N-FORMYLMETHIONINE DEFORMYLASE FROM EUGLENA GRACILIS

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We wish to report the presence of an enzyme in Euglena gracilis Z which specifically hydrolyzes N-formylmethionine and which could be indirectly involved in protein biosynthesis.

Euglena gracilis Z (Culture Collection of Algae, Indiana University, Bloomington, Indiana) was grown in the dark on a heterotrophic medium containing salts, malic acid, glutamic acid, glucose, urea, succinic acid, glycine, aspartic acid, thiamine, and vitamin B_{12}, pH 3.1-to-3.4 (Hutner et al., 1966). The cells were harvested after several days' growth in the dark without agitation. The cells were disrupted in phosphate buffer (0.1 M, pH 7.2) containing Co^{2+} (4×10^{-4}M) using a Branson Sonifier. After the sonicate was centrifuged at 12,000 rpm for 20 min, the supernatant was subjected to $(NH_4)_2SO_4$ fractionation. The protein that was precipitated between 35-to-55% saturation was dissolved in the minimum amount of chilled water and dialyzed against chilled water for 5 hr. The precipitated protein was removed and the supernatant was subjected to acetone fractionation. The fraction which precipitated between 20-to-50% was dissolved in phosphate buffer and used for further experiments. The reaction tubes contained 0.45 ml substrate (0.025 M) in phosphate buffer (0.1 M, pH 7.2) containing Co^{2+} (4×10^{-4}M). The substrate was equilibrated at 37°C and the reaction was then started by the addition of 0.05 ml enzyme (350 µg). For kinetic assays 0.5 ml substrate and 0.1 ml enzyme were used. Aliquots were withdrawn at the end of the 15 min incubation period and assayed by the standard ninhydrin method of Moore and Stein (1948). Electrophoresis on polyacrylamide gel was carried out according to the method described by Davis (1964). After electrophoresis the gel was cut into 1 mm sections (Aronson and Borris, 1967) and assayed for deformylase activity. Each 1 mm

segment was incubated in a reaction tube as described above for 5 hr at 37°C and aliquots were withdrawn and assayed as previously mentioned.

Absolutely no hydrolysis was observed for the following substrates: N-acetyl-L-methionine, N-acetyl-L-alanine, α-N-acetyl-L-ornithine, and N-formyl-L-methionyl-L-alanine. Under these conditions N-formyl-L-methionine was hydrolyzed at the rate of 80.4 μmoles per hr per mg protein nitrogen. The substrate specificity differs markedly from that reported for other deformylases (Adams, 1968; Fry and Lamborg, 1967; Lugay and Aronson, 1967; Takeda and Webster, 1968; Vogel and Bonner, 1956) and acylases (Greenstein and Winitz, 1961). The deformylase activity in the unfractionated Euglena extracts was masked, so no accurate overall purification factor can be given. Frozen preparations from which non-specific protein was removed after thawing by low-speed centrifugation had an enhanced specific activity. A cold-labile proteinaceous inhibitor has been observed with the Palo Verde acylase preparations (Lugay

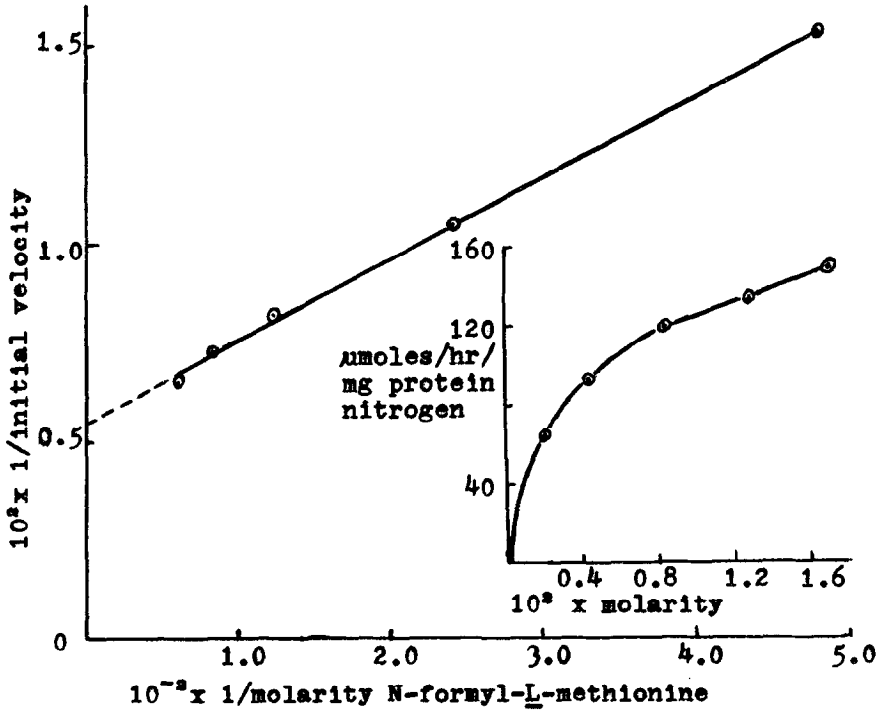

Fig. 1. Lineweaver-Burk and Michaelis-Menten (inset) plots for Euglena gracilis deformylase activity, N-formyl-L-methionine as substrate. Experimental details are given in the text.

and Aronson, unpublished results). The best Euglena preparation had a specific activity of 161 μmoles N-formyl-L-methionine hydrolyzed per hr per mg protein nitrogen. The K_m was 3.8×10^{-3} M and the V_{max} was 1.8×10^2 μmoles per hr per mg protein nitrogen as determined from the reciprocal plot shown in Fig. 1. The substrate concentration was varied from 0.00208 to 0.0167 molar.

The Euglena deformylase was seen to migrate during polyacrylamide gel electrophoresis (Figure 2) in a manner different from both the hog kidney acylase and the Palo Verde seed acylase (Lugay and Aronson, 1967), and it was less stable to purification procedures than the hog kidney and Palo Verde acyclases (Lugay and Aronson, unpublished results).

That N-formylmethionine is involved in the initiation of protein biosynthesis in Escherichia coli (Clark and Marcker, 1966) is fairly well-established (Attardi, 1967) and that it may be an initiator in Bacillus subtilis (Horikoshi and Doi, 1967; Takeda and Webster, 1968), bakers' yeast (Takeishi et al., 1968), and Euglena gracilis (Schartz et al., 1967) has also been indicated. The

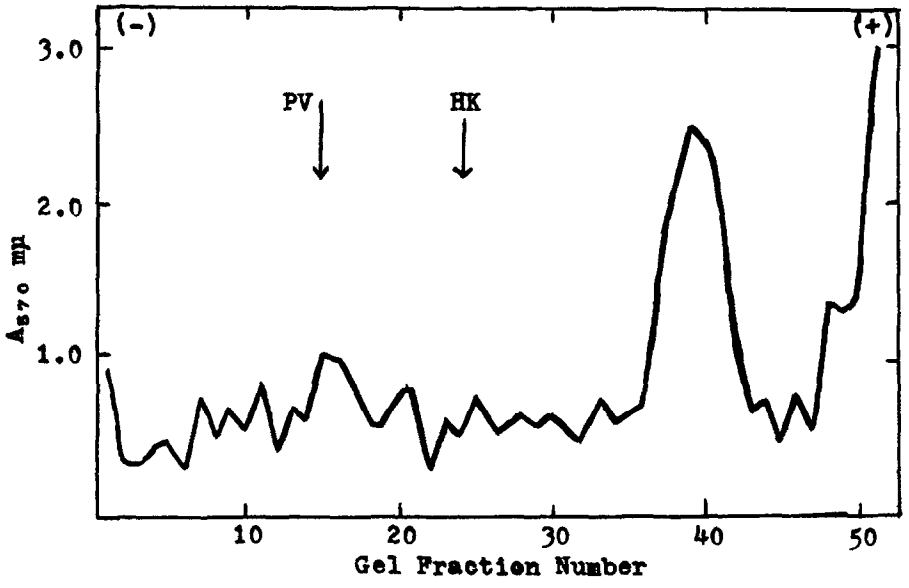

Fig. 2. Activity profile of Euglena gracilis N-formylmethionine deformylase after electrophoresis on polyacrylamide gel. Euglena deformylase activity peak appeared in fraction 39, as indicated by the ninhydrin reaction absorption at 570 mμ. Arrows point to relative positions of Palo Verde acylase activity (PV) and to hog kidney acylase activity (HK) as determined in similar experiments. The absorption peak on the anodal end of the gel was due to the free glycine in the electrophoresis buffer. Experimental details are given in the text.

Euglena gracilis deformylase may play a secondary role in protein biosynthesis by preventing the accumulation of a cellular pool of N-formyl-L-methionine formed via aberrant formylation of free methionine or via enzymatic cleavage of N-formyl-L-methionine from newly synthesized N-formyl-peptides.

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