Oximinoacids in the Inorganic Nitrogen Metabolism

There is good evidence for the occurrence of hydroxylamine (HA) as an intermediate in the assimilatory reduction of nitrate to ammonia. Free HA, a known mutagenic agent, is unlikely to be present in appreciable concentrations in the cells and very few organisms can tolerate and maintain growth on HA as sole nitrogen source, even at low concentrations ($10^{-4}M$)[1,2]. *Endomycopsis lipolytica* (= *Candida lipolytica*), however, is able to grow at $10^{-2}M$-HA without prolonged lagphase[3]. From studies of nitrite grown *Candida utilis* VIRTANEN and SARIS[4,5] suggested that HA may be bound as hydroxamates and oximes of α-keto acids. Therefore, *E. lipolytica* was considered suitable for further studies of the role of oximes in inorganic nitrogen metabolism.

E. lypolytica was grown in aerated flasks on a 9 mM HA-glycerol-mineral salt medium supplemented with growth substances[1]. Gas chromatographic analysis of the culture medium indicated the presence of 2-oximinopropanoic acid. The finding was confirmed by mass spectrometry. Also the log phase cells from HA-cultures were found to contain 2-oximinopropanoic acid, although in much lower concentrations. The oximino acid was absent in cells and medium of ammonium sulphate cultures.

A gas chromatographic method was developed for quantitative determinations of 2-oximinopropanoic acid as acetonitrile in culture media and in cells[6]. By this methhod the oxime content in the culture medium was found to increase with growth (Figure 1) until a maximum of about 130 μg oxime per ml in the middle log phase, at a culture absorbancy at 610 nm (A_{610}) of 1.0–1.2. The amount of oxime then decreased to about half the maximum in entering stationary phase (A_{610} about 1.7).

2-oximinopropanoic acid, found in HA-cultures and not in ammonium sulphate cultures, may be nonenzymic products. The pyruvic acid, a prerequisite for their formation, however, is by necessity due to an enzymatic process.

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Amounts of 2-oximinopropanoic acid (μg/g dry wt.) calculated from the amount of acetonitrile in cells of various procaryotic and eucaryotic organisms in middle log phase cultures (μg dry wt./ml) with nitrate or hydroxylamine (HA) as nitrogen source

Organism	NO_3^--cultures		HA-culture	
	Dry wt. (μg/ml)	Oxime (μg/g dry wt.)	Dry wt. (μg/ml)	Oxime (μg/g dry wt.)
Bacillus megaterium, 8291	155	8		
Pseudomonas aeruginosa, 1085	385	169		
P. aeruginosa, 701	213	207		
P. aureofaciens, 712	135	2		
Streptomyces griseus, 3475	31	5		
St. satsumaensis, ATCC 19242	53	1		
Cryptococcus albidus, J 61	320	128		
Endomycopsis lipolytica, CBS 599			390	230
Aspergillus niger	570	32		

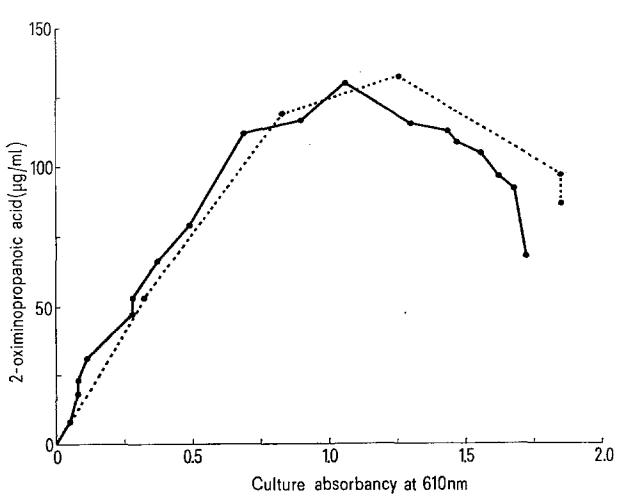

Fig. 1. Amounts of 2-oximinopropanoic acid in the culture medium (μg/ml) during growth of *Endomycopsis lipolytica*, expressed as culture absorbancy at 610 nm, in 2 different experiments (●——●; ●- - -●).

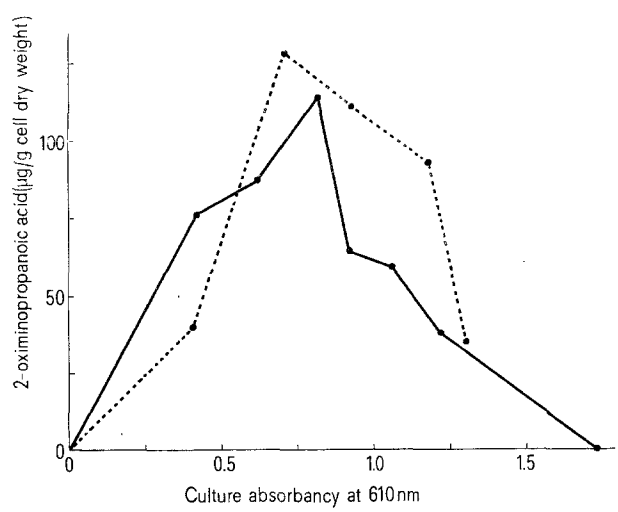

Fig. 2 Amounts of 2-oximinopropanoic acid in cells (μg/g cell dry weight) of *Cryptococcus albidus* during growth, expressed as culture absorbancy at 610 nm, in nitrate culture medium in 2 different experiments (●——●; ●- - -●).

The question whether the formation of oximes by *E. lipolytica* is only a way of detoxication or constitutes a route for direct utilization of not fully reduced nitrogen, represents points of interest. If the latter is of significance 2-oximinopropanoic acid could be expected to be present as a metabolite in cells grown on more oxidized nitrogen sources. Experiments were therefore run with some representative procaryotic and eucaryotic microorganisms, which unlike *E. lipolytica* are able to assimilate nitrate. Nitrate was substituted for HA and glucose for glycerol in the culture media. Cells from the middle log phase were analysed for oxime content using the acetonitrile method. 2-oximinopropanoic acid was found in all microorganisms tested, although in varying amounts (Table).

Analysis of the listed cells grown on ammonium sulphate always gave negative results. No oximes could be traced in chloroplasts from etiolated or non-etiolated wheat seedlings grown on nitrate.

The oxime content as a function of growth was studied in cells of *Cryptococcus albidus* grown on nitrate medium (Figure 2). It is obvious that maximum oxime content was reached to in middle log phase of growth (A_{610} about 0.8). At later stages the amount of oximino-acid decreased rapidly and became negligible when growth entered stationary phase (A_{610} about 1.7). The findings support the suggestion that the oximino-acid is used as a source of not fully reduced nitrogen in the metabolism.

The further metabolic transformation of 2-oximinopropanoic acid is not known. It has been shown that oximes may become enzymatically reduced to amino-compounds[7]. Transoximases[8] presumably play an important role.

It appears important to decide whether the formation of oximinoacids represents a bypass of minor importance or an indispensable pathway in the regular metabolic reduction of nitrogen. Work in the field is being continued at this institute.

Zusammenfassung. Es wird gezeigt, dass in Zellen von *Cryptococcus albidus* und in Kulturlösung von *Endomycopsis lipolytica* die Oxime bis zur Mitte der exponentiellen Zuwachsphase in steigenden Konzentrationen vorkommen, während sie in Ammonium-kultivierten Zellen fehlen.

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Glutamine Synthetase, an Enzyme Characteristic of Vertebrate Systems in Invertebrate Tissues

The central nervous system and sensory structures of coleoid cephalopods (e.g., squid, cuttlefish, octopi) are highly organized, and much has been made of the dramatic convergent evolution shown between these structures and those of higher vertebrates (Young[1]; Wells[2,3]; Barber[4]). It was of considerable interest to us, therefore, whether glutamine synthetase (GS), which is a characteristic enzyme of vertebrate nervous and digestive systems (Wu[5]), would also be present in large amounts in coleoid and other invertebrate tissues. Because of the intimate relationship between the structural organization of cells in the chick neural retina and the possible precocious induction of GS during development of this tissue (Piddington and Moscona[6]; Morris and Moscona[7]), we were particularly interested in knowing whether the coleoid eye, which is remarkably histotypically similar to the vertebrate eye, would also show GS activity and whether an eye of an entirely different structural type such as the typical crustacean eye would lack the enzyme.

Specimens of mature *Octopus vulgaris* and *Rossia pacifica* were obtained near the Marine Science Center of Oregon State University at Newport, Oregon. The organs were dissected in sea water and following a brief rinse in 0.01 *M* phosphate buffer (pH 7.1) were lyphilized and stored at −20 °C until assayed. For comparison, tissues of adult specimens of the commercial crab, *Cancer magister*, and the purple sea urchin, *Stronglyocentrotus purpuratus* were prepared in the same manner. Crab eyes (6 per assay) with or without eye stalks were homogenized in a porcelain mortar and pestle; all other tissues were homogenized in a glass tube with a motor-driven Teflon pestle. Assays for GS specific activity depended on the γ-glutamyltransferase properties of the enzyme and were performed according to the method of Rudnick et al.[8], as modified by Kirk[9]. The specific activity of GS was calculated as the number of micromoles of γ-glutamylhydroxamate produced per hour per mg protein in the homogenate. Protein was determined according to Lowry et al.[10].

It was found (Table) that coleoid nervous tissue in general showed relatively high GS specific activity with the optic ganglia showing the highest activity while non-nervous tissue activity was low and skin had no activity at all. This situation was analagous to that found in several vertebrates (Wu[5]). All tissues examined in the crab were notably low in GS activity, but sufficiently large amounts of tissue were used in these assays to demonstrate that the enzyme was, in fact, present. The neural ganglia, which lacked pigments, showed significantly lower GS specific activity in the crab than either coleoid species. Also, the retina of the crab was seen to show much less activity than the neural (thoracic) ganglia of this animal. It should be noted, however, that owing to the considerable amount of non-nervous tissue in the crab compound eye the specific activity of GS in the individual retinula cells may well have been considerably higher. It was also seen that the crab digestive gland revealed much less activity than the generalized nervous tissue as was the situation with coleoid tissues. The Table also

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