REPRESSION OF ARGINOSUCCINASE IN CHLAMYDOMONAS REINHARDI[1]

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Numerous studies have shown that the formation of enzymes involved in the biosynthesis of many compounds is subject to a control mechanism termed enzyme repression. For example, the enzymes of the arginine biosynthetic pathway have been shown to be repressible in Escherichia coli (Vogel, 1957; Gorini and Maas, 1958; Gorini, Gunderson and Burger, 1961; Maas, 1961). Enzymes of this pathway have been shown to be repressible by endogenously formed arginine in E. coli (Gorini and Maas, 1957; Novick and Maas, 1961). Repression by endogenous arginine is not observed in mutants partially blocked in arginine biosynthesis. The work reported here demonstrates that arginosuccinase is repressible in the unicellular green alga Chlamydomonas reinhardi and indicates that repression by endogenous arginine may occur.

Organism and Growth Conditions

The organisms used in this study were wild type, strain 137c, of C. reinhardi and the arginine requiring mutant of this strain, arg-2. The nutritional requirement of arg-2 is satisfied only by arginine. It is unable to convert citrulline to argino-succinic acid (Hudock, 1962). Cultures for experimental study

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were grown in the high salt medium of Sueoka (1960) or in this medium supplemented with 20 μg/ml of arginine. Cultures were grown at a temperature of 25° and a light intensity of 4000 lux. Wild type cultures were grown in the high salt medium supplemented with arginine and then permitted to undergo a known number of divisions in the absence of exogenous arginine. Cells of arg-2 were studied after they had been cultured on high salt medium for 24 hours. The growth rate of arg-2 on high salt medium is about ten per cent that of wild type. When grown under conditions of arginine limitation, it would be expected that arg-2 would be completely derepressed.

Preparation of Cell Extracts

Cells were harvested by centrifugation, washed once and then suspended in a sodium phosphate-citric acid buffer at pH 7.5 (McIlvaine's standard), the reported optimum pH for ox liver arginosuccinase activity (Ratner et al., 1953). Cells were disrupted by sonic oscillation and filtered through a pad of celite. The slightly turbid filtrate was clarified by centrifugation for 20 minutes at 27,000 x g at 0°. The supernatant fluid was used as a crude enzyme preparation. Protein concentration was determined by the biuret method.

Preparation of Sodium Arginosuccinate

Arginosuccinic acid was purchased as the barium salt from the California Corporation for Biochemical Research. The sodium salt was obtained by ion exchange on a Dowex-50 column. A sample of each solution was tested with ninhydrin and in all cases found to be positive. The solution was stored frozen at -10° and was discarded if not used within two weeks.

Enzyme Assay

The subtrate of arginosuccinase, arginosuccinic acid, is negative to the Sakaguchi reaction, specific for the guanidino

group of arginine (Ratner et al., 1953). This reaction was used to assay for the appearence of arginine in the enzyme catalyzed reaction. Kinetic studies were made in test tubes at 35°. Each tube contained in a total volume of 5.0 ml; 5.5 μmoles of sodium arginosuccinate and between 0.2 and 0.5 mg of protein from the enzyme preparation. The reaction mixture was buffered with the sodium phosphate-citric acid buffer. Reactions were initiated by the addition of enzyme and terminated by heating in a boiling water bath fpr one minute. After cooling, the reaction mixture was centrifuged for 20 minutes at 27,000 x g and the volume of the supernatant fluid was brought to 5.3 ml with distilled water. To each tube, 0.20 ml of two per cent ∝napthol in 0.5 N NaOH and 0.50 ml of five per cent NaOCl were added. The optical density at 540 mμ was read immediately after the addition of NaOCl. Comparison of the value obtained with a standard arginine curve established under the same conditions permitted a determination of the arginine concentration.

A unit of arginosuccinase activity is defined as that amount of enzyme which will produce one umole of arginine per hour. Specific activity is given as units/mg protein.

Results and Discussion

The activity of arginosuccinase was determined in extracts of wild type that had grown for two days in high salt medium supplemented with 20 μg/ml of arginine, wild type that had grown for two days on high salt medium, and arg-2 subjected to an arginine limitation on high salt medium for 24 hours. These results are given in Table I. They demonstrate that arginosuccinase is repressible by arginine in C. reinhardi.

The specific activity of the enzyme was then determined in extracts of wild type following various periods of growth in the absence of exogenous arginine. These results are presented in Table II and in Figure I.

Table I

Specific Activity of Arginosuccinase in Extracts of wild type and of arg-2 under Various Growth Conditions

Culture	Growth Conditions	Specific Activity
arg-2	24 hours, high salt medium	19.0
wild type	48 hours, high salt medium	10.2
wild type	48 hours, high salt medium plus 20 μg arginine/ml	1.1

Table II

Specific Activity of Arginosuccinase in Extracts of wild type Following Growth for Various Periods in the Absence of Exogenous Arginine

Divisions in Absence of Exogenous Arginine	Specific Activity
0.00	1.1
0.07	1.4
0.44	2.7
0.86	2.9
1.60	5.5
2.20	8.6
2.80	10.2

From the data in Table I it is apparent that the specific activity of arginosuccinase in extracts of C. reinhardi is a function of the presence of arginine in the growth medium. The activity of the enzyme in extracts of wild type grown in the absence of exogenous arginine is ten times as great as that found in extracts of cells grown in the presence of arginine. The activity in extracts of arg-2 cultured under conditions of arginine limitation for 24 hours is almost 20 times as great as the level in repressed wild type. It is also clear from these data that extracts of arg-2 contain high levels of arginosuccinase activity. This observation confirms the conclusion (Hudock, 1962) that arg-2 is unable to convert citrulline to arginosuccinic acid.

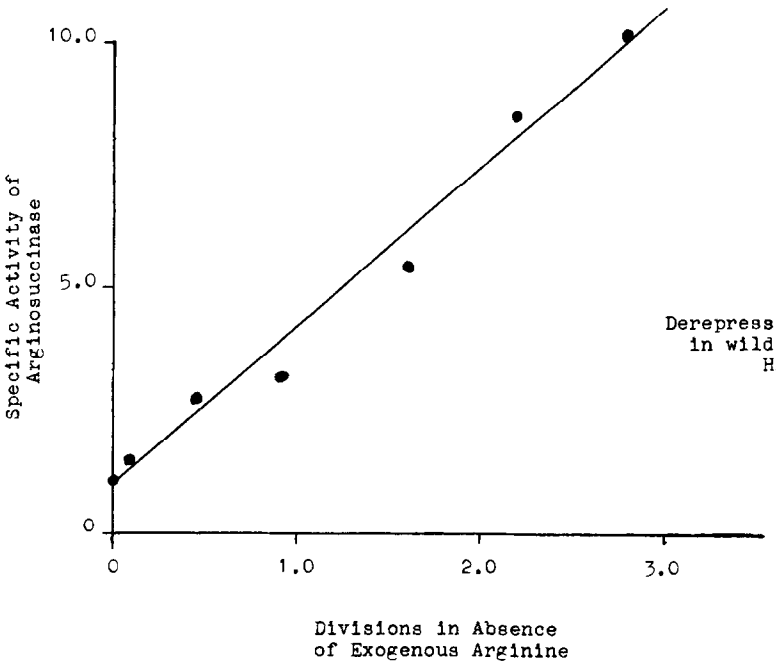

Figure I

Derepression of Arginosuccinase in wild type C. reinhardi on High Salt Medium

The data following the derepression of arginosuccinase in wild type are given in Figure I. It can be seen that the increase in enzyme activity is roughly linear over the period of nearly three generations in the absence of exogenous arginine.

Gorini and Maas (1957) and Novick and Maas (1961) have demonstrated that prototrophs of E. coli synthesize arginine at a rate which is so high that endogenously formed arginine represses ornithine transcarbamylase. From Table I, it can be seen that the arginosuccinase activity in derepressed wild type is only one-half that found in arginine-deprived arg-2. This may indicate that there is partial repression by endogenous arginine in wild type C. reinhardi or it may represent an uncontrolled response resulting from the starvation of an auxotroph. A choice between these alternatives is not possible on the basis of this work.

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