OCCURRENCE OF HIGH AND LOW M_r FORMS OF GLYCOGEN PHOSPHORYLASE IN EXTRACTS OF HUMAN BRAIN

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Received 29 July 1981

1. Introduction

Although glycogen phosphorylase (EC 2.4.1.1) from rabbit muscle has been subjected to the most detailed analysis of structure, mechanism and function, other glycogen phosphorylases have received less attention. The human brain enzyme in particular, has been neglected in many respects. It is known that this tissue contains three isoenzymes which correspond to a predominant brain type, muscle type, and a hybrid of the two [1,2]. Here, we have investigated the characteristics of human brain phosphorylase by means of gel-exclusion chromatography and sucrose density gradient sedimentation. Both phosphorylase *a* and *b* appear to exist in a high M_r form of ~400 000 and a low M_r form whose apparent size differs with the method of determination. Although the nature of the high M_r form is not known, we believe that it reflects a previously unknown property of human brain phosphorylase. The low M_r form is probably an equilibrium mixture of dimer and monomer which gives different apparent M_r-values depending upon the position of equilibrium.

2. Materials and methods

Samples of human cerebral cortex were kindly supplied by Dr Lucien Coté and by the Neuro-Tissue Facility (Department of Pathology, Columbia University NY). Tissues were obtained at autopsy and stored frozen for up to several years, except for one sample which was used without prior freezing. Tissue extracts were prepared by homogenizing at 5°C with 2.5 vol. 15 mM 2-mercaptoethanol and 0.1 mM phenylmethanesulfonyl fluoride using a motor-driven teflon pestle, and centrifuging at 20 000 × *g* for 20 min.

For gel-exclusion chromatography the extract was treated as described in the text and a 1.5 ml aliquot containing 300 mg sucrose was layered on a 1.4 cm × 94 cm column of Sephacryl S-300 (Pharmacia, Uppsala) equilibrated with a buffer containing 0.20 M Tris–HCl (pH 7.2), 0.20 M NaCl, and 5 mM 2-mercaptoethanol. The column was eluted with the same buffer at 5°C with a flow rate of 12 ml/h and fractions of 1.5 ml were collected.

Sucrose density gradient sedimentation was done as in [3] with a SW 50.1 rotor, using 4.6 ml gradients of 5–20% sucrose containing 5 mM 2-mercaptoethanol and indicated concentrations of Tris–HCl (pH 7.2) and NaCl. The gradients were collected in 0.20 ml fractions.

Phosphorylase was assayed as in [4] in a final volume of 50 μl; 2 mM AMP was included for the assay of phosphorylase *b*. Orthophosphate was determined as in [5].

3. Results

Chromatography of an untreated extract of human brain on a column of Sephacryl S-300 resulted in the appearance of two sharp, symmetrical peaks of phosphorylase *b* activity (fig.1). The high M_r component (Ph I) had ~10–15% phosphorylase *a* activity, while the low M_r component (Ph II) was free of phosphorylase *a*. Calibration was done with standard proteins; Ph I had an apparent M_r of 380 000, while that of Ph II was 71 000. The same pattern was observed in samples from 4 different individuals, including one in which the tissue was extracted without prior freezing. Extraction of tissue with buffer containing 5 mM mercaptoethanol, 0.2 M Tris–HCl (pH 7.2) and 0.2 M NaCl gave the same result.

Published by Elsevier/North-Holland Biomedical Press
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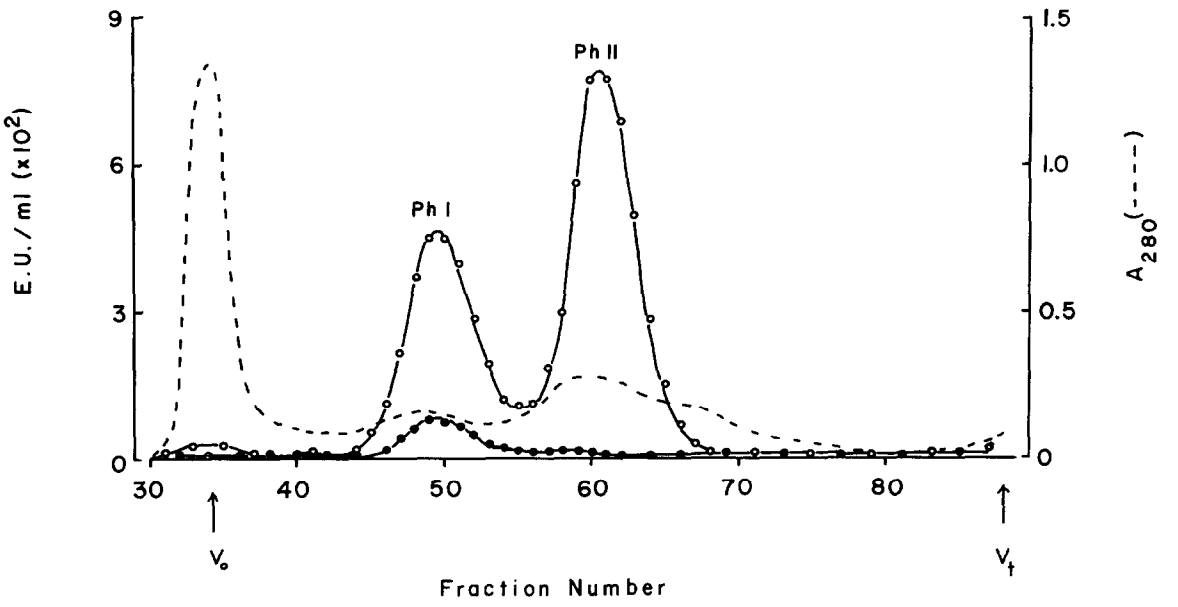

Fig.1. Chromatogram of untreated human brain extract on Sephacryl S-300. Assay was with 20 μl aliquots for 1 h at 37°C. Total phosphorylase activity (○); phosphorylase *a* (●); A_{280}-values (– – –).

A similar extract prepared from frozen rabbit brain gave a single peak of phosphorylase activity with M_r 87 000 in this system. Extracts of rabbit muscle and liver and purified rabbit muscle phosphorylase *b* all gave single peaks of phosphorylase activity with closely similar M_r-values (130 000). Human muscle extracts showed a single component with M_r of ~ 110 000. In view of the results obtained in sedimentation experiments and the known M_r of rabbit muscle phosphorylase *b* (dimer, 194 664) [6], it seems likely that some of the M_r-values obtained in this way are low, possibly because of interactions of the phosphorylases with the gel matrix of Sephacryl. The very low apparent M_r-values for Ph II and the rabbit brain enzyme point to significant dissociation to monomer at these low concentrations (~1 EU/ml extract).

Sucrose density gradient sedimentation experiments were carried out with the untreated extract, using 0.20 M Tris–HCl and 0.20 M NaCl in the gradient. The two forms of phosphorylase from human brain extract are well separated (fig.2) and show approximate $s_{20,w}$ values of 14.3 S and 7.0 S. These correspond to M_r of ~ 400 000 and 136 000, respectively. Pure rabbit muscle phosphorylase *b* gave an $s_{20,w}$ of 8.3 in this system; reported values of $s_{20,w}$ for rabbit muscle phosphorylase *a* (tetramer) and *b* (dimer) are 13.5 S and 8.42 S, respectively [7]. Phosphorylase *a* activity coincided with the high molecular weight form (Ph I) and, in this case, represented 40% of the total activity of the peak. When the experiment was done using only 50 mM Tris–HCl in the gradient, the high M_r form was absent.

Incubation of the crude human brain extract at 37°C for 10 min resulted in loss of the Ph I component in both gel-exclusion and sedimentation experiments. However, the Ph II peak in the sucrose gradient increased in activity, suggesting conversion of Ph I to Ph II. The increase in Ph II activity was greater than the loss of Ph I activity, so the conclusion must be considered tentative. When fractions from gel-exclusion chromatography containing Ph I were combined, precipitated with $(NH_4)_2SO_4$ (50% of saturation), and rechromatographed, Ph I appeared in its characteristic position but there was no trace of Ph II. This suggests that the loss of Ph I is a result of enzymatic action during incubation, rather than simple dissociation to Ph II. Loss of Ph I was not prevented by inclusion of 50 mM NaF or 10 mM EDTA during extraction and incubation.

Incubation of crude brain extract at 4°C for 1 h with porcine pancreatic α-amylase and Diazyme (mix-

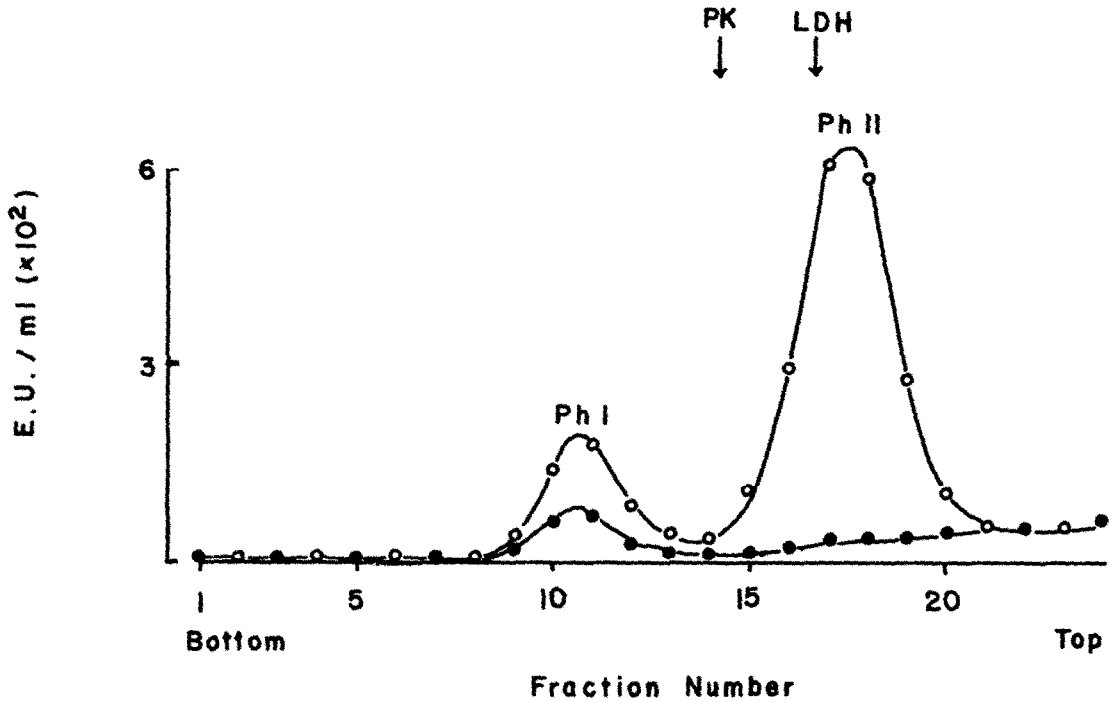

Fig.2. Sucrose density gradient sedimentation of untreated human brain extract. Sample was 0.20 ml and run was 17 h at 27 000 rev./min. Assay was with 10 μl aliquots for 1 h at 30°C. Total phosphorylase activity (○); phosphorylase *a* (●). Gradient contains 0.20 M Tris–HCl (pH 7.2) and 0.20 M NaCl. Arrows indicate peak positions of internal standards: PK, rabbit muscle pyruvate kinase, M_r 237 000, $s_{20,w}$ 10.4 S; LDH, rabbit muscle lactate dehydrogenase, M_r 144 000, $s_{20,w}$ 7.6 S.

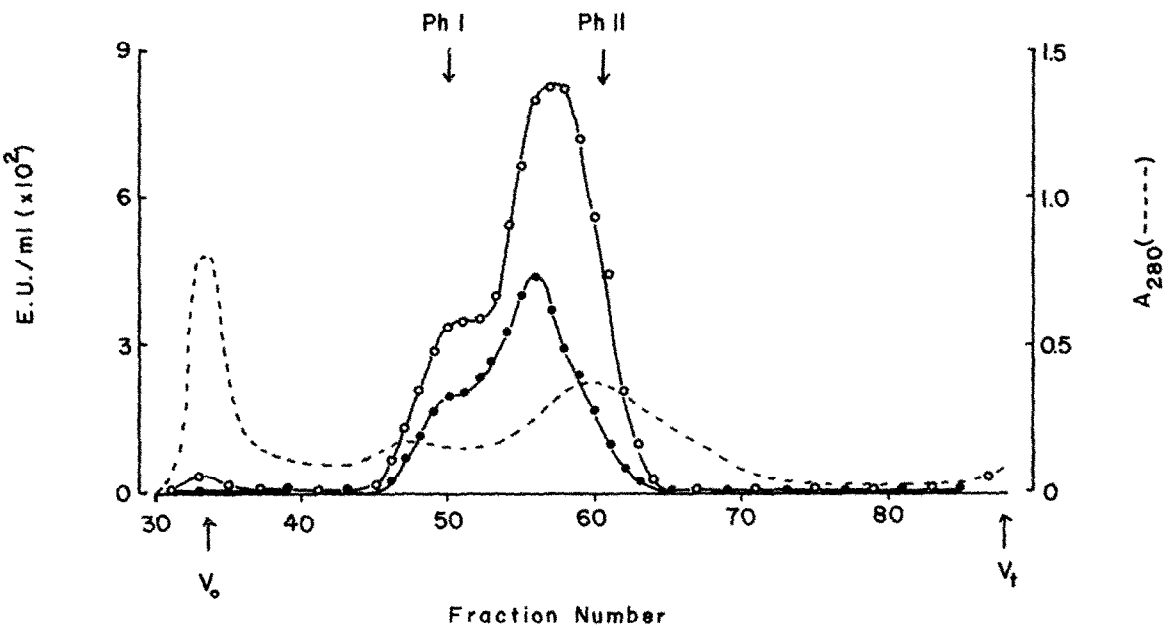

Fig.3. Sephacryl S-300 chromatogram of human brain extract treated with phosphorylase kinase. Assay was with 20 μl aliquots for 1 h at 37°C. Total phosphorylase activity (○); phosphorylase *a* (●).

ture of glucosidases [8]) caused no change in the chromatographic pattern. This argues against Ph I being a complex containing an α-glucan, since both enzymes are capable of digesting α-glucans.

An attempt was made to activate the crude human brain phosphorylase with endogenous kinases by incubating the crude extract for 10 min at 37°C in the presence of 10 mM 2-glycerophosphate (pH 6.8), 5 mM $Mg(Ac)_2$, 10 μM cyclic AMP, 0.6 mM ATP, 7.5 mM theophylline and 20 mM NaF. Chromatography revealed the loss of Ph I and the appearance of a small peak of phosphorylase *a* activity with M_r 115 000; Ph II was unaffected. A similar experiment carried out with added rabbit muscle phosphorylase kinase (Sigma) (3 EU/ml), 10 mM 2-glycerophosphate (pH 6.8), 5 mM $Mg(Ac)_2$, 3 mM ATP and 20 mM NaF is shown in fig.3. Phosphorylase *a* activity appeared as a double peak: one peak was in the position of Ph I while the second showed $M_r \sim 120\,000$. There was no separate peak corresponding to Ph II.

4. Discussion

Human brain phosphorylase can exist in the high M_r species designated Ph I in either the *a* or *b* forms. Although the apparent M_r of Ph I suggests that it is a tetramer, it remains possible that Ph I is a complex between phosphorylase dimer (or monomer) and other proteins. Conversion of Ph I to Ph II during incubation of the crude brain extract is probably catalyzed by an endogenous enzyme acting upon the phosphorylase polypeptide or other components of the complex. There is no evidence for the presence of a high M_r form of phosphorylase *b* in rabbit brain or any other tissue.

Brain phosphorylase *a* can also exist in a form the M_r of which suggests it to be dimeric. Phosphorylase *b*, besides occurring in the Ph I form, exists in a low M_r form (Ph II) which is probably mostly monomer. Phosphorylase *b* monomer has been demonstrated in extract of rat brain [9]; isolated monomer reverted to a mixture of dimer and monomer [9]. The monomer of rabbit muscle phosphorylase is inactive under conditions where it cannot reassociate to dimer [10]. In the case of human brain phosphorylase *b* we cannot conclude from our results whether the monomer is active as such, is in equilibrium with active dimer, or forms an active dimer in the presence of the substrates.

The distribution of phosphorylase isoenzymes [1,2] between Ph I and Ph II is under investigation.

Acknowledgements

We wish to thank the Muscular Dystrophy Association for their generous support of this work through a Research Grant and a Postdoctorate Fellowship to C. P. Z.

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