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UREASE OF *SPIRULINA MAXIMA*

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Abstract—Urease (EC 3.5.1.5) was purified from *Spirulina maxima* by ammonium sulfate precipitation, DEAE-cellulose chromatography and gel filtration on Sephadex G-200. The enzyme had maximum activity at pH 8.7, a K_m for urea of 0.12 mM and a MW of *ca* 232 000. A MW of 38 000 was determined for the subunits. The enzyme was inactivated by *p*-hydroxymercuribenzoate.

INTRODUCTION

Spirulina maxima is a large, spiral-shaped, blue-green alga which grows naturally on the surface of ponds of high salinity and alkalinity. This alga is a useful potential source of proteins for human and (or) animal consumption, since it has a high protein content [1], contains all essential amino acids in proportions comparable to other conventional protein foods [2] and has a good digestibility [3]. For this reason, the production of the alga has attracted great interest and the attention of many workers [4–6]. Faucher *et al.* [6] observed that urea is able to serve as the sole nitrogen source for the growth of *Spirulina maxima*. This observation prompted us to investigate the mechanism by which the alga utilize urea. It is known that one of the pathways of urea assimilation involves the direct hydrolysis to NH_4^+ and carbon dioxide catalysed by urease. The other pathway, in which the cleavage of urea to NH_4^+ and carbon dioxide is concomitant with the breakdown of ATP, is catalysed by ATP: urea amidolyase [7]. This second enzyme is found in yeasts and unicellular green algae that contain no detactable urease activity. In this paper, we describe the purification and partial characterization of the urease and some of its properties.

RESULTS AND DISCUSSION

Hydrolysis of urea in crude extracts

Urea was hydrolysed by crude extracts of *Spirulina maxima* prepared as described in the Experimental. No requirement for ATP, Mg^{2+} or bicarbonate could be demonstrated for this hydrolysis. Therefore, the mechanism by which the alga utilize urea is hydrolysis by urease.

Purification of urease

The crude extract was brought to 65% of saturation with ammonium sulfate (see Table 1) and the enzyme in the sediment was extracted with a solution of ammonium sulfate of 20% saturation. After dialysis, the solution was applied to a column of DEAE-cellulose (1 × 15 cm) equilibrated with 5 mM Tris–maleate buffer, pH 7.0, containing 2 mM 2-mercaptoethanol. The elution started with this buffer and continued with a linear gradient of 0.1–0.4 M Tris–maleate. The urease activity eluted as a single symmetrical peak at a Tris–maleate concentration of 0.14 M. Active fractions were pooled, concentrated by lyophilization and dialysed against 10 mM Tris–HCl buffer, pH 7.5, containing 20 mM potassium chloride and 2 mM 2-mercaptoethanol. From a Sephadex G-200 column equilibrated with this buffer, the enzyme activity eluted as a single symmetrical peak. The final enzyme preparation (Table 1) appeared homogenous on electrophoresis in 7.5% polyacrylamide gels with and without sodium dodecyl sulfate. This purified enzyme was used in the following experiments.

General properties

The apparent MW of the native enzyme estimated by gel filtration through a calibrated Sephadex G-200 column was *ca* 232 000. This result was obtained both in the absence and presence of 2 mM 2-mercaptoethanol. On the other hand, electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate, gave a subunit band with a MW of 38 000. Therefore, the enzyme from *Spirulina maxima* appears to consist of six subunits. These are similar in size to the species obtained by treatment of jack bean urease with performic acid [8]. The MW determined for the native enzyme is similar to the values reported for the 'monomer' of jack bean urease [9], the soybean enzyme at low ionic strength [10] and the urease from *Otala lactea* in the presence of thiols [11].

The pH activity curve of the purified enzyme shows a well-defined optimum at pH 8.7 (half maximal activity at pH 7.3 and 9.6). In contrast with this result, other ureases are maximally active over a wide range of pH [12] or exhibit different optima depending on the type of buffer used [13]. One exception is the enzyme from bovine rumen, which exhibits a sharp optimum at pH 8.0 [14].

The enzyme exhibited Michaelis–Menten kinetics with a K_m value of 0.12 mM at the optimum pH. This

Table 1. Purification of urease from *Spirulina maxima*

Purification step	Total enzyme (units)	Specific activity	Recovery (%)
Crude extract	19.25	0.21	100
Ammonium sulfate fractionation	15.00	0.33	78
DEAE-cellulose chromatography	12.30	0.75	63.9
Sephadex G-200 chromatography	8.44	9.27	43.8

The crude extract was prepared from an amount of freshly harvested cells equivalent to 0.9 g on a dry wt basis. Other details are given in the Experimental. Specific activity is expressed as μmol urea hydrolysed/min·mg protein. One unit of enzyme will hydrolyse 1 μmol urea/min at 37° and pH 8.7.

value is significantly lower than the values obtained for most of the bacterial and vegetable ureases [15]. Finally, and in common with other ureases [12–14, 16], the enzyme from *Spirulina maxima* was inactivated by *p*-hydroxymercuribenzoate, indicating that thiol groups are in some way involved in the action of the enzyme. The urease from the alga was completely inactivated by the sulfhydryl reagent at a concentration of 10^{-5} M and at a concentration of 10^{-6} M the reagent caused a 60% inactivation of the enzyme.

EXPERIMENTAL

Cultivation of Spirulina maxima *and preparation of crude extract.* The alga was cultivated and maintained under the conditions described by Faucher *et al.* [6]. The synthetic medium of Zarrouk [17] was used. The cells were harvested by centrifugation and washed × 3–4 with 10 mM Tris–HCl (pH 7.5) containing 100 mM KCl. Direct examination and cultures revealed that washed cells were free of bacteria or other micro-organisms. The washed cells were resuspended and homogenized cells were centrifuged at 15 000 *g* for 15 min and the pellet was discarded. Throughout this work the supernatant will be referred to as 'crude extract'.

Assays. Enzyme assays were performed at 37°; at intervals, reactions were stopped by adding 0.1 ml 10% trichloroacetic acid to 1 ml incubation mixture. In the assay for urease activity the substrate buffer soln contained: 30 mM urea and 60 mM Tris–maleate buffer (pH 8.0 or 8.7). The amount of ammonia formed was determined with Nessler's reagent [18]. The media for ATP: urea amidolyase contained: 30 mM urea, 1 mM Na_2ATP, 8 mM $MgSO_4$, 8 mM $KHCO_3$ and 60 mM Tris–HCl buffer (pH 8.0 or 8.7). Ammonia was determined by a Conway microdiffusion technique [19] followed by estimation of evolved ammonia with Nessler's reagent. The amount of urea was determined colorimetrically with α-isonitrosopropiophenone [20]. Protein was determined by the method of Lowry *et al.* [21] employing bovine serum albumin as the standard.

Electrophoresis on polyacrylamide gels. Native enzyme and subunits were analysed by electrophoresis on disc gel containing 7.5% (w/v) acrylamide at pH 8.3 (Tris–glycine). Gels were stained with Coomassie brilliant blue and destained and stored in 10% HOAc. Methods described by Davis [22] were used for electrophoresis of native enzyme. Sodium dodecyl sulfate gel electrophoresis was performed by the method of Weber and Osborn [23] with horse heart cytochrome *c*, ovalbumin and bovine serum albumin as MW markers.

MW determination by gel filtration. The method of Andrews [24] was used and the columns of Sephadex G-200 were calibrated with thyroglobulin, apoferritin, catalase, lactic dehydrogenase and bovine serum albumin.

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