polycephalum

The slime mold, *Physarum polycephalum*, is one of those unusual organisms which can pass from an acellular, plasmodial stage to a cellular, spore stage during its life cycle (*1*), and as such represents an ultimate in morphogenetic expression. The sequential nuclear events accompanying this change in morphology include synchronous, mitotic, nuclear divisions (*2*) and probably meiosis when uninucleate spores are formed in the mature sporangium (*3*). Since both stages can be obtained at will in the laboratory, this organism lends itself well to a comparative metabolic study capable of yielding information relating to those physiological factors which may control or trigger the process of nuclear division and concomitant spore formation. Such an introductory metabolic study is, therefore, the purpose of this report and is concerned with the comparison of the activities of two oxidases in both stages of the organism (*4*).

Preparation of active homogenates from the plasmodial stage of this organism has been described previously (*5*).

Table 1. Comparison of oxidase activities in plasmodial and spore stages in the slime mold, *Physarum polycephalum*.

Cytochrome oxidase		Ascorbic acid oxidase	
K*/mg of protein N	Spore/plasmodium	K†/mg of protein N	Spore/plasmodium
Spore			
0.081		0.041	
Plasmodium			
0.028	2.9/1	0.252	1/6.1

* K = 2.3 log [reduced cytochrome c] per second.
† K = 2.3 log [ascorbic acid] per second.

The use of sonic oscillations for the disruption of spores was found to be effective in the preparation of spore homogenates (*6*).

Allen and Price (*7*) first suspected the presence of cytochrome oxidase in *Physarum polycephalum* on the basis of inhibitor studies on whole plasmodia and a positive Nadi reaction on crushed plasmodia. We have been able to demonstrate that cytochrome oxidase is indeed present in homogenates of both plasmodial and spore stages by following the enzymic oxidation of reduced cytochrome *c* at 550 mμ in the Beckman spectrophotometer. Although the kinetics of mammalian cytochrome *c*–cytochrome oxidase reaction has been reported as obeying a combination of first-order and zero-order reactions (*8*), the conditions of our experiments reveal only first-order concurrence with the identical system in *Physarum*. This is in agreement with the cytochrome *c*–cytochrome oxidase first-order reaction rates in other preparations (*9*). First-order velocity constants were, therefore, calculated from the slope of the plots of 2.3 log reduced cytochrome *c* versus time. These constants were observed to be directly proportional to the amount of enzyme used in the reaction mixture; and when they are related to protein nitrogen content, they can be used as a true measure of cytochrome oxidase activity in each cell-free stage of the life cycle of the organism.

In an attempt to measure cytochrome oxidase activity in the plasmodial stage, Holter and Pollock (*10*) used the indirect manometric method of Schneider and Potter (*11*), which requires the use of ascorbic acid as a reductant of cytochrome *c*. Upon subsequent examination of this system, it was shown that the electrons from ascorbic acid not only pass through the cytochrome system but are also mediated to oxygen by an atypical ascorbic acid oxidase (*5*). These two systems can be separated by differential centrifugation, since the ascorbic acid oxidase is not associated with a particulate fraction and can be retained in the supernatant of a 25,000 *g* centrifugation; this supernatant is in turn devoid of cytochrome oxidase activity. The ascorbic acid oxidase activity is atypical, since it is resistant to the usual metallorespiratory inhibitors, forms hydrogen peroxide as an oxidation end product, and requires the presence of an unknown inherent sulfhydryl compound for which diethyldithiocarbamate or its disulfide can be substituted. Since it has been shown previously that the ascorbic acid–ascorbic acid oxidase system in *Physarum* obeys first-order reaction kinetics (*5*), then first-order velocity constants can also be obtained and validated as a measure of enzyme activity, as is described above.

A comparison of the oxidase activities in both the plasmodial and spore stages, shown in Table 1, reveals a shift in oxidases as the organism undergoes spore formation. Whereas there is approximately three times as much cytochrome oxidase activity in spores as in plasmodia, there is approximately six times as much ascorbic acid oxidase activity in the plasmodia as in spores.

The physiological interpretation of this shift is obscure at present, since information regarding metabolic patterns in this organism is lacking. However, these results do indicate that the atypical ascorbic acid oxidase, heretofore not rigidly established as a terminal oxidase, is not without definite function but by virtue of this shift may play an important role in the metabolism of the organism. Further, since energy is required for the nuclear divisions and formation of spore walls, a possible explanation for the increase of cytochrome oxidase activity can be offered on an energetic basis; those phosphorylation reactions necessary for the production of high-energy phosphate compounds have been demonstrated to occur in discreet stages of the cytochrome system participating in cellular oxidations (*12*).

This shift in oxidases may be fundamental to all organisms but may well be masked in organisms which undergo cellular division but which are constantly in the cellular state.

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