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THE DEMONSTRATION OF A COOPERATIVE ACTION OF BACTERIAL AND INTESTINAL MUCOSA ENZYMES IN THE ACTIVATION OF MUTAGENS

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SUMMARY

While 2-aminoanthracene and 2-aminofluorene are converted to frameshift mutagens by microsomal preparations from rat livers, the microsomes from the intestinal mucosa of the same animals, under the experimental conditions used herein, either have little such activity or lack it altogether. Cell-free extracts of the colon anaerobe Bacteroides fragilis may exhibit such activity to varying degrees depending upon the conditions of incubation. However mixtures consisting of cell-free extracts from B. fragilis and microsomes from intestinal mucosa demonstrate significant- more than additive- activity in converting these chemicals to mutagens.

INTRODUCTION

Previous reports from our laboratory have suggested a cooperative and sequential action by bacteria and mammalian microsomal enzymes in the conversion of human bile as well as components thereof into substances mutagenic for Salmonella typhimurium (1,2). In view of the possible importance of this novel concept in the etiology of cancer of the colon, we wished to investigate this phenomenon further using carcinogens which have been shown to be mutagens following activation by rat hepatic microsomal enzymes.

In the present study, we demonstrate the conversion of 2-aminofluorene and 2-aminoanthracene to mutagens by the concerted action of microsomes derived from the intestinal mucosa and of cell-free extracts prepared from a human strain of Bacteroides fragilis, under conditions which allow no or only minimal activity when either of the preparations is used singly. Moreover, we show that this concerted action alone can occur aerobically under which condition extracts of

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anaerobes have already been shown to possess only minimal activity in converting precarcinogens to mutagens (3).

MATERIALS AND METHODS

2-Aminoanthracene was obtained from Aldrich Chemical Co., and 2-aminofluorene and 2-nitrofluorene from the National Cancer Institute Chemical Repository, IIT Research Institute, Chicago, Ill.

Bacterial extracts from Bacteroides fragilis strain 4841 were prepared as previously described (3). Adult male Fischer F344 rats served as the source of the microsomes derived from the livers and the mucosa of the large intestine. Liver microsomes were prepared according to the procedure of Ames et al (4) while mucosal preparations were obtained by the procedure of Fang and Strobel (5).

Mutagenic activity for Salmonella typhimurium TA1538 was determined by the procedure of Ames et al (4) or by the pre-incubation modification thereof (6, 7). Where indicated the usual rat liver microsome preparation was replaced by microsomes from the intestinal mucosa or by a cell-free extract derived from B. fragilis. Anaerobiosis was achieved by placing plates containing the test chemical, bacteria and, if required, microsomes or bacterial extracts into Gas-pak jars (BBL, Cockeysville, Md.). Following 16 hours of such incubation at 37°C, the plates were removed from the anaerobic chambers and incubated for an additional 32 hours aerobically.

Table 1

Activation of Carcinogens to Mutagens by Enzymes from the Intestinal Mucosa or from Bacteroides fragilis

Expt.		Additions	Incubation	Revertants per Plate				
				No enzyme	RL	IM	Bf	IM+Bf
I		None	Anaerobic	9	5	6	10	16
		2-Nitrofluorene	Anaerobic	513				
		2-Aminofluorene	Anaerobic	217	1081	356	363	919
		2-Aminoanthracene	Anaerobic	11	739	12	31	237
II		None	Aerobic	3	6	6	8	
		2-Nitrofluorene	Aerobic	600				
		2-Aminofluorene	Aerobic	172	942	198	157	498
		2-Aminoanthracene	Aerobic	9	475	65	12	493
III	A	None	Pre-Incubation/Aerobic	4		12	10	13
	B	None	Pre-Incubation/Aerobic				9	9
	A	2-Aminoanthracene	Pre-Incubation/Aerobic	8		32	39	72
	B	2-Aminoanthracene	Pre-Incubation/Aerobic				97	221
		2-Nitrofluorene	Pre-Incubation/Aerobic	729				
IV	A	None	Aerobic	9		6	8	7
	B	None	Aerobic				10	10
	A	2-Aminoanthracene	Aerobic	11	12	30	12	125
	B	2-Aminoanthracene	Aerobic				31	237

Abbreviations: RL, microsomes from rat liver; IM, microsomes from intestinal mucosa; Bf, cell-free extract from B. fragilis.
The amounts of 2-nitrofluorene, 2-aminofluorene and 2-aminoanthracene were 100, 250 and 10 µg per plate, respectively. 2-Nitrofluorene was included as a positive control for strain TA1538.
The protein contents of the enzyme preparations were 240 µg per plate of IM for all the experiments. For Bf, the values were 380 µg per plate for experiments I and II, 132 and 264 µg per plate for parts A and B of experiment III, and 190 and 380 µg per plate for parts A and B of experiment IV, respectively.

Table 2

Effect of Heat Inactivation of the Enzyme Preparations on the Conversion of 2-Aminoanthracene to a Mutagen.

Additions	Enzyme	Revertants per plate
None	None	3
2-Aminoanthracene	None	9
None	RL	6
2-Aminoanthracene	RL	475
None	Bf #1	12
2-Aminoanthracene	Bf #1	65
None	Bf #2	8
2-Aminoanthracene	Bf #2	103
None	IM	6
2-Aminoanthracene	IM	12
2-Aminoanthracene	IM + Bf #1	493
2-Aminoanthracene	IM + Bf #2	580
None	Heated IM + Bf #1	11
2-Aminoanthracene	Heated IM + Bf #1	56
None	Heated IM + BF #2	6
2-Aminoanthracene	Heated IM + BF #2	150
None	IM + Heated Bf #1	7
2-Aminoanthracene	IM + Heated Bf #1	8
None	IM + Heated Bf #2	6
2-Aminoanthracene	IM + Heated Bf #2	12

Abbreviations: RL, microsomes from rat liver; IM, microsomes from intestinal mucosa; Bf, cell-free extact from B. fragilis.
The final concentration of 2-aminoanthracene was 10 µg per plate while the protein contents for IM, Bf #1 and Bf #2 were 242, 380 and 590 µg per plate.
The plates were incubated aerobically throughout the duration of the experiment. Heat inactivation of the enzymes was accomplished by heating at 80°C for 5 minutes.

RESULTS AND DISCUSSION

Although both of the test chemicals were readily converted to mutagens by rat liver microsomes, the microsomes prepared from the intestinal mucosa of these animals possessed little or no such activity (Tables 1 and 2). Cell-free extracts from B. fragilis, depending upon the experimental conditions used, exhibited varying levels of activity (Tables 1 and 2). However, mixtures of the enzyme preparations consistently showed activities that were not only significantly higher than that of either preparation alone but actually were more than additive, presumably demonstrating a synergistic action. That this is not merely due to co-factors present in either enzyme preparations but may reflect enzyme activity was demonstrated by thermal denaturation experiments. Heating either preparation

at 80°C for 5 minutes resulted in a loss of enzyme activity of the mixtures (Table 2).

These findings which indicate an actual concerted action by bacterial and mammalian enzymes are undoubtedly germane to an understanding of the etiology of colon cancer. It has been shown that on a weight basis, there is a 1000-fold excess of anaerobic over aerobic bacteria of demonstrated metabolic versatility in the colon (8). These may convert a colon-specific carcinogen to a penultimate metabolite which could then be transformed further to the ultimate form by intestinal enzymes. Studies which are proceeding in our laboratory, may provide additional information regarding to the significance and mechanism of the observations reported herein.

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