PHOSPHORYLASE AND URIDINEDIPHOSPHOGLUCOSE-GLYCOGEN TRANSFERASE IN PYRIDOXINE DEFICIENCY

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SUMMARY

The total phosphorylase activity of the skeletal muscle of rats maintained on a pyridoxine deficient diet has been found to fall to 35 % of the normal value. The phosphorylase *a* activity of the tissue of these rats has the normal value, and the glycogen content of the muscles of such deficient rats is not different from that of control animals. The apparent activity of uridine diphosphoglucose–glycogen transferase is not changed from the normal level in pyridoxine deficiency.

INTRODUCTION

It has been shown that phosphorylase *a* contains 4 moles of pyridoxal-5′-phosphate per mole of enzyme, and that the apoenzyme prepared by ammonium sulfate precipitation from acid solution is inactive in the absence of added pyridoxal-5′-phosphate[1,2]. Since other enzymes requiring pyridoxal-5′-phosphate have been shown to decline in activity in Vitamin B_6 deficient animals[3], it was of interest to investigate the activity of phosphorylase in the muscle of rats made deficient in B_6. The activity of UDPG-glycogen transferase was also measured, since this enzyme has been shown to be active *in vivo* in lengthening a preexisting glycogen chain[4,5], and, thus, to have an action like that of phosphorylase.

MATERIALS, METHODS AND RESULTS

In the first series of experiments weanling albino rats (Sprague-Dawley) were divided into two groups. The control group was fed laboratory chow, and the experimental group was given a pyridoxine-deficient diet supplied by Nutritional Biochemicals Corporation. After 7 to 8 weeks the animals were anesthetized and muscle samples were removed as follows. Both gastrocnemii were exposed *in situ* and then cut out in rapid succession. A 100–200 mg sample of each was taken to determine its glycogen content in duplicate. The remaining tissue was weighed and homogenized for 3 min in a Virtis homogenizer with 9 volumes of ice-cold 0.05 *M* Tris–0.001 *M* EDTA,

Abbreviations: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediamine-tetraacetic acid; UDPG, uridine diphosphoglucose; UDP, uridine diphosphate.

pH 7.5. For phosphorylase determinations an aliquot of this 1:10 homogenate was immediately diluted five-fold in ice-cold 0.001 *M* EDTA–0.02 *M* NaF, pH 6.0. The fluid medium used for homogenization was chosen so that phosphorylase and UDPG–glycogen transferase could both be determined[6]. For the analysis of the latter enzyme, an aliquot of the uncentrifuged homogenate was incubated for 15 min at 30° in the presence of $1.5 \cdot 10^{-3}$ *M* UDPG, 1.0% of added glycogen, $7.5 \cdot 10^{-3}$ *M* cysteine, $1.2 \cdot 10^{-2}$ *M* $MgCl_2$, $1.0 \cdot 10^{-3}$ *M* EDTA, $1 \cdot 10^{-1}$ *M* Tris, pH 7.5. The reaction was stopped by heating the mixture for 2 min in boiling water, followed by cooling in ice, centrifugation, and determination of the amount of UDP formed *via* the pyruvate kinase–lactic dehydrogenase system[7].

The immediate dilution of the homogenate with EDTA–NaF served to stop subsequent action of the specific phosphatase (PR-enzyme) and specific kinase known to change the relative proportion of phosphorylases *a* and *b*. Once diluted in this manner, the ratio of *a* to *b* remained constant during a period of several days. The phosphorylase *a* activity was determined at 30° at pH 6.0 in the presence of 0.016 *M* glucose-1-phosphate and 1% of added glycogen. Total phosphorylase activity was determined as above, with the addition of $1 \cdot 10^{-3}$ *M* 5′-adenylic acid (5′-AMP).

The data in Table I show that the only significant change exhibited by the deficient animals was a decrease in the total phosphorylase activity to 35% of that found in the control rats. Incubation of the homogenate under conditions which lead to the reactivation of the apoenzyme of phosphorylase[8], did not result in any increase of total activity. When the original 1:10 homogenates of the muscles from deficient animals were stored overnight at 5° or incubated at 30° for short periods of time, a decrease in phosphorylase activity in the absence of added 5′-adenylic acid was observed, showing that the PR-enzyme (acting to change $a \rightarrow b$) was active. When the fresh homogenates from the deficient animals were supplemented with ATP and

TABLE I

EFFECT OF PYRIDOXINE DEFICIENCY ON ENZYME ACTIVITY AND GLYCOGEN CONTENT

Animal	*Glycogen content (% wet wt.)*	*Phosphorylase activity (units*/g tissue)*		*UDPG-glycogen transferase activity (units**/g tissue)*
		— 5′-AMP	*+ 5′-AMP*	
Control group				
1	0.56	1060	9990	17.0
2	0.73	487	8910	22.9
3	0.57	165	7890	17.9
4	0.50	1290	8940	20.0
Deficient group				
5	0.58	822	4185	20.2
6	0.44	876	3195	17.0
7	0.66	540	2580	18.8
8	0.50	1040	3510	12.0
9	0.54	675	2525	17.6
10	—	595	2400	19.7

* For detailed description of assay see ref. 2.

** One unit of activity is the number of μmoles of UDPG which are converted to UDP under the assay conditions described in the text.

Mg^{++}, there occurred an increase in phosphorylase *a* activity, indicating that phosphorylase *b* kinase was active.

In a second series of experiments the weanling rats were divided into two groups. One group was fed the pyridoxine-deficient diet used above. The second group was fed the "Vitamin B Complex Test Diet (with complete Vitamin Fortification)", supplied by Nutritional Biochemicals Corporation. Thus, each group of rats received food of similar physical appearance. The first rats of each group were killed after two weeks, at which time the level of phosphorylase in the deficient rats was already depressed. For these measurements and for all subsequent ones, the initial homogenization of the excised muscle was done in EDTA–NaF.

At 3.5 weeks, four rats were changed from the B_6 deficient diet to the complete diet. These rats were sacrificed at intervals following resumption of B_6 intake. As shown by the data in Table II, these rats showed an increasing level of total phosphorylase activity which approached normal values after 4 weeks of access to the complete diet.

TABLE II

TIME COURSE OF PHOSPHORYLASE ACTIVITY CHANGE IN PYRIDOXINE DEFICIENCY

Animal	*Rat weight (g)*	*Days on diet*	*Phosphorylase activity (units*/g tissue)*	
			− 5′-AMP	*+ 5′-AMP*
Control group				
11	86	15	928	7320
12	130	24	460	8200
13	164	35	820	9260
14	178	45	—	8520
15	166	51	1160	9440
16	170	51	1140	9240
Deficient group				
17	54	15	828	3750
18	66	15	595	3360
19	70	24	1135	3350
20	72	24	860	3140
21	72	35	1020	2980
22	90	45	930	2190
23	89	51	720	2300
Deficient group after supplementation**				
24	110	11**	960	6000
25	152	21	700	6000
26	146	21	660	4000
27	148	27	930	9930

* For detailed description of assay see ref. 2.
** On deficient diet for 24 days and then on complete diet (see text) for time shown.

DISCUSSION

The data in Table II show that the level of phosphorylase *a* in pyridoxine-deficient rats remains relatively constant while that of total phosphorylase (*a* + *b*) shows a marked drop. To some extent this shifting of the *a* to *b* ratio in the muscle

appears to be a mechanism for maintaining a nearly normal potentiality for glycogen metabolism in the B_6 deficient animal. The extent of decline in the total phosphorylase level is about that which has been seen in other enzymes having pyridoxal-5'-phosphate as a prosthetic group[3].

The UDPG–glycogen transferase activity of the deficient animals was not significantly different from that of the control group. This finding does not suggest any role of pyridoxal-5'-phosphate in the reaction catalyzed by this enzyme. It should be pointed out, however, that if only the data for phosphorylase *a* activity and not those for total phosphorylase activity as well, were available from the present experiments, a similar conclusion would seem justified. Of course, in the light of other facts[1,2], such a conclusion is known to be erroneous. It is conceivable that the UDPG-glycogen transferase may exist partly in an inactive form in skeletal muscle, although no such finding has been reported. If such be the case, it must be emphasized that the data in this paper do not give any information about changes in the amount of such a protein in pyridoxine deficiency. Thus, the nutritional experiments described here do not afford conclusive evidence against a role of pyridoxal-5'-phosphate in the activity of UDPG–glycogen transferase. However, the effect of B_6 deficiency on total phosphorylase activity is explicable in the light of knowledge of the structure of the enzyme.

NOTE ADDED IN PROOF

The UDPG-glycogen transferase activity of the normal rat gastrocnemius, excised and homogenized as described above, is increased only 32 % by assaying the enzyme in the presence of $5 \cdot 10^{-3}$ *M* glucose-6-phosphate The concentration of glucose-6-phosphate plus fructose-6-phosphate in the standard transferase assay, due to the endogenous presence of these esters in the homogenate itself, is only $1 \cdot 10^{-5}$ *M*.

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