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Acylase Activity of Ascites Tumour Cells on Dichloroacetyl Derivatives of Amino-acids

While investigating the specificity of acylase activity of soil bacteria on acyl derivatives of amino-acids[1–3], we noted that the acetone powder of Ehrlich ascites carcinoma cells was able to hydrolyse dichloroacetyl-DL-aspartic acid but not (or only to a small extent) the dichloroacetyl derivatives of some amino-acids such as DL-glutamic acid, DL-serine, DL-methionine and DL-phenylalanine[4].

We have now investigated the acylase activity of lyophilized powder of ascites tumour cells such as Ehrlich ascites carcinoma, sarcoma 180, leukæmia *SN* 36, ascites hepatoma *MH* 134 and *MH* 129P in *C3H* mice, and *AH* 7974, *AH* 130, and Yoshida sarcoma in rats on dichloroacetyl derivatives of the following twelve amino-acids: DL-aspartic and DL-glutamic acids, glycine, DL-alanine, DL-valine, DL-leucine, DL-phenylalanine, DL-tryptophan, DL-serine, DL-threonine, DL-methionine, and ε-*N*-benzoyl-DL-lysine.

Lyophilized powder of ascites tumour cells was prepared as follows: intraperitoneally transplanted ascites tumour cells were aspirated at the fifth day after transplantation, centrifuged at 1,000 r.p.m. for 10 min., washed three times with phosphate buffer solution, and then lyophilized. It was confirmed that the cells contained almost 85–95 per cent tumour cells.

Five mgm. of lyophilized powder of ascites tumour cells were added to 1 c.c. of neutralized 0·1 *M* substrate and 1 c.c. of *tris* buffer at *p*H 8·2, and the mixture was incubated at 37° C. for 2 or 18 hr. in the presence of toluene, and then titrated by Grassmann–Heyde's method.

The results obtained in the experiments carried out, under comparable conditions, on the acylase activity of lyophilized powder of eight ascites tumours are presented in Table 1. Ehrlich carcinoma, sarcoma 180 and leukæmia *SH* 36 were all able to hydrolyse dichloroacetyl-DL-aspartic acid but not (or only to a small extent) the dichloroacetyl derivatives of the other eleven amino-acids. It is of interest that *MH* 134, *MH* 129*P*, *AH* 7974, *AH* 130, and Yoshida sarcoma differed from Ehrlich carcinoma, sarcoma 180, and leukæmia *SN* 36 in failing to hydrolyse dichloroacetyl-DL-aspartic acid. Incidentally, *MH* 134, *MH* 129P, *AH* 7974 and *AH* 130 were hepatoma. The low acylase activities of *AH* 7974 and *AH* 130 seem worth noting when considered together with the fact that the rat liver could hydrolyse dichloroacetyl-DL-aspartic acid[5].

Thus, our experiment showed that there is a significant difference among ascites tumours in their capability of hydrolysing dichloroacetyl derivatives of amino-acids, and that acylase activity may be available as a tool for studying biochemical specificity of living material[1–3].

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BIOLOGY

Chloride Regulation in *Triops*

Little physiological work on the branchiopods has been reported because of their relative scarcity and the unpredictability of supplies of animals. Among the freshwater species Krogh[1] reports on some preliminary experiments with *Branchipus* and *Triops* (*Apus*), Panikkar[2] gives some figures for the blood concentration of *Chirocephalus*, and Fritzche[3] gives a blood concentration for *Daphnia*.

An opportunity arose for me to use a few specimens of *Triops cancriformis* (Bosc) bred in laboratory conditions by Mr. A. R. Longhurst. The level of

Table 1. Acylase Activity of Ascites Tumour Cells toward *N*-Dichloroacetyl Amino-acids

Substrate	Percentage of hydrolysed substrate for 2 and 18 hr. at 37° C.															
	Ehrlich carcinoma		Sarcoma 180		Leukæmia *SN* 36		*MH* 134		*MH* 129*P*		*AH* 7974		*AH* 130		Yoshida sarcoma	
	2	18	2	18	2	18	2	18	2	18	2	18	2	18	2	18
Dichloroacetyl-DL-aspartic acid	27	56	28	55	22	52	0	7	0	3	1	3	2	3	1	2
Dichloroacetyl-DL-glutamic acid	0	1	0	0	0	1	1	0	0	0	0	2	2	2	0	0
Dichloroacetylglycine	0	3	2	3	0	1	1	3	0	1	2	3	0	1	1	2
Dichloroacetyl-DL-alanine	1	4	1	4	0	2	1	5	0	1	2	2	2	2	1	4
Dichloroacetyl-DL-valine	0	2	0	3	0	0	0	2	1	3	1	2	1	2	1	1
Dichloroacetyl-DL-leucine	0	2	0	3	1	2	1	4	0	3	2	2	1	0	0	2
Dichloroacetyl-DL-phenylalanine	0	2	0	4	1	1	0	1	1	1	0	1	2	6	2	3
Dichloroacetyl-DL-tryptophan	0	1	0	1	0	0	1	0	0	2	0	1	1	5	2	3
Dichloroacetyl-DL-serine	0	2	1	2	0	1	0	2	0	1	2	3	1	3	0	2
Dichloroacetyl-DL-threonine	2	2	0	2	0	1	0	0	0	0	0	1	2	2	0	1
Dichloroacetyl-DL-methionine	0	3	1	2	0	3	0	4	0	0	2	1	1	2	1	2
α-*N*-Dichloroacetyl-ε-*N*-benzoyl-DL-lysine	0	2	0	1	1	1	0	0	0	0	1	2	1	0	0	1

The digests consist of 5 mgm. of lyophilized sample, 1 c.c. of neutralized 0·1 *M* substrate, and 1 c.c. of *tris* buffer at *p*H 8·2 at 37° C. Hydrolytic activity was measured by Grassmann–Heyde's alcoholic titration method.