Octopine dehydrogenase in gastropods from different environments

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Summary. Lactate dehydrogenase was found in all species studied, whilst octopine dehydrogenase was present in prosobranchs, but absent from opisthobranchs and pulmonates.

Octopine dehydrogenase (EC 1.5.1.11) catalyses the reductive condensation of pyruvate and arginine to yield octopine. Functionally this enzyme replaces lactate dehydrogenase and is widely distributed in molluscs, having been demonstrated in representatives of the gastropods, bivalves, scaphopods and cephalopods[1,2]. Lactate dehydrogenase may be absent from tissues containing octopine dehydrogenase or lactate and octopine dehydrogenases may occur together. There has been considerable work on the possible physiological role of octopine dehydrogenase in cephalopods and bivalves (for review, Gäde[3]), but, as yet, there is no satisfactory explanation as to why some molluscs possess octopine dehydrogenase, whilst others do not. Gastropods are found in marine, freshwater and terrestrial environments, by investigating the distribution of octopine dehydrogenase in a range of species it should be possible to see if there was any correlation between environment and the occurrence of octopine dehydrogenase.

All the molluscs were collected locally and used within 24 h. Octopine and lactate dehydrogenases were assayed as previously described[4]. The results are summarized in the table. Lactate dehydrogenase was detected in all of the gastropods studied, whilst octopine dehydrogenase activity ranged from 0 to 675 nmoles/min/mg protein. The ratio of lactate dehydrogenase to octopine dehydrogenase was fairly constant for the 3 tissues from any one species. So octopine dehydrogenase did not appear to be present in some tissues but absent from others.

Where octopine dehydrogenase and lactate dehydrogenase occur in the same tissue, they must both be competing for the available pyruvate. Octopine and lactate dehydrogenase are not allosteric enzymes, so how the flux through the two alternative pathways is controlled is unknown.

Octopine dehydrogenase appears to be absent from the terrestrial and fresh water pulmonates investigated (fresh water pulmonates are thought to be derived from terrestrial forms). The enzyme had previously been reported absent from another fresh water pulmonate *Limnea stagnalis*[5]. It was suggested[4] that the presence of octopine dehydrogenase may be related to the use of free amino acids as osmotic effectors in marine organisms. Octopine dehydrogenase was not, however, present in all of the marine gastropods studied. Octopine dehydrogenase has also been found in the fresh water bivalve *Anodonta cygnea*[6], although here

Lactate dehydrogenase and octopine dehydrogenase in the tissues of gastropods

Species	Activity (mean and range)* Lactate dehydrogenase	Octopine dehydrogenase
Prosobranchia, Archaeogastropoda		
Patella vulgata		
Mantle	48 (27-63)	5.1 (4-7)
Foot	30 (25-68)	4.8 (4-6)
Retractor	36 (19-41)	5.9 (3-8)
Patella aspera		
Mantle	83 (77-89)	22 (16-32)
Foot	89 (84-94)	12 (9-14)
Retractor	41 (30-51)	19 (13-25)
Monodonta lineata		
Mantle	8.8 (0-18)	138 (112-162)
Foot	8.3 (0-17)	268 (162-332)
Retractor	7.5 (0-15)	675 (54-1,014)
Gibulla umbilicalis		
Mantle	6.3 (0-12)	83 (54-119)
Foot	3.6 (0-7)	289 (110-334)
Retractor	3.1 (0-6)	515 (380-583)
Prosobranchia, Mesogastropoda		
Littorina littorea		
Mantle	123 (120-150)	0**
Foot	289 (221-294)	0
Retractor	169 (156-190)	0
Littorina obtusata		
Mantle	47 (24-75)	0
Foot	70 (45-102)	0
Retractor	32 (20-41)	0
Littorina rudis		
Mantle	95 (50-140)	0
Foot	71 (29-158)	0
Retractor	36 (25-56)	0
Prosobranchia, Neogastropoda		
Buccinum undatum		
Mantle	33 (27-39)	183 (163-202)
Foot	44 (29-45)	195 (175-222)
Retractor	27 (25-31)	216 (203-228)
Nucella lapillus		
Mantle	12 (11-16)	93 (58-137)
Foot	10 (7-15)	248 (181-315)
Retractor	19 (16-21)	633 (548-748)
Opisthobranchia		
Archidoris pseudoargus		
Mantle	54 (25-60)	0
Foot	196 (160-200)	0
Retractor	104 (100-107)	0
Pulmonata		
Helix aspersa		
Mantle	396 (380-455)	0
Foot	382 (289-438)	0
Retractor	667 (200-1200)	0
Cepaea nemoralis		
Mantle	93 (53-135)	0
Foot	81 (68-92)	0
Retractor	85 (76-91)	0
Agriolimax reticulatus		
Mantle	7.7 (5-10)	0
Foot	24 (15-32)	0
Planorbis corneus		
Mantle	8.8 (6.1-11.5)	0
Foot	5.3 (4.5-6.6)	0
Retractor	3.1 (2.9-3.4)	0

*nmoles/min/mg protein at 30 °C; **less than 0.05 nmoles/min/mg protein.

fresh water bivales are probably derived from marine forms.

Of the species studied here and by other authors[1,2], octopine dehydrogenase was absent from the opisthobranchs, whilst in the prosobranchs the enzyme occurred widely in the Archaeogastropoda and Neogastropoda but has rarely been reported from the Mesogastropoda. The pulmonates and the opisthobranchs are thought to have split off early in evolution from the prosobranch line, possibly from the mesogastropod stock[7]. Whether this correlation between the distribution of octopine dehydrogenase and the taxonomy of the gastropods is correct will require data from many more species. Interestingly the primitive polyplacophoran *Lepidochitona cinerareus* showed no octopine dehydrogenase activity, only lactate dehydrogenase being detected (290 nmoles/min/mg protein). These results suggest that octopine dehydrogenase may have arisen on more than one occasion during the evolution of the Mollusca.

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Incorporation of base analogues for detection of unusual base compositions in plant metaphase chromosomes (*Vicia faba*, Leguminosae)

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Summary. Bromodeoxyuridine and bromodeoxycytidine with fluorescence-plus-Giemsa staining resulted in similar patterns of asymmetric bands in *Vicia faba* chromosomes. Limitations as to the use of methods for identification of A + T- and G + C-rich regions are discussed.

During the last years many attempts have been made to visualize, at the microscopic level of resolution, metaphase chromosome regions with unusual base composition. One method for the detection of A + T- or G + C-rich regions is the comparison of banding patterns induced by base-specific fluorochromes, which in vitro show preferential affinity for A + T- or G + C-rich DNA sequences[2-6]. Another method is in situ hybridization with radioactively labelled homopolynucleotide sequences. A 3rd, and perhaps more direct method is the autoradiographic detection of incorporated radioactively-labelled DNA precursors. The power of resolution of this method has been improved by introducing differential staining techniques for the demonstration of halogenated nucleoside analogues (e.g., by means of the fluorescence-plus-Giemsa technique[7]).

A pulse treatment with either bromodeoxyuridine (BrdU), bromodeoxycytidine (BrdC) or azacytidine (AzaC) during the end of S-phase results in pale bands after Giemsa-staining, fluorescence-plus-Giemsa staining (BrdU, BrdC) or Feulgen staining (AzaC) of metaphase chromosomes of man[8-10] and *Vicia faba*[11,12]. These bands seemed to be identically positioned (fig. 1) and are therefore interpreted by most authors as representing late replicating DNA rather than as being the result of base-specific incorporation of the analogues in these regions, which, after appropriate pretreatment, in most cases show up with dark Giemsa bands (fig. 1). This interpretation gains additional support from autoradiographic labelling of the same chromosome regions after pulse treatment with ^{3}H-thymidine during late S-phase[12].

In *V. faba*[13] (as in animals: man, mouse, *Cricetulus triton, Dipodomys ordii,* and *Drosophila nasuta*), incubation of cells for 1 cell-cycle in BrdU just prior to fixation resulted in asymmetric dark bands after application of a fluorescence-plus-Giemsa technique. This banding pattern has generally been interpreted as giving evidence of regions with uneven distribution of adenine and thymidine between the 2 strands of the DNA double helix. Therefore it was of interest to study the effects of treatment with BrdC or AzaC instead of BrdU. Unfortunately, AzaC did not result in chromosome differentiation after treatment for 1 or 2 cell cycles, neither after Feulgen staining nor by the use of the fluorescence-plus-Giemsa technique. Probably, the mechanism leading to AzaC banding subsequent to pulse treatment and Feulgen staining is quite different (AzaC inhibits normal methylation of the cytidine moiety[14,15]) from that giving rise to corresponding bands after

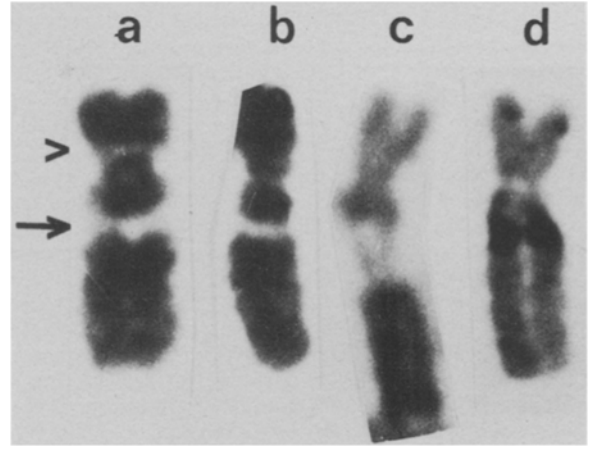

Figure 1. A sample of 4 chromosomes V of the reconstructed *Vicia faba* karyotype ACB (with a pericentric inversion in standard chromosome V). *a* DNA late replication bands (arrows) after a short pulse with BrdU and subsequent fluorescence-plus-Giemsa staining[12]; *b* the same after incubation in BrdC; *c* 'segment extension' after pulse treatment with AzaC and subsequent Feulgen staining[11]; *d* Giemsa-bands of the same chromosome[12].