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ALLOSTERIC TRANSITIONS AND MEMBRANE-BOUND ATPase FROM RAT TISSUES: THE EFFECT OF FAT DEPRIVATION ON THE ALLOSTERIC INHIBITION BY FLUORIDE*,**

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SUMMARY

In rats red a fat-sufficient diet, ATPases (ATP phosphohydrolase, EC 3.6.1.3) from heart, kidney and brain microsomes showed allosteric kinetics for the inhibition by F^-, with values of $n = -2.0$. In rats fed a fat-free diet, the values of n for the ATPases changed from -2.0 to -1.0 in heart and kidney microsomes. When these animals were then fed a fat-sufficient diet the values of n reached the control values. In brain microsomal ATPases no modification of the values of n were found between both groups of animals. The regulatory properties of the membrane on bound ATPases are discussed.

INTRODUCTION

A diet low in essential fatty acids provokes alterations in the fatty acid composition of most tissues, and these changes are well documented[1–5]. The occurrence of membrane-bound (Na^+–K^+)-ATPase and Mg^{2+}-ATPase in many different tissues from various species has been reported[6]. Changes in the allosteric behavior of the rat erythrocyte ATPases[7,8], *p*-nitrophenylphosphatase[9] and acetylcholinesterase[10] were observed in rats fed a fat-deficient diet. The present work extends these studies to heart, kidney and brain microsomal ATPases of the same animals. A possible

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relationship between the lipid composition of the tissues in rats deficient in essential fatty acids and allosteric modifications will be discussed here.

MATERIALS AND METHODS

Animals and diets

Sprague–Dawley rats were fed a fat-free diet in order to produce a deficiency in essential fatty acids. The diets for fat-deficient and fat-sufficient animals have been described previously[8].

Enzyme preparation

The methods of preparation of microsomal ATPases from kidney described by Chignell and Titus[11] were used with some modifications. The histidine buffer (pH 6.8) was changed to Tris–HCl buffer (pH 7.2). Heart and brain microsomal ATPases were obtained by the method of Brown *et al.*[12] without dialysis of the 600 $\times$ g supernatant.

Enzyme assay

The determinations of the ATPases were performed with freshly prepared microsomal membranes or with membranes kept frozen at -70 °C for 2–3 weeks. ATPase activity was estimated by determining the $^{32}P_i$ liberated from [γ-^{32}P]-ATP by the method described previously[7]. For brain and kidney microsomal total ATPases the following incubation mixture was used: 25 mM Tris–HCl buffer (pH 7.4); 1 mM [γ-^{32}P]-ATP (Tris salt; 50000 cpm); 2 mM $MgCl_2$; 80 mM NaCl; 33 mM KCl. Total ATPase activity from heart microsomes was determined according to Brown *et al.*[12]; the reaction mixture contained: 25 mM Tris–HCl buffer (pH 7.2), 1 mM [γ:^{32}P]ATP (Tris salt; 50000 cpm), 1 mM $MgCl_2$; 1 mM NaCl; 2 mM KCl. Mg^{2+}-ATPase in all microsomal preparations was determined with the same incubation mixture but in the absence of NaCl and KCl. The assays of heart, kidney and brain microsomal ATPase activities were performed at 37 °C for 1 h, 8 min and 30 min, respectively. Inhibition by F^- was investigated by measuring the enzymatic activity in the presence of NaF at a concentration of 0.3–3.0 mM. In the case of heart microsomal ATPases Tris–HF was used.

Protein determinations

These determinations were carried out according to Lowry *et al*[13] with bovine serum albumin as standard.

RESULTS AND DISCUSSION

Typical inhibition curves of heart, kidney and brain microsomal ATPases from rats fed a fat-sufficient diet obtained with the effector F^- are shown in Fig. 1. The curves showed cooperative shapes with values of $n = -2.0$ determined from the Hill equation[14]. When the same experiments were carried out with heart and kidney microsomal ATPases from rats fed a fat-deficient diet, values of $n = -1.0$ were obtained for both Mg^{2+}-ATPases and total ATPases (Figs 2A and 2B). As can be seen, cooperative shapes of the inhibition curves for F^- were found in brain

microsomal ATPases from fat-deficient animals and the Hill coefficient was −2.0 in both ATPases. The results for several microsomal ATPases from both groups of animals are summarized in Table I. As can be observed, the values of n for heart and kidney microsomal ATPase of control animals are significantly higher ($P < 0.001$) than those of the corresponding ATPase activities of the rats fed a fat-free diet. The values of K_i for the inhibition by F^- effector of the ATPases from rat tissues were similar in both groups (Figs 1 and 2). The presence or absence of fat in the diet did not influence the specific activity of the ATPases. The statistical treatment of the data from corresponding cases presented in Table I showed no significant difference in the values of K_i from both groups of animals, nor in specific activity (not shown). Similar results were obtained with the other enzymatic systems studied in erythrocyte from rats fed a fat-deficient diet. In addition, the saturation curves of the substrate ligand for erythrocyte membrane ATPases, *p*-nitrophenylphosphatase and acetylcholinesterase showed no difference between both groups. In all cases the decrease of the values of n was observed in the allosteric response only for the positive or the negative effector, in fat-deficient animals[7–10].

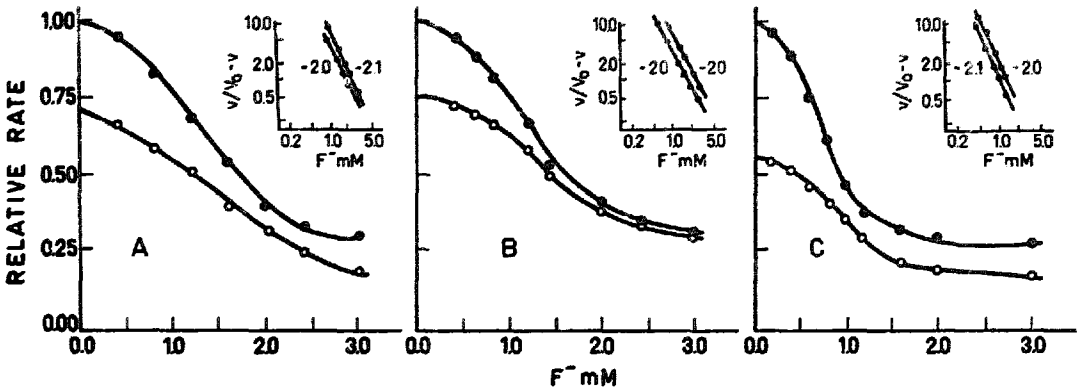

Fig. 1. Effect of concentration of F^- on the reaction rate of Mg^{2+}-ATPase (○—○) and total ATPase (●—●) from rats fed a fat-sufficient diet. (A) Heart microsomal ATPases; (B) kidney microsomal ATPases; (C) brain microsomal ATPases. Inset: the same data were replotted according to Hill; the slope of each line is indicated in the figures. The Mg^{2+}-ATPase and total ATPase specific activities, expressed as μmoles PO_4^{3-}/h per mg protein of rat microsomes, were 16.6 and 26.2 for heart; 16.0 and 21.0 for kidney; and 12.0 and 21.4 for brain.

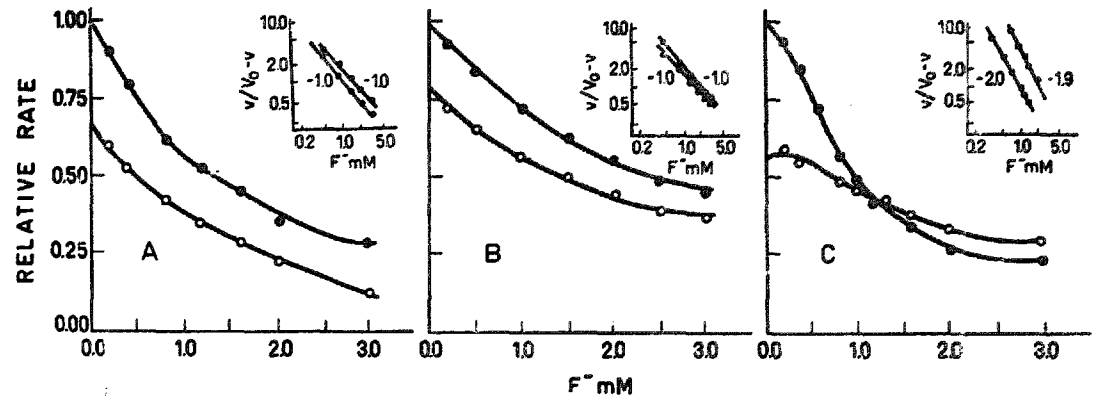

Fig. 2. Effect of concentration of F^- on the reaction rate of Mg^{2+}-ATPase (○—○) and total ATPase (●—●) from rats fed a fat-deficient diet. (A) Heart microsomal ATPases, (B) kidney microsomal ATPases, (C) brain microsomal ATPases. Inset: the same data were replotted according to Hill; the slope of each line is indicated in the figures. The Mg^{2+}-ATPase and total ATPase specific activities, expressed as μmoles PO_4^{3-}/h per mg protein of rat microsomes, were 17.2 and 25.6 for heart; 14.8 and 19.0 for kidney; and 10.0 and 17.6 for brain.

TABLE I

VALUES OF n FOR THE ATPASES FROM RATS FED FAT-SUFFICIENT AND FAT-DEFICIENT DIET

Results are given as means ± S.E. Figures in parentheses are the number of animals used.

Microsomal	*Diet*			
	Sufficient		*Deficient*	
	Mg^{2+}-ATPase	*Total ATPase*	*Mg^{2+}-ATPase*	*Total ATPase*
Heart*	1.88 ± 0.05 (8)	1.98 ± 0.10 (6)	1.00 ± 0.01 (7)	1.12 ± 0.06 (6)
Kidney*	1.94 ± 0.07 (6)	2.00 ± 0.12 (7)	1.00 ± 0.03 (3)	1.06 ± 0.05 (8)
Brain	1.60 ± 0.06 (3)	1.95 ± 0.12 (7)	1.80 ± 0.05 (7)	1.97 ± 0.11 (5)

* The values of n for rat fed fat-sufficient and fat-deficient diet are significantly different, $P < 0.001$.

As was previously found with ATPases[8] and acetylcholinesterase[10] of erythrocytes the values of n for heart and kidney microsomal ATPases equalled the values of the control animals after 16 days of refeeding the fat-starved animals. The need for a "*de novo*" synthesis of proteins was ruled out in the allosteric modification in erythrocytes from fat-deficient animals[8,10]. In these cases, changes in the values of n were obtained after 8–12 days of feeding the deficient animals a fat-sufficient diet.

Initially the decrease in the allosteric character was observed in erythrocyte membrane ATPases from deficient animals[7,8]. This behavior is found now in heart and kidney microsomal ATPases. These facts suggested that the behavior of membrane-bound ATPases from rats fed a fat-free diet is not confined to one tissue. In addition, very recently, we communicated changes in allosteric kinetics from membrane ATPase of bacterial systems when the fatty acid composition of the membrane was modified[15]. The mechanism through which changes in the fatty acids of the membrane regulate the "phenotypic allosteric desensitization phenomenon" of the ATPases is not clear at present. Several features of the changes in fatty acids in the fat-deficient tissue systems distinguish them from the corresponding changes in bacterial systems. However, one would be tempted to suggest that all these facts observed appear as a response to the general regulatory property of the membrane on bound enzymes. The response is due in each case to the particular environments originated by modifications in fatty acids.

In the case of brain microsomal ATPases no changes were observed in the allosteric kinetics between both groups as can be seen in Fig. 2C and Table I. Brain lipids were less susceptible to dietary changes than lipids of other tissues in essential fatty acid deficiency[5,16–18]. These facts indicated that the non-influence on allosteric behavior in brain membrane-bound ATPases might be caused by a very low metabolic turnover in essential fatty acids. The structural state of the cell membrane that might affect the enzyme kinetics could not be acquired in brain membrane under these experimental conditions.

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