starvation process, the number of colony forming units increased. Taking into account the similar trend in the survival of both bacteria when lacking aeration in DMM at 30 °C, there is nevertheless a substantial difference in the changes of degradation rates and concentrations of the cell components. This is caused by differences in the characteristics of the 2 species, primarily in the regulation of metabolism and in the utilization of different metabolic pathways under starvation conditions.

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The presence and partial characterization of carbohydrase enzymes in the gut of *Callosobruchus maculatus*

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Summary. Carbohydrase activity has been demonstrated in homogenates of the alimentary tract of *C. maculatus:* β-D galactosidase > α-D glucosidase > α-D galactosidase > β-D glucosidase activity. The effects of pH, temperature and substrate concentration on β-D galactosidase, α-D glucosidase and α-D galactosidase activities are described.

Callosobruchus maculatus F. is a major storage pest of the cowpea, *Vigna unguiculata*[1]. To date studies on this insect have tended to concentrate on ecological and behavioral aspects[2-7] whilst the physiology and biochemistry of *C. maculatus* and indeed the bruchids as a whole, has been virtually ignored.

Whilst digestive glycosidases have been reported in many insect species[8], very little information of a quantitative nature is available concerning their biochemical properties[9]. Hydrolysis of various carbohydrates by insects can be explained by supposing that carbohydrate specificity is dependent on the nature of the substrate, in particular its glycosidic bond and the α or β form of the linkage[10]. This hypothesis postulates the existence of 5 basic enzymes capable of hydrolyzing all oligosaccharides and glycosides based on glucose, galactose and fructose. However, one or more of these glycosides may be absent from the digestive fluid of a given species[11]. Fraenkel[12] used Weidenhagen's[10] hypothesis to explain digestion of all di, tri-saccharides and glucosides, utilized by adult *Calliphora vicina,* by the presence of only 2 gut enzymes (α-glucosidase and α-galactosidase). Whilst this hypothesis has been criticized[11,13-15], Bongers[16] and Wenzyl[17] have more recently used the idea of an unspecific α-D-glucosidase to explain their results from *Oncopeltus fasciatus* and *Calliphora erythrocephala.* On this basis it was considered valid to assay the activities of the 4 main classes of oligosaccharidases (α- and β-D-glucosidase, and α- and β-D-galactosidase) using p-nitrophenyl glycosides as 'generalized' substrates.

The present paper describes the presence and partial characterization of oligosaccharidase enzymes from the alimentary tract of *C. maculatus* and is intended as a basis for future physiological and biochemical studies.

Materials and methods. A culture of *Callosobruchus maculatus* originating from Campinas, Brazil, was reared and maintained on seeds of *Vigna unguiculata* at 28 ± 0.5 °C and 60% relative humidity. The photoperiod was arranged to provide 12 h light and 12 h dark.

Sexually mature animals of both sexes were killed by decapitation and their alimentary tracts removed by dissection under ice-cold (ca. 4 °C) distilled water. Homogenization was carried out in distilled water in a Potter-Elvehjem homogenizer with a Teflon pestle (clearance 0.1–0.15 mm) with 20 passes of the plunger at 1000 rev/min; the homogenization tube was surrounded by ice throughout this procedure. The resulting homogenate was then centrifuged at 9000 × g for 5 min at 4 °C in a Haematocrit centrifuge and the supernatant retained for enzyme assay. In general the alimentary tracts from 110 animals were homogenized in 1.5 ml distilled water. All homogenates were freshly prepared.

The activities of α-D and β-D glucosidase and α-D and β-D galactosidase were determined by the estimation of p-nitrophenol liberated by hydrolysis of the corresponding p-nitrophenyl glycoside[18]. The composition of the reaction mixtures was as follows:

Aryl α-D glucosidase 200 µl 0.1 M McIlvane's buffer, pH 5.8[19], 50 µl 51.3 mM p-nitrophenyl α-D glucopyranoside (to give a final concentration of 9.5 mM) and 20 µl homoge-

Table 1. Activation energies of oligosaccharidases present in the gut of *C. maculatus*

Enzyme	Temperature range (°C)	Activation energy (kJ · mole^{-1})
α-D-Glucosidase	18.0–57.2	35.80[a] 31.40[b]
α-D-Galactosidase	18.0–35.0	39.40[a] 38.30[b]
	35.0–51.0	7.85[a] 8.42[b]
β-D-Galactosidase	18.0–57.2	43.84[a] 38.30[b]

[a] and [b] represent independent experiments

nate were incubated at 35±0.1 °C for 30 min in sealed Eppendorf tubes, unless otherwise stated in the text. The reaction was terminated by the addition of 1 ml 50 mM NaOH and the absorbance read at 405 nm.

Aryl β-D glucosidase as for aryl α-D glucosidase except for the use of the substrate p-nitrophenyl β-D glucopyranoside.

Aryl α-D galactosidase as for aryl α-D glucosidase except for the use of the substrate p-nitrophenyl α-D galactopyranoside (35 mM: to give a final concentration of 6.5 mM) and pH 5.6.

Aryl β-D galactosidase as for aryl α-D galactosidase except for the use of the substrate p-nitrophenyl β-D galactopyranoside and pH 4.6.

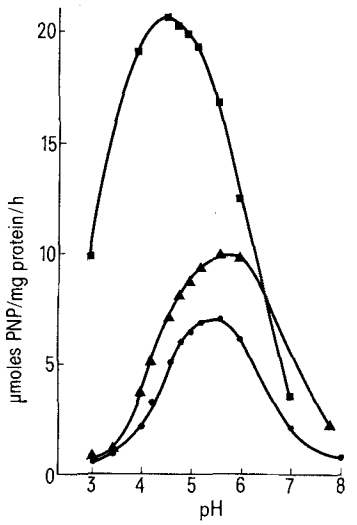

Figure 1. pH profiles of oligosaccharidases present in the gut of *C. maculatus*. −■−, β-D-Galactosidase activity; −▲−, α-D-glucosidase activity; −●−, α-D-galactosidase activity. Typical experiment representative of 2 experiments.

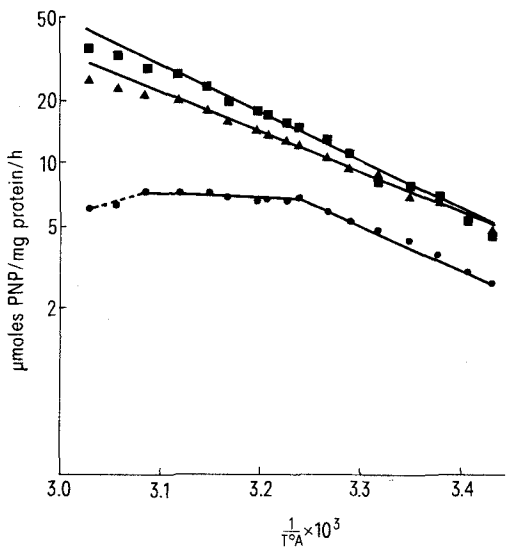

Figure 2. Arrhenius plot of the effect of temperature on the reactions catalyzed by oligosaccharidases present in the gut of *C. maculatus* in the temperature range 18.0–57.2 °C. −■−, β-D-Galactosidase activity; −▲−, α-D-glucosidase activity; −●−, α-D-galactosidase activity. Typical experiment representative of 2 experiments.

Protein determinations were carried out using the method of Lowry et al.[20] with bovine serum albumen fraction V (Sigma Chemical Co.) as standard. All solutions were made up in glass distilled water, buffer components and reagents were obtained from BDH Chemicals Ltd, and were AnalaR grade or the best commercially available. Enzyme substrates were obtained from Sigma Chemical Co.

Results and discussion. The oligosaccharidases α-D and β-D glucosidase and α-D and β-D galactosidase were present in homogenates of the alimentary tract of *Callosobruchus maculatus*. The most active enzyme was β-D galactosidase (sp. act.±SEM being 15.25±2.37 µmoles p-nitrophenol released/mg protein/h; n=5) followed by α-D glucosidase (8.61±1.15 µmoles p-nitrophenol released/mg protein/h; n=5) and α-D galactosidase (4.83±0.74 µmoles p-nitrophenol released/mg protein/h; n=5). The level of β-D glucosidase activity (1.16 µmoles p-nitrophenol released/mg protein/h; n=2) was extremely low and consequently this enzyme was excluded from subsequent studies.

The effect of pH on the activity of the 3 main enzymes was studied using McIlvane's citrate-phosphate buffer to produce a stable range of pH from 3.0 to 8.0. The results obtained are shown in figure 1. Maximal activity was observed at pH 4.6 with β-D galactosidase and at pH 5.6 with α-D glucosidase and α-D galactosidase. These values agree fairly well with those reported for digestive carbohydrase enzymes from a variety of different insects[12,21-26]. For example, all the digestive enzymes in the gut of *Tribolium castaneum* are reported to show maximum activity at pH 5.5[26].

In studying the effect of temperature on enzyme activity, pairs of tubes (control and experimental) were pre-equilibrated at temperatures in the range 18–57.2 °C for 5 min. The reaction was allowed to proceed for 45 min at temperatures of 28 °C and below, 30 min from 28 to 48 °C and 20 min at temperatures above 48 °C. Preliminary studies showed that the reactions were linear over these time periods. The activity of α-D galactosidase was maximal at 51 °C whilst that of β-D galactosidase and α-D glucosidase was still increasing with temperature at 57.2 °C. Both β-D galactosidase and α-D glucosidase yielded more-or-less linear Arrhenius plots (fig. 2) although α-D galactosidase gave an essentially curvilinear plot. For convenience the latter has been treated as 2 straight lines (determined by linear regression analysis of the data over the temperature ranges 18–35 °C and 35–51 °C). The activation energies calculated from the Arrhenius plots of the 3 enzymes are shown in table 1. The apparent discontinuity in the Arrhenius plot of α-D galactosidase at 34 °C is similar to that reported in plots of β-glucosidase from the head of *Triner-*

Table 2. Apparent K_m and V_{max} values for oligosaccharidases present in the gut of *C. maculatus*

Enzyme	Range of substrate concentrations (mM)	K_m (mM)	V_{max} (µmoles PNP released/mg protein/h)
α-D-Glucosidase	0.19–1.27	0.77[a]	5.37[a]
		0.79[b]	6.49[b]
	1.90–9.50	1.86[a]	8.93[a]
		1.81[b]	10.30[b]
α-D-Galactosidase	0.33–6.48	3.02[a]	6.41[a]
		3.80[b]	5.65[b]
β-D-Galactosidase	0.15–6.48	0.42[a]	15.60[a]
		0.56[b]	20.80[b]

[a] and [b] represent independent experiments; PNP, p-nitrophenol.

vitermes trinervoides[27] and α-D galactosidase, β-D galactosidase and β-D glucosidase from the alimentary tract of *Locusta migratoria*[23].

The effect of substrate concentration on the activity of α-D and β-D galactosidase and α-D glucosidase was determined under optimal pH conditions. Care was taken to ensure that substrate availability did not become rate-limiting during the course of the reactions. The apparent Michaelis constant (K_m) and V_{max} were calculated from Lineweaver-Burk plots of the data for each enzyme (table 2). The affinities of the 3 enzymes for their respective substrates compare reasonably well with values reported elsewhere in various other insect species[21,23,25,28]. In the present study the activity of α-D glucosidase did not show simple Michaelis-Menton kinetics (table 2); the Lineweaver-Burk plot being bi-phasic. Similar bi-phasic plots have been reported for β-D glucosidase and β-D galactosidase from the gut of *Locusta* with similar substrates to those used here, although in the locust the plot for α-D glucosidase was linear[23]. A number of alternative explanations may be advanced. There may be 2 different enzymes or isoenzymes present, each with different affinities for the substrate. Some support for this comes from the fact that 3 β-glucosidase components have been reported in the crop of *Locusta*[29] and biphasic Lineweaver-Burk plots have also been reported for β-glucosidase activity in this species[23]. A second possibility is that a single enzyme is involved but that there is some change in the kinetic parameters of the system at different substrate concentrations. Clearly further studies are necessary before the significance of such bi-phasic plots can be explained in relation to the physiology of digestion in *Callosobruchus maculatus*.

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Nonhistone protein with high affinity for histone H1 and HMG 14 protein

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Summary. Specific interaction of a nonhistone protein from mouse spleen chromatin with histones HI, H2A and HMG 14 protein is shown. Some implications of these findings are briefly discussed.

Interactions of nonhistone proteins with particular components of chromatin have drawn the attention of many investigators, as these proteins are the most probable candidates for the role of elements controlling the genome's expression. So, any nonhistone component specifically interacting with some chromatin elements and not interacting with others is important. The more so if such a protein belongs to the transcriptionally active chromatin fraction.

In the present paper I report the results of the investigation of the interaction of one particular nonhistone protein, PS_1, described by us earlier[1,2]. This protein is selectively released from the nuclei of mouse spleen under conditions of mild hydrolysis with micrococcal nuclease or DNAse I, i.e. when preferential hydrolysis of actively transcribed genes[3-5] takes place. This fact makes this protein a very interesting subject of investigation. Moreover, this protein, according to its properties[1] is quite different from other nonhistone proteins, namely HMG proteins, which are also selectively released from nuclei under similar conditions[6]. Earlier, we showed that PS_1 protein does not interact with DNA[7]. In the work described here, I found that this component interacts very specifically with histones H1, H2A and HMG protein 14.

Materials and methods. PS_1 protein was purified by preparative electrophoresis in the presence of SDS and electro-eluted from the gel as described earlier[2]. Purified protein was extensively dialyzed against 1000 vols of 0.5 M sodium phosphate buffer pH 7.5 containing 1% of Triton × 100 (Merck), followed by dialysis against another 1000 vs of the same buffer without Triton × 100. After the dialysis PS_1 protein was iodinated with NaI^{125} (USSR) using the chloramine T procedure, as described[8]. The specific radioactivity of the iodinated protein was $2.5-3.5 \times 10^6$ cpm/μg.