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Isoenzyme Studies on Toxoplasma gondii Isolates Using Isoelectric Focusing

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Abstract

Zymogram analysis using isoelectric focusing on polyacrylamide gels was performed to characterize and distinguish two *Toxoplasma gondii* isolates ("strains" BK and RH). The activity of the following 14 enzymes in the cell lysates was investigated: IDH, MDH, ME, 6PG, G6P, LDH, IPO, HEX, PGM, EST, ALP, ACP, LAP, and PGI. Nine enzymes (IDH, G6P, LDH, HEX, PGM, EST, ALP, ACP, and PGI) showed distinct and reproducible banding patterns, and four of them (IDH, G6P, EST, PGI) enabled a reliable distinction of the two *Toxoplasma gondii* isolates. A contamination of the parasite extracts with host proteins could be excluded by comparison of the enzyme activities of the Toxoplasma isolates with mouse peritoneal exudate cells. Isoenzyme analysis proved to be a helpful method for a characterization and a distinction of *Toxoplasma gondii* isolates.

Zusammenfassung

Zymogramm-Analysen mittels Isoelektrischer Focusierung in Polyacrylamid-Gelen wurden zur Charakterisierung und zur Unterscheidung von zwei *Toxoplasma* gondii-Isolaten („Stämme" BK und RH) eingesetzt. Die Aktivität der folgenden 14 Enzyme wurde untersucht: IDH, MDH, ME, 6PG, G6P, LDH, IPO, HEX, PGM, EST, ALP, ACP, LAP und PGI. Neun Enzyme (IDH, G6P, LDH, HEX, PGM, EST, ALP, ACP und PGI) zeigten deutliche und reproduzierbare Bandenmuster, vier davon (IDH, G6P, EST, PGI) ermöglichten eine klare Unterscheidung der *Toxoplasma* gondii-Isolate. Eine Verunreinigung der Parasiten-Extrakte mit Wirtsproteinen wurde durch einen Vergleich zwischen den Enzymaktivitäten der Toxoplasma-Isolate mit jenen von Zellen des Peritoneal-Exudates ausgeschlossen. Die Isoenzym-Analyse erwies sich als eine hilfreiche Methode zur Charakterisierung und zur Unterscheidung von *Toxoplasma* gondii-Isolaten.

Introduction

Electrophoretic separation of isoenzymes has been applied in recent years to differentiate morphologically indistinguishable strains of several species of Coccidians (5,

12). Above that, such isoenzyme studies lead to a better understanding of the metabolic properties of these organisms and contribute to their biochemical characterization (7, 1 16). We examined the isoenzyme profiles of *Toxoplasma gondii,* a widespread parasite of mammals, including man, and birds. The aim of this study was to determine which of the enzymes reported to be found in related species can be detected in *Toxoplasma gondii* isolates and whether these isolates can be distinguished by their zvmograms.

Materials and Methods

Cultivation of parasites and preparation of extracts

Toxoplasma gondii trophozoites of the BK "strain" (1) and of the RH "strain" (11) were maintained by continuous passages in mice (Him: OF1 (Swiss) SPF). The mice were intraperitoneally injected with 1 x 10^6 trophozoites each. The parasites were collected from the peritoneal cavity two days p.i. and washed three times in 145 mM sterile NaCl (10 min 3000 g). The cell pellet was resuspended with distilled water (10^8 cells/ml) and cooled with ice while being sonicated (Sonifer Cell Disruptor B-30, Branson Sonic Power Co./U.S.A., step 4, 5 min). The homogenate was centrifuged at 3000 g for 20 min at 4°C. Thereafter, the supernatant was centrifuged at 24000 g for 120 min at 4 °C. The protein content of this supernatant was determined (Protein Assay, Bio-Rad Lab., Vienna, Austria) and adjusted to a concentration of 3 mg/ml. The material was stored in small quantities at -70 °C until required for electrophoresis.

In addition, a homogenate of mouse peritoneal exudate cells was tested for enzyme activity. The cells were obtained by i.p. injection of Sephacryl S-300 (Pharmacia Ges.m.b.H., Vienna, Austria). They were collected from the peritoneal cavity of the mice 5 days after the injection of the gel particles. A lysate of the mouse peritoneal exudate cells was prepared in the same manner as the parasite samples.

Isoelectric focusing

Isoelectric focusing (IEF) was performed on polyacrylamide gels (PAA, T5/C3) of 1 mm thickness in the pH ranges 4—6.5 and 3—10 according to the manufacturer's recommendations (9). In short, IEF was performed in the following way: a prefocusation was carried out with a constant power of 20W for 500 Vh. Subsequently, aliquots of samples (20 = 60 µg protein) were applied to the gel on filter paper pieces. IEF was performed at a constant power of 20 W for 2500 Vh. At the end of the run, the pH-gradient of the gel was measured with a surface pH-electrode (Lot 403-30-M8, Ingold, Zürich, Switzerland) at 5 mm intervals. A pH-gradient curve was constructed for each run separately.

Enzyme staining

The activities of the following 14 enzymes were investigated (in parenthesis: abbreviation of enzyme, Enzyme Nomenclature number, appropriate pH-range, reference for the staining procedure): isocitrate dehydrogenase (IDH, E.C.1.1.1.42, 4—6.5, 14), malate dehydrogenase (MDH, E.C.1.1.1.37, 3—10, 8), malic enzyme (ME, E.C.1.1.1.40, 3—10, 14), 6-phosphogluconate dehydrogenase (6PG, E.C.1.1.1.44, 3—10, 8), glucose 6-phosphate dehydrogenase (G6P, E.C.1.1.1.49, 4—6.5, 14), L-lactate dehydrogenase (LDH, E.C.1.1.1.27, 3—10, 14), indophenol oxidase (IPO, E.C.1.15.1.1, 3—10, 14), hexokinase (HEX, E.C.2.7.1.1, 3—10, 14), phosphoglucomutase (PGM, E.C.2.7.5.1, 3—10, 14), esterases (EST, E.C.3.1.1.1, 3—10, 14), alkaline phosphatase (ALP, E.C.3.1.3.1, 3—10, 14), acid phosphatase (ACP, E.C.3.1.3.2, 3—10, 14), leucine aminopeptidase (LAP, E.C.3.4.1.1, 3—10, 14), phosphoglu-

Table 1. pI values of the isoenzymes of isocitrate dehydrogenase (IDH), glucose 6-phosphate dehydrogenase (GO), L-lactate dehydrogenase (LDH), hexokinase (HEX), phosphoglucomutase (PGM), esterases (EST), alkaline phosphatase (ALP), acid phosphatase (ACP), and phosphoglucose isomerase (PGI) extracted from *Toxoplasma gondii* isolates and mouse peritoneal exudate cells (mc)

	"Strain"				"Strain"		
Enzyme	BK	RH	mc	Enzyme	BK	RH	mc
IDH	-	4.39	4.39	PGM	-	-	5.14
	-	-	4.48		5.18	5.18	5.18
	-	-	4.68		5.25	5.25	5.25
	-	-	4.71		5.40	5.40	5.40
	-	4.98	-		5.64	5.64	-
	5.15	5.15	-		7.28	7.28	-
	5.30	5.30	-				
	5.34	5.34	-	EST	-	4.78	-
	5.38	5.38	-		-	4.90	-
	5.48	5.48	-		-	5.10	-
					5.24	5.24	5.24
G6P	-	5.20	5.20		5.38	5.38	5.38
	5.26	-	-		-	-	5.53
	5.39	5.39	5.39		-	-	5.85
	5.55	-	-		5.95	5.95	5.95
					6.04	6.04	6.04
LDH	-	-	5.04				
	-	-	5.12	ALP	5.24	5.24	
	-	-	5.20		5.40	5.40	
	-	-	5.30		5.60	5.60	
	-	-	5.52		5.82	5.82	
	5.60	-	-		6.10	6.10	not
			5.64		6.30	6.30	done
	5.70	5.70	-		6.46	6.46	
	5.80	5.80	-		6.65	6.65	
	-	-	5.86		6.87	6.87	
	5.95	5.95			7.19	-	
	-	-	6.08				
	-	-	6.15	ACP	5.19	-	
	6.17	6.17	-		5.36	5.36	
	-	-	6.20		5.60	5.60	
	6.28	6.28	6.28		5.82	5.82	not
	6.40				6.10	6.10	done
	-	-	6.45		6.40	6.40	
	-	-	6.56		6.48	6.48	
	-	-			6.74	6.74	
HEX	-	-	5.26				
	-	-	5.28	PGI	-	4.17	-
	5.34	-	5.34		-	-	6.06
	5.39	5.39	5.39		6.84	6.84	-
					7.45	7.45	7.45
					7.80	7.80	7.80

cose isomerase (PGI, E.C.5.3.1.9, 4—6.5, 8). All extracts were focused and stained at least tw ice for each enzyme.

The isoelectric point (pI) of each isoenzyme was determined by refering the band to the pH-gradient curve.

Results

The results of this study showed that nine of the 14 enzymes examined gave distinct and reproducible patterns (IDH, G6P, LDH, HEX, PGM, EST, ALP, ACP, and PGI). The pI values of the stained bands of each of these enzymes are presented in Table 1. The remaining five enzymes, MDH, ME, 6PG, IPO, and LAP showed no or only poor enzyme activities.

Discussion

Isoenzyme electrophoresis has been successfully applied for the characterization and differentiation of protozoan parasites (3, 4, 6, 12, 13). In particular, the technique of isoelectric focusing has proven to be a sensitive and effective method in studying isoenzyme differences in protozoan isolates (2, 3, 8, 16). Therefore, this technique was chosen for the characterization and differentiation of *Toxoplasma gondii* isolates, which differ physiologically (e.g. pathogenicity) indeed, but not morphologically (11).

Among the nine enzymes which revealed discrete bands of enzyme activity in our *Toxoplasma gondii* isolates, four enzymes (LDH, G6P, IDH, and PGI) had been reported from related families (Eimeriidae and Sarcocystidae) of the suborder Eimeriina before (5, 15, 16). The technique of IEF had, however, only been applied to characterize isolates and species of Eimeria. Therefore, a comparison of the zymograms between this genus and *Toxoplasma gondii* could be drawn for LDH only. The number of bands showing LDH activity and the location of these within the pH-range point out some similarities in the metabolic properties of both species, although they belong to different families. Eleven bands of LDH activity extending from pH 5.0—6.5 were apparent in *Eimeria tenella* extracts; *Toxoplasma gondii* extracts showed bands of activity in the pH-range 5.6—6.4, both species showed a similar pattern. This interesting feature may be an expression of a close relationship of these genera.

The lack of activity of the enzymes MDH, ME, 6PG, IPO, and LAP may either be due to inadequate handling during the electrophoresis or the staining procedure, or to the lack of these enzymes in the metabolism of *Toxoplasma gondii.* The lack of such important metabolic enzymes in protozoan parasites has also recently been discussed for Trypanosomes in which LDH was not detected (10).

A comparison between the isoenzyme profiles of *Toxoplasma gondii* and mouse peritoneal exudate cells leads to the assumption that the banding patterns appearing in the parasite isolates are specific for the parasite. The reason for this assertion becomes evident by examination of the profiles of IDH and LDH. The *Toxoplasma gondii* isolates do not take over the complete spectrum of host bands in their patterns; this proves that *Toxoplasma gondii* must synthesize enzymes or at least some isoenzymes on its own. Thus it is very unlikely that *Toxoplasma gondii* can selectively take over distinct isoenzymes from the enzyme set of the host cells, it can therefore be presumed

that all enzymes of the parasite and of the host revealing identical isoelectric points, are isoenzymes which coincidently appear at the same pH. We can therefore assume that the parasite extracts were not contaminated with host proteins. Hence, the isoenzyme profiles of our *Toxoplasma gondii* isolates could be compared. The following enzymes proved to be useful for this purpose: IDH, G6P, LDH, HEX, EST, ALP, ACP, and PGI. Only indistinct differences (faint bands) in the isoenzyme patterns of four enzymes (LDH, HEX, ALP, and ACP) were observed. Other enzymes, however, showed a very clear heterogeneity between the *Toxoplasma gondii* isolates. These enzymes (IDH, G6P, EST, and PGI) allowed a reliable and reproducible differentiation of our *Toxoplasma gondii* isolates and should therefore be used for further investigations of "strain" differentiation. One enzyme (PGM) showed identical banding patterns in both isolates and may thus be considered as a candidate for enzymes which allow a species characterization.

As far as we know, isoenzyme studies on *Toxoplasma gondii* isolates are reported here for the first time. Clear and reproducible banding patterns could be obtained with extracts of *Toxoplasma gondii* cells with a number of metabolic enzymes, thus showing that isoenzyme analysis using isoelectric focusing is a useful technique for the characterization and distinction of *Toxoplasma gondii* isolates.

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