FORMATION OF TETRAMER PHOSPHORYLASE *A IN VIVO*

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1. Introduction

It is known that the breakdown of glycogen during muscle stimulation is caused by the conversion of inactive phosphorylase *b* into active phosphorylase *a*. But phosphorylase *a* itself can exist in two forms *in vitro*: in a dimer form with two subunits and a tetramer state with four subunits [1,2]. According to Metzger et al. only the dimer form of phosphorylase *a* is capable of binding glycogen, the tetramer is not [3], therefore phosphorylase *a* in the tetramer state is enzymatically inactive, or rather it possesses only slight activity [4].

It has been assumed that phosphorylase *a* can exist in tetramer form also *in vivo*, or rather the dimer and tetramer forms could be converted into each other *in vivo*, too. The possibility of this is increased by the fact that the concentration of the phosphorylase *a* in the muscle is great enough to become tetramer. In this case the glycogen could not break down, even if the interconversion cascade of the muscle phosphorylase has taken place, i.e. the production of cyclic 3′,5′-AMP, the activation of protein kinase and phosphorylase kinase, furthermore the conversion of phosphorylase *b* into phosphorylase *a*. The interconversion of the dimer and tetramer forms may play a role in the biological control. This possibility had been suggested by Metzger et al. on the basis of their observations *in vitro* [3,5].

In our present work we wish to prove that muscle phosphorylase may get into tetramer state *in vivo* also in consequence of the decreasing body temperature.

2. Methods

In our experiments we used frogs (*R. pipiens*) of different body temperatures. The conversion of muscle phosphorylase *b* into phosphorylase *a* was induced by epinephrine given intraperitoneally (100 μg/kg body weight).

The homogenization of the muscle was carried out by means of a method which fixed the phosphorylase *a* formed in the muscle *in vivo*, in the originally dimer–tetramer states during the extraction and determination. The homogenization was started with a buffer containing glycogen of body temperature, and the apparatus and muscle pulp was gradually cooled to 0° within 2 min. Glycogen becomes bound to the dimer form of phosphorylase *a*, thereby inhibiting the subsequent tetramerisation of the original dimer *a* during the cooling. On the other hand, the cooling applied during the homogenization inhibits the subsequent dissociation of tetramer phosphorylase *a* into dimer form.

For the determination of phosphorylase *a* fixed in dimer and tetramer forms (see above) we utilized the observation [6] that phosphorylase phosphatase converts only the dimer phosphorylase *a* into inactive phosphorylase *b*. After treatment with phosphorylase phosphatase, the tetramer remained in *a* form. Its amount can be determined after diluting and incubating for 10 min at 30°, which caused the dissociation of tetramer into dimer state. Therefore the activity of the originally tetramer *a* would be measurable according to the method of Cori et al. [7].

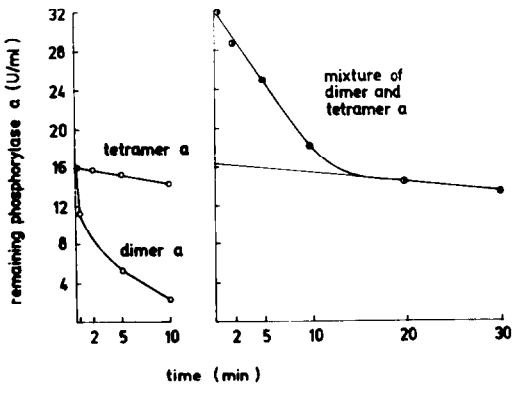

Fig. 1. Effect of phosphorylase phosphatase on dimer and tetramer phosphorylase *a* preparations. 16 Units (0.32 mg) crystalline phosphorylase *a* were incubated with phosphatase in a buffer consisting of 0.04 M Tris, 0.01 M mercaptoethanol, 0.002 M EDTA, pH 6.8 at 18°. Samples were removed and diluted to 1:40 with buffer containing 0.02 M NaF for stopping the phosphatase reaction and for determining the activity of remaining phosphorylase *a* according to the method of Cori et al. [7]. One unit of activity is defined as the amount of phosphorylase causing the release of 1 μmole of P_i from glucose-1-phosphate per min at 30°.

Dimer phosphorylase *a* was prepared from crystalline phosphorylase *a* by diluting with buffer containing 0.5% glycogen then incubated at 36° for 10 min and cooled to 18°. Tetramer phosphorylase *a* was obtained by diluting with buffer containing no glycogen and cooling to 18°. Only then was 0.5% glycogen added to the solution which did not cause dissociation of tetramer at 18° [3,10,11].

3. Results

The homogenization and the assay procedure described in Methods was first checked with crystalline phosphorylase *a* prepared according to the method of Krebs et al. [8]. Samples were brought into dimer and tetramer state, and the 1:1 mixture of these were incubated with phosphorylase phosphatase (prepared according to Stalmans et al. [9]), and the decrease of the phosphorylase *a* activity was determined (fig. 1).

Fig. 1 shows that dimer *a* lost its activity rapidly when incubated with phosphorylase phosphatase, while the tetramer form remained practically unchanged. In the mixture of dimer and tetramer *a*, only the dimer phosphorylase *a* was converted into inactive phosphorylase *b*, while the tetramer form remained unchanged even after longer incubation. Its amount can easily be determined graphically.

After the above control of the extraction and determination procedure we made a study of the ratio of dimer and tetramer phosphorylase *a* formed *in vivo* in the skeletal muscle of frogs after the adminstration of epinephrine.

Frogs were kept at 30, 18, 9 and 5° body temperatures before and during epinephrine treatment. After 5–15 min, when epinephrine effect reached a maximum at different body temperatures, homogenates were made from the muscles of narcotised animals in the above-mentioned manner (see Methods) and the ratio of dimer and tetramer *a* was determined.

The muscle's own phosphatase served as phosphorylase phosphatase, which always proved sufficient for the conversion of dimer *a* in 5–10 min. Our results are presented by figs. 2 and 3.

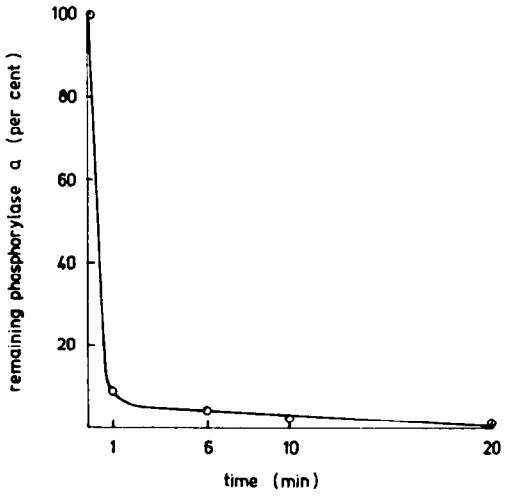

Fig. 2. Effect of endogenous phosphorylase phosphatase on phosphorylase *a* formed in frog muscle at 30° body temperature. Frogs were kept at 30° and treated by epinephrine given intraperitoneally. Homogenates were made from their muscle as described in Methods, with a buffer consisting of 0.04 M Tris, 0.01 M mercaptoethanol, 0.002 M EDTA and 0.5% glycogen, pH 6.8. The homogenate was centrifuged then incubated at 18°. Samples were removed and diluted with buffer containing 0.02 M NaF to such a degree that the activity of the remaining phosphorylase *a* could be determined. The amount of dimer and tetramer phosphorylase *a* is given in per cent of the total phosphorylase *a*, determined separately in another muscle sample frozen in liquid air, homogenization being carried out in a buffer containing 0.02 M NaF, also.

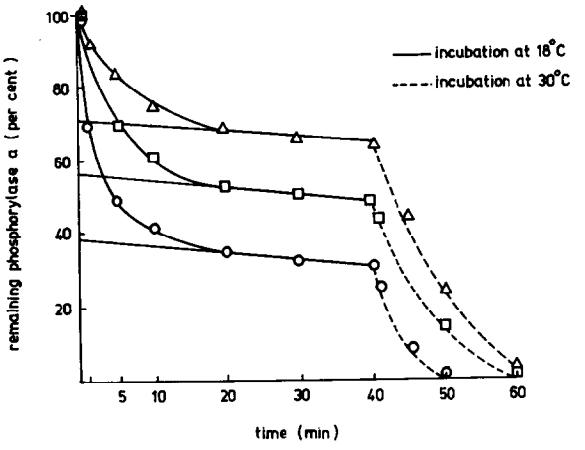

Fig. 3. Inactivation of phosphorylase *a* formed in frog muscle at body temperature 18°, 9° and 5°. Treatment of frogs kept at 18°, 9° and 5° by epinephrine and investigation on muscle phosphorylase *a* as given in the text of fig. 2 and the Methods. From the 40-th min on the incubation was continued at 30°. Decrease of phosphorylase *a* content formed in frog muscle at body temperature 18° (o–o–o); at body temperature 9° (□–□–□) and at body temperature 5° (△–△–△).

Fig. 2 shows that phosphorylase *a* formed after epinephrine injection in the frog muscle maintained at body temperature 30° is inactivated by its phosphorylase phosphatase during the incubation of the homogenate at 18°. This indicates that the whole amount of phosphorylase *a* (36.5 U/g muscle) is in dimer state in the frog muscle at body temperature 30°.

Fig. 3 shows the phosphorylase *a* formed in frog muscle at body temperature 18°, 9°, 5° and its inactivation during the incubation of muscle homogenate.

In frog muscle kept at 18° only 61% of the phosphorylase *a* (32.4 U/g muscle) is inactivated by the endogenous phosphatase, i.e. 61% is in dimer form. 39% of phosphorylase *a* remained active after incubation which indicates that this part of phosphorylase *a* was in tetramer state.

In frog muscle at body temperature 9° 57%, and at body temperature 5° 71% of phosphorylase *a* remained unchanged during incubation.

The remaining tetramer *a* became convertible by the phosphatase only after being heated to 30° as a result of the dissociation of tetramer into dimer.

Table 1 shows the ratio of dimer and tetramer phosphorylase *a* formed in frog muscle of different body temperatures.

Table 1
Amount of dimer and tetramer phosphorylase *a* in frog muscles at different body temperatures.

	Body temperature			
	30°	18°	9°	5°
Tetramer*	0	39	57	71
Dimer*	100	61	43	29

* In per cent of total phosphorylase *a*.

4. Discussion

From the results presented in this paper phosphorylase *a* can get into tetramer state *in vivo* at low body temperature.

The interconversion of dimer and tetramer forms has a regulating role in the glycogen breakdown of poikilothermic animals, and it can result in the preservation of the glycogen reserve at low body temperature.

Phosphorylase phosphatase can convert only the dimer phosphorylase *a* into phosphorylase *b*, demonstrated by experiments with purified enzyme preparations and muscle homogenates. Phosphorylase phosphatase can not affect tetramer phosphorylase *a*, therefore the halving of its molecular weight can not be attributed to the effect of phosphatase. In contrast to numerous preliminary observations the function of phosphorylase phosphatase can be interpreted as follows:

$$\underset{\text{Tetramer}}{\text{Phosphorylase } a} \rightleftharpoons 2\ \underset{\text{Dimer}}{\text{Phosphorylase } a} \xrightarrow[\text{Phosphatase}]{\text{Phosphorylase}} 2\ \text{Phosphorylase } b + 4\ P_i$$

The conversion of tetramer *a* into dimer *a* is influenced by numerous effectors, but this dissociation always precedes the phosphorylase phosphatase effect.

References

[1] J.H. Wang and D.J. Graves, J. Biol. Chem. 238 (1963) 2386.
[2] J.H. Wang and D.J. Graves, Biochemistry 3 (1964) 1437.

[3] B. Metzger, E. Helmreich and L. Glaser, Proc. Natl. Acad. Sci. U.S. 57 (1967) 994.
[4] C.H. Huang and D.J. Graves, Biochemistry 9 (1970) 660.
[5] B. Metzger, L. Glaser and E. Helmreich, Biochemistry 7 (1968) 2021.
[6] G. Bot and I. Dósa, Acta Biochim. Biophys. Acad. Sci. Hung. 6 (1971) 73.
[7] G.T. Cori, B. Illingworth and P.J. Keller, in: Methods in Enzymology, Vol. 1, eds. S.P. Colowick and N.O. Kaplan (Academic Press, N.Y., 1955) p. 200.
[8] E.G. Krebs, D.S. Love, G.E. Bratvold, K.A. Trayser, W.L. Meyer and E.H. Fischer, Biochemistry 3 (1964) 1022.
[9] W. Stalmans, H. De Wulf, B. Lederer and H.G. Hers, European J. Biochem. 15 (1970) 9.
[10] J.H. Wang, M.L. Shonka and D.J. Graves, Biochemistry 4 (1965) 2296.
[11] J.H. Wang, M.L. Shonka and D.J. Graves, Biochem. Biophys. Res. Commun. 18 (1965) 131.