MULTIPLE FORMS OF PHOSPHORYLASE KINASE IN RED AND WHITE SKELETAL MUSCLE

H. P. JENNISSEN and L. M. G. HEILMEYER Jr.
Physiologisch-Chemisches Institut der Universität
8700 Würzburg, Koellikerstraße 2, Germany

Received 17 January 1974

1. Introduction

Phosphorylase kinase isolated from rabbit skeletal muscle has been reported to contain three types of subunits called A, B and C [1] or α, β and γ [2]. Molecular weights have been determined by SDS-gel electrophoresis to be 118 000 or 145 000 for A or α, 108 000 or 128 000 for B or β, and 41 000 for C or γ. The molar ratio for A : B : C has been determined to be 0.85 : 1.0 : 1.83 [1] whereas for $\alpha : \beta : \gamma$ a ratio of 0.83 : 1.0 : 1.06 was found [2]. Based on a molecular weight of 1.33×10^6 or 1.28×10^6 for the native enzyme determined by [1] or [2] respectively a subunit structure of $A_4B_4C_8$ or $\alpha_4\beta_4\gamma_4$ was proposed [1, 2]. Cohen showed, that the α chain is contaminated with a protein, called α' which was thought to be a proteolytic breakdown product of α. Application of affinity chromatography [3] to the purification of this enzyme from the physiological mixture of white and red muscle yields the same subunit pattern α, α', β and γ as that described for the homogeneous enzyme by Cohen [2]. The present publication shows that white and red muscle phosphorylase kinase differ in their subunit composition.

2. Methods and materials

The adsorption and elution of phosphorylase kinase on an affinity column is described in [3]. Further purification of the red muscle enzyme was achieved by affinity chromatography on a casein–Sepharose column is analogy to the purification of protein kinase described by [4]. For the isolation of the red muscle enzyme, muscles were excised according to [5]. The psoas was used as white muscle. Phosphorylase and phosphorylase kinase assays were performed on an Auto analyzer according to [7] and [8] respectively. Protein was determined according to the method of Lowry et al [9]. Zone electrophoresis in the presence of SDS was performed according to [10] or in the discontinuous system according to [11]. As standards for the calculation of the molecular weights the following peptides were used: Chymotrypsinogen (25 000), lactate dehydrogenase (36 000), catalase (60 000), bovine serum albumin (68 000), phosphorylase *b* (100 000) and myosin (200 000). All reagents used were analytical grade.

3. Results and discussion

Fig. 1A shows the separation of the subunits of purified, homogeneous rabbit skeletal muscle phosphorylase kinase by zone electrophoresis on 5% polyacrylamide SDS gels according to Weber and Osborn [10]. As can be seen, this preparation purified by affinity chromatography contains the main polypeptide chains α, β, γ and the minor component α' as described by Cohen [2].

Since the method of purifying the enzyme has circumvented the pH 6.1 acidification step of the classical procedure, which might have been expected to activate those cathepsins with pH-optima in the acid range, the continued presence of α' argues against it having resulted from proteolytic breakdown. There is however, a correlation between the weight ratio of

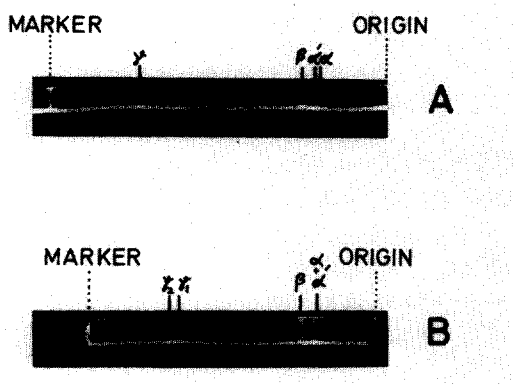

Fig. 1. SDS gel electrophoresis of phosphorylase kinase. 15 μg of the enzyme were applied to both gels. A represents results obtained with the zone electrophoresis system (5% gel) according to Weber and Osborn [10] and B those obtained with the discontinuous system (10% gel) according to Neville [11]. Protein was stained with Coomasie Brilliant Blue and destained by diffusion into Dowex AG 501 X 8.

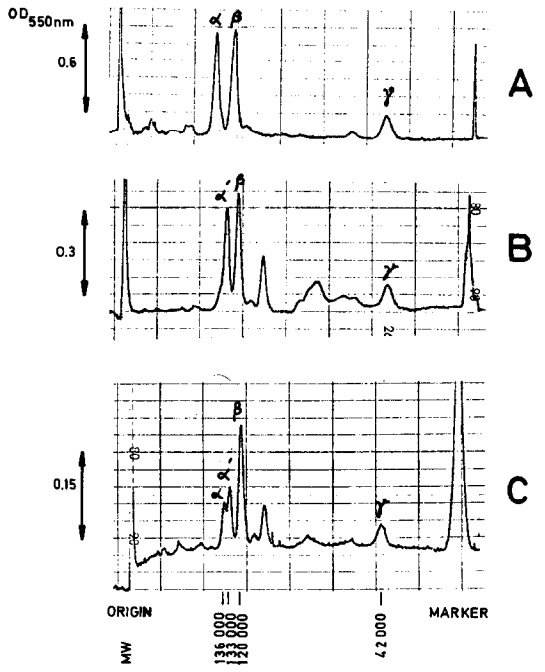

Fig. 2. Electrophoresis of white and red muscle phosphorylase kinase on 5% polyacrylamide SDS gels according to Weber and Osborn [10]. The optical density of the destained gels was recorded with a Gilford automatic densitometer. A contains 10 μg white muscle enzyme (enzyme *w*) and B 6 μg red muscle enzyme (enzyme *r*). C contains a mixture of 1 μg enzyme *w* and 3 μg enzyme *r*. For other conditions see legend to fig. 1.

α to α′ of approx. 10:1 and the weight ratio of white to red muscle in the mixture of the back, hind and fore leg muscles employed for the usual purification. Thus, phosphorylase kinase was purified approx. 150-fold from white and approx. 340-fold from red muscle (Methods). The densitometric tracings of the polypeptide chains of both enzymes following separation in the Weber-Osborn system are shown in fig. 2, A and B. It can be clearly seen, that the white muscle enzyme (named enzyme *w*) contains only the subunit α with an apparent molecular weight of 136 000, whereas the component α′ with an apparent molecular weight of 133 000 is derived from red muscle (enzyme *r*). When the red and white muscle enzymes are mixed, the subunits α and α′ are well separated, whereas the components β and γ give single bands of an intensity corresponding to the sum of the chains from both enzymes (see fig. 2C). The red muscle enzyme is still contaminated by a small amount of polypeptides of molecular weights 100 000, 64 000 and 52 000. From the area under the peaks, a molar ratio of 0.78 : 1.0 : 1.18 for α : β : γ of white muscle and 0.86 : 1.0 : 0.84 for α′ : β : γ of red muscle is calculated. As demonstrated in fig. 3, both enzymes, *w* and *r*, are eluted from a column containing Sepharose 4 B with identical elution volumes. Since the enzyme isolated from the mixture of both types of muscles shows the same behaviour, the molecular weights of both enzymes, w and r, must be the same (approx. 1.3×10^6). From the molar ratios of the polypeptide chains (see above), the subunit structure $\alpha_4\beta_4\gamma_4$ can be assigned to the white muscle enzyme and $\alpha'_4\beta_4\gamma_4$ to the red muscle enzyme. The pH 6.8/8.2 activity ratio was determined to be 0.02 for the enzyme *w* and 0.05 for the enzyme *r*. This again argues against proteolytic degradation which is known to increase this ratio by a factor of 10 to 20 [12]. Unless specific proteolysis of the α component to form α′ has occurred in the intact red muscle cell without affecting the pH 6.8/8.2 activity ratio, one may define the white and red muscle phosphorylase kinase as isoenzymes.

A further resolution of the subunits of phosphorylase kinase is obtained in the discontinuous SDS gel system described by Neville [11]. (See fig. 1B). The γ component which seems to be homogeneous in the

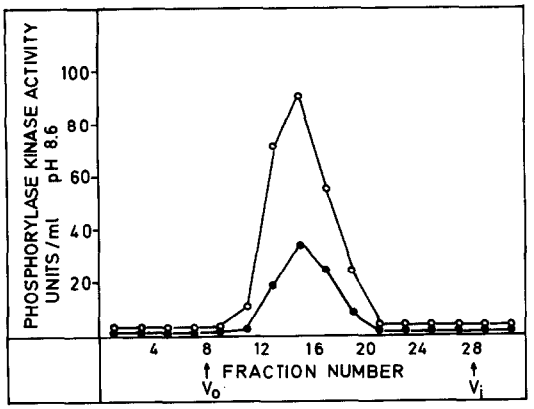

Fig. 3. Comparison of the elution of red and white muscle phosphorylase kinase from a Sepharose 4 B column. A column of 0.9 cm diameter and 55 cm bed height was used. The Sepharose was equilibrated and eluted at a rate of 6 ml/hr with a buffer containing 50 mM β-glycerophosphate, 2 mM EDTA and 1 mM dithiothreitol pH 7.0. Samples of 0.5 ml containing 160 units of purified enzyme w (●——●) and 360 units of purified enzyme *r* (○——○) were subsequently applied and eluted. Fractions of 1.5 ml were collected.

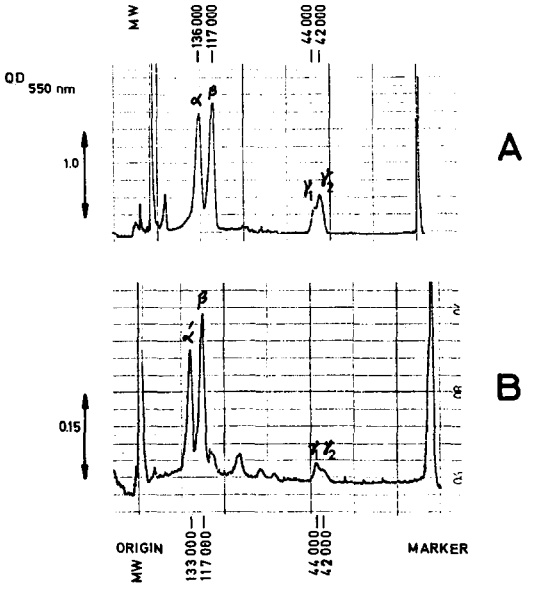

Fig. 4. Discontinuous electrophoresis according to Neville [11] in 10% polyacrylamide SDS gels. To gel A the enzyme (12 μg) prepared from the usual mixture of muscles (mainly white muscles) was applied. Gel B contains 3 μg enzyme *r*. For other conditions see legend to figs. 1 and 2.

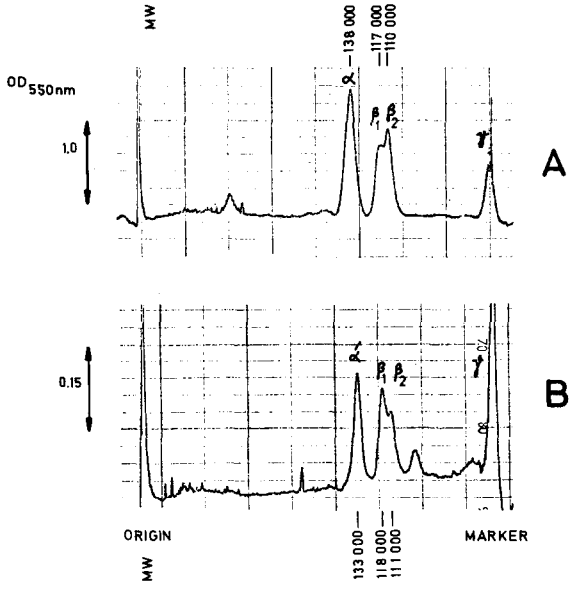

Fig. 5. Discontinuous electrophoresis according to Neville [11] in 5% polyacrylamide SDS gels. The conditions are the same as described in the legend to fig. 3.

zone electrophoresis can be resolved into two bands (γ_1 and γ_2) (see fig. 1B and fig. 4). Both enzymes, the one from red muscle and that prepared from the usual mixture of red and white muscles, contain both types of subunits, γ_1 and γ_2 (fig. 4). If the polyacrylamide concentration is lowered to 5%, the β subunit can also be resolved into two bands, β_1 and β_2 (fig. 5). Two proteins used as controls, beef liver glutamate dehydrogenase and chymotrypsinogen A, known to have a unique sequence, gave single bands in this discontinuous system. The observed multiciplicity of the β and γ subunits of phosphorylase kinase could result from a variety of factors e.g. true differences in the primary structure of the polypeptide chains, the β and γ chains being glycopeptides [13], or the existence of different complexes of the same polypeptide chain with sodium dodecyl sulphate [14, 15].

Recently it has been shown, that myosin isoenzymes exist in red and white muscle [5]. The light chains of red muscle are higher in molecular weight than their counterparts in white muscle [16]. These differences as well as different types of lactate dehydrogenase isoenzymes [17] and as shown here, phosphorylase

kinase isoenzymes probably reflect the different metabolism and function of these two types of muscle.

Acknowledgements

We wish to thank Mrs Christa Pöhlmann for excellent technical help and Mr K. H. Klinger for the excision and preparation of muscles. We are grateful to Dr F. A. Sreter for his advice in the preparation of red muscles. For stimulating discussions we wish to thank Dr E. Helmreich. This work was supported by grants from the Deutsche Forschungsgemeinschaft, The Fonds der Chemie and from the Federal Ministry for Science and Technology.

References

[1] Hayakawa, T., Perkins, J. P., Walsh, D. A. and Krebs, E. G. (1973) Biochemistry 12, 567.
[2] Cohen, P. (1973) Eur. J. Biochem. 34, 1.
[3] Jennissen, H. P., Hörl, W. H. and Heilmeyer Jr., L. M. G. (1973) Hoppe-Seyler's Z. Physiol. Chem. 354, 236.
[4] Corbin, J. D., Brostrom, C. O., King, C. A. and Krebs, E. G. (1972) J. Biol. Chem. 247, 7790.
[5] Sreter, F. A., Sarkar, S. and Gergely, J. (1972) Nature New Biol. 239, 124.
[6] Fischer, E. H. and Krebs, E. G. (1958) J. Biol. Chem. 231, 65.
[7] Haschke, R. H. and Heilmeyer Jr., L. M. G. (1972) Anal. Biochem. 47, 451.
[8] Jennissen, H. P. and Heilmeyer Jr., L. M. G. (1974) Anal. Biochem., in press.
[9] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265.
[10] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406.
[11] Nevelle Jr., D. M. (1971) J. Biol. Chem. 246, 6328.
[12] Krebs, E. G., Love, D. S., Bratvold, G. E., Trayser, K. A., Meyer, W. L. and Fischer, E. H. (1964) Biochemistry 3, 1022.
[13] Glossmann, H. and Neville Jr., D. M. (1971) J. Biol. Chem. 246, 6339.
[14] Reynolds, J. A., Tanford, C. (1970) Proc. Natl. Acad. Sci. U.S. 66, 1002.
[15] Katz, S., Shaw, M. E., Chillag, S., Miller, J. E. (1972) J. Biol. Chem. 247, 5228.
[16] Sarkar, S., Sreter, F. A., Gergely, J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 946.
[17] Pette, D. and Staudte, H. W. (1973) in: Limiting Factors of Physical Performance, Keul, J. (ed.), p. 23, G. Thieme Verlag, Stuttgart.