Molecular and Cellular Biochemistry **110**: 113–121, 1992.
© 1992 *Kluwer Academic Publishers. Printed in the Netherlands.*

Phosphorylation-induced conformational changes in the phosphorylase *ab* hybrid as revealed by resolution of pyridoxal 5'-phosphate with imidazole citrate and cysteine

György Vereb, Edina Pallagi and Pál Gergely
Department of Medical Chemistry, University School of Medicine, Debrecen, Hungary

Received 3 May 1991; accepted 15 October 1991

Summary

The accessibility of pyridoxal 5'-phosphates of the phosphorylase *ab* hybrid to resolution by imidazole citrate and cysteine was studied and compared with that of the *b* and *a* forms. Promotion of resolution of phosphorylated forms by raising the temperature or in the presence of glycogen indicates that the resistance of phosphorylase *a* and *ab* to resolution at 0° C is due rather to their tetrameric state than their phosphorylation-related active conformation. The pattern of resolution of the *ab* hybrid was similar to that of the *a* and differed from that of the *b* forms in that it occurred at 30° C and 37° C but not at 0° C, moreover, it did not show first-order kinetics. On the other hand, inhibition of resolution by ligands binding to the nucleotide site of phosphorylase reflected an intermediate sensitivity of the *ab* form between that of the *b* and *a* forms. We conclude that partial phosphorylation of phosphorylase *b* elicits conformational change(s) in both subunits which influence the monomer-monomer interactions and resolution of pyridoxal 5'-phosphates. Resistance of *ab* hybrid to monomerizing agents as imidazole citrate, comparable to that of other forms, argues for its stability, ruling out its reshuffling into mixtures of phosphorylase *b* and *a*. (Mol Cell Biochem **110:** 113–121, 1992)

Key words: phosphorylase *ab,* protein phosphorylation, PLP resolution, sedimentation, skeletal muscle (rabbit)

Introduction

Enzyme catalyzed interconversions of glycogen phosphorylase (EC 2.4.1.1) occur via a partially phosphorylated dimeric molecule termed phosphorylase *ab* or *ab* hybrid consisting of phosphorylated and non-phosphorylated subunits [for review see 1–3]. The formation of phosphorylase *ab* was demonstrated both *in vitro* [4, 5] and *in vivo* [6–10].

Previous enzyme kinetic experiments suggested identical conformation of active sites in phosphorylated and non-phosphorylated subunits [11] which can be attributed to homotropic and heterotropic interactions between substrate and nucleotide binding sites. To study conformational changes of subunits elicited by partial phosphorylation under conditions different from those of activity measurements (e.g., in the absence of substrates), the resolution of the coenzyme pyridoxal 5'-

Address for offprints: P. Gergely, Department of Medical Chemistry, University School of Medicine, Debrecen, Bem tér 18/B, H-4026, Hungary

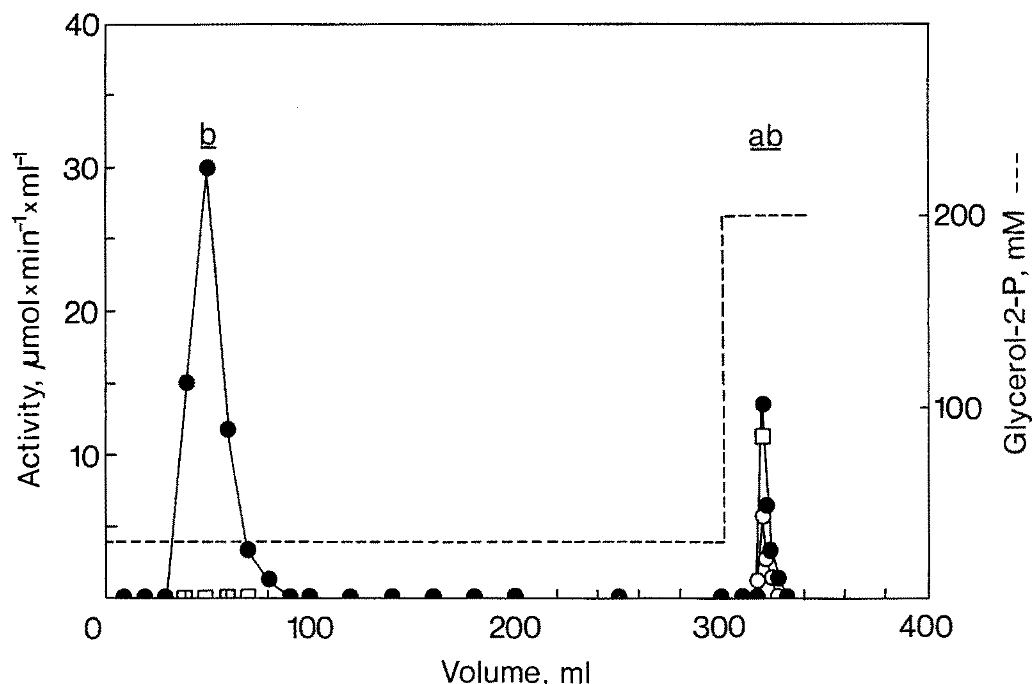

Fig. 1. Isolation of phosphorylase *ab* from the phosphorylase kinase catalyzed reaction mixture by DEAE-Sephacel column chromatography. 1.2 ml reaction mixture containing 10 mg/ml phosphorylase *b* 2.6 μg/ml phosphorylase kinase, 1 mM ATP, 10 mM Mg-acetate, 40 mM Tris·HCl, 25 mM disodium glycerol-2-P and 25 mM 2-mercaptoethanol, pH 8.2 was incubated at 30° C until the phosphorylase activity ratio: – AMP/+ AMP remained constantly 0.5. This corresponded to about 20% conversion of phosphorylase *b* when the reaction was stopped by 2.4 ml 10 mM EDTA, 1 mM EGTA, 5 mM disodium glycerol-2-P, 25 mM 2-mercaptoethanol and the pH was adjusted to 6.8. The mixture was loaded on a DEAE-Sephacel (Pharmacia) column (2.5 × 6.5 cm) equilibrated with 30 mM disodium glycerol-2-P, 1 mM EDTA and 10 mM 2-mercaptoethanol, pH 6.8. After elution of phosphorylase *b* with 10 volumes of equilibrating buffer phosphorylase *ab* was eluted with 200 mM disodium glycerol-2-P, 1 mM EDTA and 10 mM 2-mercaptoethanol. Phosphorylase activity was measured without AMP (○), with 1 mM AMP (●) and with 1 mM AMP + 5 mM caffeine (□). Dashed line shows the concentration of disodium glycerol-2-P.

phosphate (PLP) by imidazole citrate and cysteine seemed especially suitable. This process was reported to depend on phosphorylation state resulting in inactive apoenzyme monomers of phosphorylase *b*, but not of phosphorylase *a* [12, 13]. In addition, resolution of phosphorylase *b* was sensitive to a variety of allosteric effectors which show different affinity for the *b*, *ab* and *a* forms [14]. Using this method, Harris *et al.* [15] could demonstrate the involvement of the N-terminal tail of phosphorylase *b* in the monomer-monomer interaction.

In the present work we report that at 30° C or 37° C not only the *b*, but also the *ab* and *a* forms of phosphorylase are inactivated with imidazole citrate and cysteine at a comparable rate. We demonstrate an identical reactivity of the subunits of the *ab* hybrid and also its characteristic allosteric sensitivity by comparing the resolution of the *b*, *ab* and *a* forms performed in the presence of allosteric effectors.

Materials and methods

Materials

Reagents were of analytical grade. Phosphorylase *b* was prepared from rabbit skeletal muscle according to Fischer and Krebs [16]. Phosphorylase *ab* and *a* were obtained by phosphorylation of phosphorylase *b* with phosphorylase kinase and treated with Norit A as described previously [11]. The preparation of the *ab* form makes use of the finding that at the beginning of the *in vitro* phosphorylase *b* into *a* conversion catalyzed by phosphorylase kinase apparently only *ab* form is produced. This holds until about 20% of phosphorylase *b* becomes phosphorylated. After having stopped the reaction in this phase the *b* and *ab* forms were separated on DEAE-Sephacel column. A typical separation procedure, shown in Fig. 1, demonstrates that phosphorylase *b* can be eluted by 30 mM disodium glycerol-2-P, while the *ab* form was eluted with a solution containing 200 mM disodium glycerol-2-P. The hybrid nature of

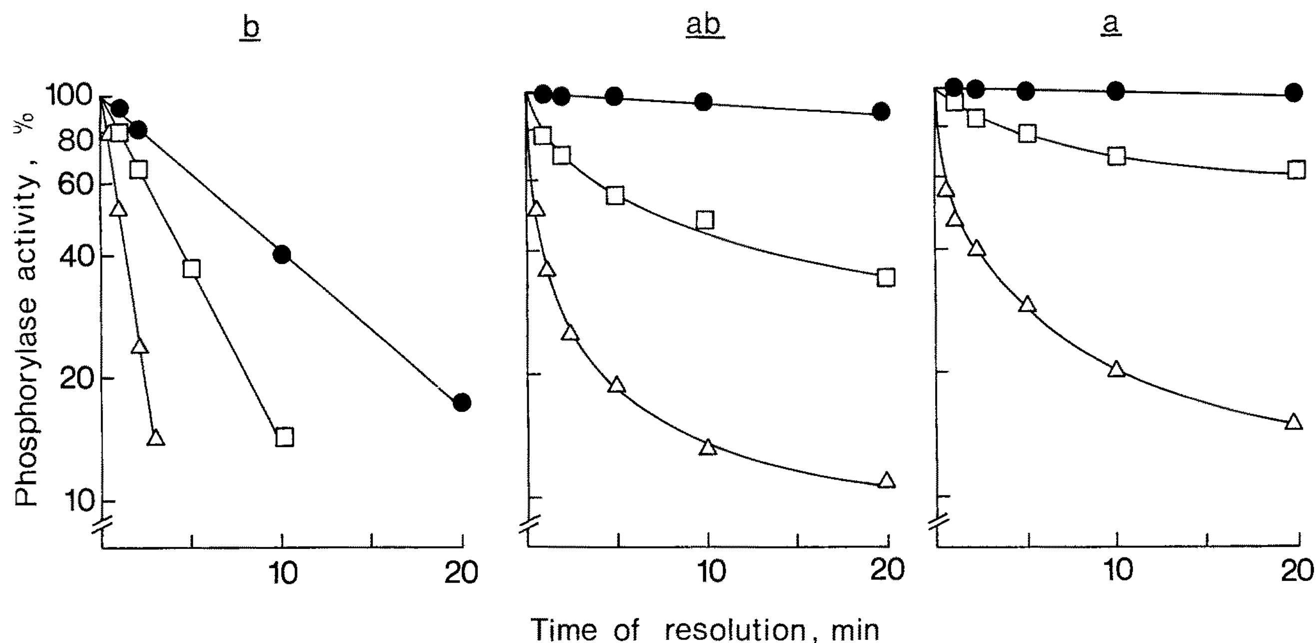

Fig. 2. Effect of various temperatures on the resolution of phosphorylase *b, ab* and *a*. 1 mg/ml phosphorylase was incubated with imidazole citrate and cysteine at 0° C (●), 30° C (□) or 37° C (△) as described in methods. The probes at 0° C contained 0.4 M imidazole, 0.1 M citrate, 0.1 M cysteine and 5 mM dithiothreitol, pH 6.5. In probes at 30° C and 37° C resolution was performed in the presence of 0.2 M imidazole, 0.05 M citrate, 0.05 M cysteine and 2.5 mM dithiothreitol, pH 6.5 after 30 min preincubation with 5 mM disodium glycerol-2-P and 5 mM 2-mercaptoethanol, pH 6.8. The residual phosphorylase activities were determined from diluted samples (1 : 40) taken at times indicated, and were plotted on a logarithmic scale as percent of appropriate controls.

this enzyme is indicated by its allosteric sensitivity [5, 11], in that its activity measured in the absence of AMP is doubled in the presence of 1 mM AMP plus 5 mM caffeine, reaching nearly the activity measured in the presence of 1 mM AMP. On the other hand, 5 mM caffeine inhibits totally the activity of phosphorylase *b* measured in the presence of 1 mM AMP and the − AMP/+ AMP activity ratio reaches a value of 0.95 for phosphorylase *a*.

Phosphorylase activity

Phosphorylase was assayed in the presence of 16 mM glucose-1-P, 1% glycogen, 25 mM disodium glycerol-2-P, 12 mM 2-mercaptoethanol, 0.2–0.5 mg/ml bovine serum albumin at pH 6.8 and 30° C. The reaction mixture contained also 1 mM AMP for phosphorylase *b* or 5 mM caffeine + 1 mM AMP when assaying phosphorylase *ab* and *a* [5]. The amount of inorganic phosphate, released from glucose-1-P, was determined according to Taussky and Shorr [17].

Resolution of PLP from phosphorylase

The resolution of PLP by imidazole citrate and cysteine was performed on the basis of previous observations of Fischer *et al.* [12], Shaltiel *et al.* [13] and Harris *et al.* [15]. In standard experiments 2 mg/ml phosphorylase *b*, *ab* or *a* was preincubated with 1% glycogen at 30° C for 30 min in 5 mM disodium glycerol-2-P, 10 mM 2-mercaptoethanol, pH 6.8 and resolution was started by addition of an equal volume of freshly prepared reagent containing 0.4 M imidazole, 0.1 M citric acid, 0.1 M L-cysteine, 5 mM dithiothreitol adjusted to pH 6.5 with HCl. It was prewarmed to 30° C or 37° C immediately before addition. Probes at 0° C contained two-fold amount of imidazole, citrate and cysteine. From the reaction mixtures (total volume 160 μl) samples taken at various time intervals were diluted (1 : 40) with 50 mM Tris·HCl, 50 mM 2-mercaptoethanol, 1 mg/ml bovine serum albumin, pH 6.8 for phosphorylase activity measurement. Inactivation reactions were run in duplicate and the residual activities (expressed in percent of unreacted sample) were plotted on a logarithmic scale vs. time. The first-order rate constant (k) was calculated from the half-life time of process using the equation of $t_{0.5} = 0.693/k$.

Sedimentation experiments

2 mg/ml phosphorylase *ab* or *a* was incubated at 30° C

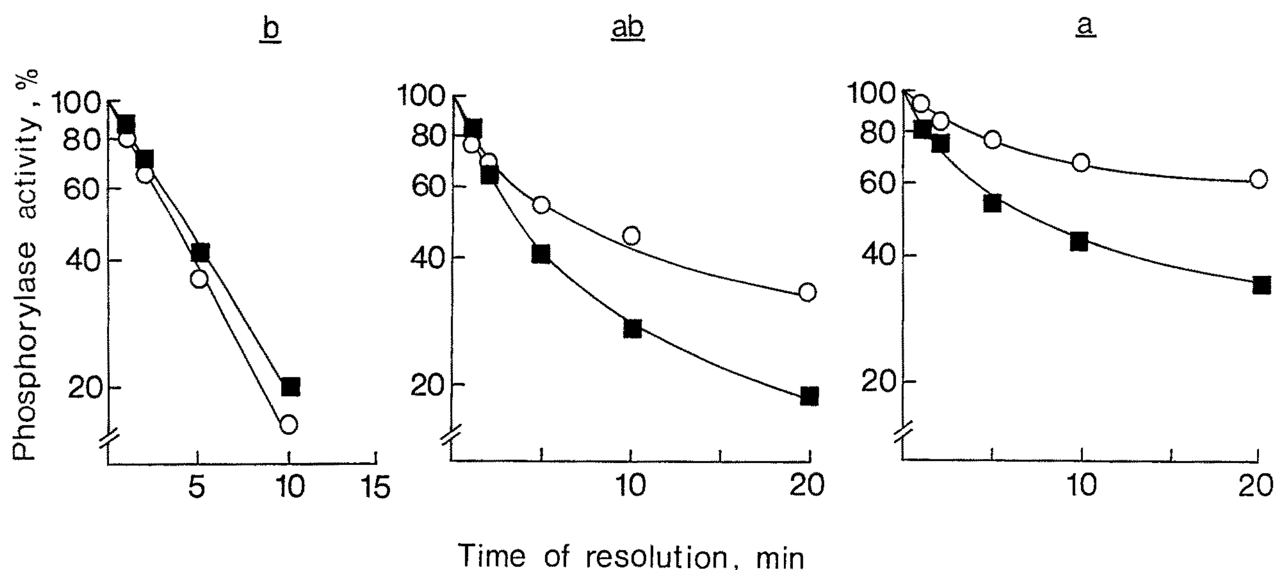

Fig. 3. Effect of glycogen on the resolution of phosphorylase *b, ab* and *a*. 2 mg/ml phosphorylase was incubated (30° C, 30 min) without (○) or with 1% glycogen (■) in 5 mM disodium glycerol-2-P and 5 mM 2-mercapto-ethanol, pH 6.8. Resolution was performed at 30° C in the presence of 1 mg/ml phosphorylase, 0.5% glycogen, 0.2 M imidazole, 0.05 M citrate, 0.05 M cysteine, 2.5 mM dithiothreitol, pH 6.5 as described in the legend of Fig. 2. For details see methods.

for 30 min in 5 mM disodium glycerol-2-P, 5 mM 2-mercaptoethanol, pH 6.8. After a second incubation (1 mg/ml phosphorylase *ab* or *a,* 0.2 M imidazole, 0.05 M citrate, pH 6.5, 30° C, 30 min) this mixture was centrifuged at 30° C. Sedimentation coefficients were determined in a MOM 3180 analytical ultracentrifuge (Budapest, Hungary) at a speed of 50,000 rpm. Movement of boundaries was calculated from microcomparator measurements of the Schlieren diagrams.

Results

The role of quaternary structure in the inactivation of phosphorylase b, ab *and* a *by imidazole citrate and cysteine*

Figure 2 shows the inactivation patterns of phosphorylase *b, ab* and *a* at various temperatures in the presence of imidazole citrate and cysteine. At 0° C the inactivation of phosphorylase *b* shows an apparent first-order kinetics with a rate constant of 0.13×10^{-2} s^{-1}. The reactivities of *ab* and *a* forms are negligible at 0° C yielding rate constants of 9.6×10^{-5} s^{-1} and 7×10^{-5} s^{-1}, respectively. However, a rise in the temperature to 30° C or 37° C accelerates not only the inactivation of phosphorylase *b* ($k_{30°C} = 0.36 \times 10^{-2}$ s^{-1} and $k_{37°C} = 1.15 \times 10^{-2}$ s^{-1}), but also a temperature dependent inactivation of the *ab* and *a* forms can be observed. Their initial reaction rates are commensurable with that of phosphorylase *b* but they do not show first-order kinetics. The propensity of the *ab* form to inactivate at 30° C seems to be intermediate between those of the *b* and *a* forms and it fails to show two different reactivities which would characterize the dephospho and phosphorylated subunits. The temperature-dependence and kinetics of its inactivation resembles rather those of the *a* form. Our results can be interpreted that tetramer formation characteristic of the phosphorylated forms at low temperature [4] counteracts the resolution but a shift to dimers occurring at 30° C or 37° C [18, 19] allows their monomerization and resolution. In accord with this, phosphorylase *b* is predominantly dimer even at 0° C under conditions used for inactivation (without AMP and below the concentrations of 4–5 mg/ml) [13, 20]. Since a considerable amount of the phosphorylated forms can be tetrameric even at higher temperatures used, depending on the conditions [21], the inactivation was studied in the presence of well-known dimerizing agents as glycogen and glucose. Figure 3 shows that preincubation with 1% glycogen at 30° C essentially does not affect the inactivation of phosphorylase *b* but increases the rate of inactivation of the *ab* and *a* forms.

The effect of glucose and glycogen on the inactivation of phosphorylase *a* is compared in Fig. 4. Though preincubation with 50 mM glucose at 30° C induces a com-

plete dimerization of phosphorylase *a* [19], after such a preincubation 25 mM glucose seems to inhibit slightly the resolution as compared to the control reaction. In addition, the resolution of phosphorylase *a* preincubated with glycogen is also inhibited by 25 mM glucose resulting in nearly the same inactivation rates in the presence or absence of 0.5% glycogen.

Ligand effects on the resolution of various phosphorylase forms

Considering the different affinities of the *b, ab* and *a* forms for various ligands, we tested their effect on the resolution in the concentrations they proved efficient in activity measurements [11]. Experiments were always performed in the presence of 0.5% glycogen to obtain optimal conditions for inactivation.

Figure 5 shows the effect of various AMP concentrations on the inactivation process. Phosphorylase *b* can be protected only slightly by 1 mM AMP, while even 10^{-4} M AMP exerts partial protection for the *ab* form and fully suppresses the inactivation of phosphorylase *a*. For quantitative evaluation the protective effects were expressed in relative efficiency values. In case of phosphorylase *b* and *ab* these values were calculated as the ratios of reaction rate constants in the absence and presence of ligands (Table 1). Rate constants for the *ab* hybrid were determined from the apparently linear part of inactivation. For phosphorylase *a*, the ratios of inactivation percents without and with ligands were given after 5 min inactivation, since comparable rate constants could not be determined. Table 1 also shows the sensitivities of various forms to IMP, glucose-6-P, caffeine and glucose, based on similar measurements presented in Fig. 5.

Among the ligands tested, AMP distinguished the *b, ab* and *a* forms at a concentration of 10^{-4} M, giving relative efficiencies of 1.05, 2.78 and 11.50, respectively. Similar differences were found under the protective effect of 5×10^{-4} M IMP. Glucose-6-P proved to be effective only at 5×10^{-3} M, exerting the highest protective effect on phosphorylase *b*. Phosphorylase *a* was insensitive to glucose-6-P, just as in activity measurements. Finally, caffeine and glucose hardly distinguished between the *b* and *ab* forms, and exerted the smallest protection for the *a* form.

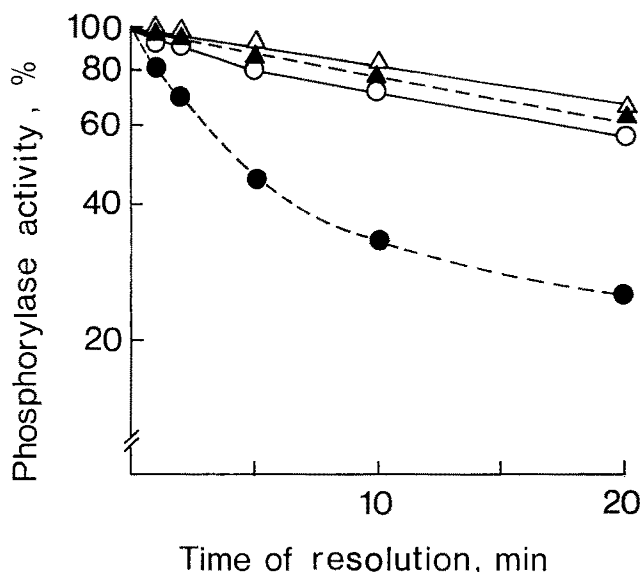

Fig. 4. Effect of glucose on the resolution of phosphorylase *a* in the absence and presence of glycogen. 2 mg/ml phosphorylase *a* was preincubated (30° C, 30 min) in 5 mM disodium glycerol-2-P and 5 mM 2-mercaptoethanol without additions (○), with 50 mM glucose (△), with 1% glycogen (●), or with 1% glycogen + 50 mM glucose (▲). Preincubated enzymes were diluted with an equal volume of reagent to obtain 0.2 M imidazole, 0.05 M citrate, 0.05 M cysteine, 2.5 mM dithiothreitol, pH 6.5. Resolution was measured at 30° C as described in methods.

Dissociation of phosphorylase ab *and* a *in the presence of imidazole citrate as determined by sedimentation*

Relationship between resolution and monomerization was previously established only for phosphorylase *b* [13, 14]. To ascertain that imidazole citrate dissociates the *ab* and *a* forms into monomers like the *b* form, sedimentation experiments were carried out in the absence of cysteine (Fig. 6). On the basis of $S_{20,w}$ values phosphorylase *a* and *ab* partly yielded monomers in the presence of 0.2 M imidazole, 0.05 M citrate and 0.05 M 2-mercaptoethanol, supporting the occurrence and putative role of monomerization during resolution of *ab* and *a* forms, too. Interestingly, both *ab* and *a* forms showed a second peak of 13-14S, corresponding to their tetramer forms. Such a phenomenon was previously observed by Helmreich *et al.* [19] and was attributed to the high ionic strength. It was found to occur even in the presence of 0.15–0.2 M glucose [22]. However, preincubation with caffeine counteracted this tetramer formation and prevented monomerization, too (not documented).

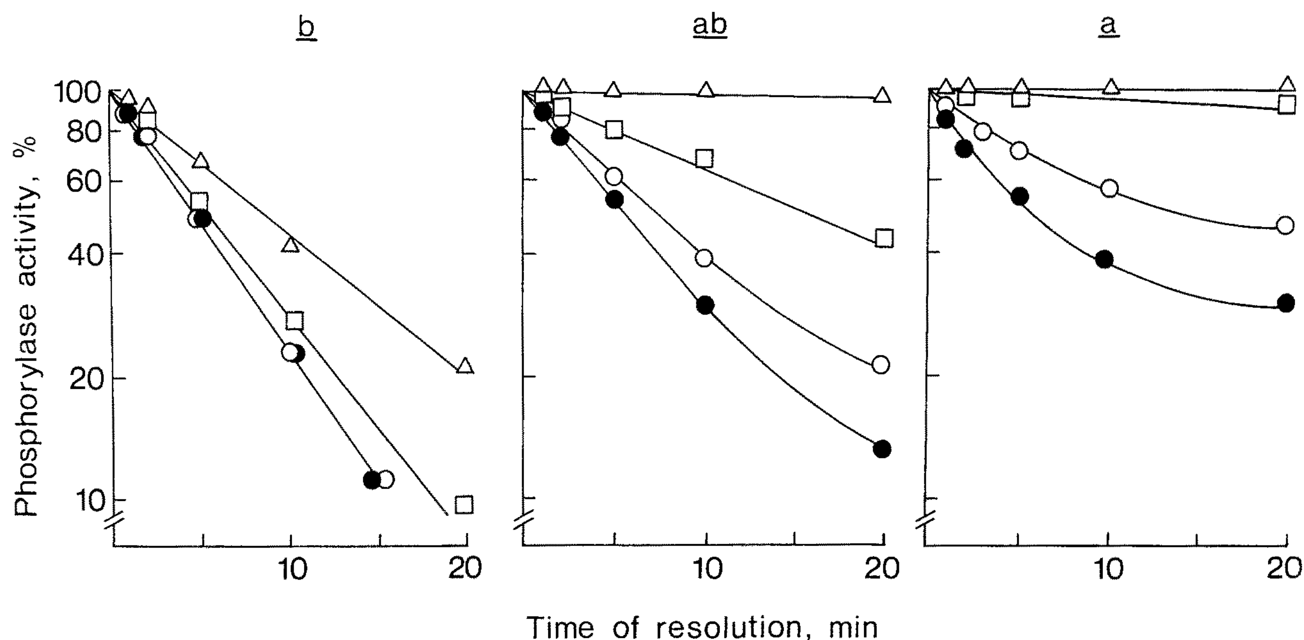

Fig. 5. Effect of AMP on the resolution of various phosphorylase forms. After preincubation with 1% glycogen (30° C, 30 min) the phosphorylase solutions (2 mg/ml) were diluted with an equal volume of reagent yielding 0.2 M imidazole, 0.05 M citrate, 0.05 M cysteine, 2.5 mM dithiothreitol, pH 6.5 and the following AMP concentrations: none (●), 10^{-5} M (○), 10^{-4} M (□) or 10^{-3} M (△). Resolution was measured at 30° C as described in methods.

Determination of the rate-limiting step in the resolution of phosphorylase ab

For phosphorylase *b* a rate-limiting monomerization and a fast reaction with cysteine was previously reported [14, 15]. To determine the rate-limiting step in the resolution of phosphorylase *ab,* the composition of phosphorylase obtained after about 50% inactivation of phosphorylase *ab* was analyzed. In case of a rate-limiting reaction of PLP with cysteine reassociated *b* and *a* dimers are expected in addition to the unreacted *ab* dimers in te diluted reaction mixture. On the other hand, a very slow reaction of phosphorylated monomers with cysteine would result in reassociated phosphorylase *a* besides the unreacted *ab* form.

Analysis of the partially reacted phosphorylase *ab* by DEAE-Sephacel chromatography (Fig. 7) shows essentially no phosphorylase *b* activity eluted by 30 mM disodium glycerol-2-P. Moreover, the enzyme species eluted by 200 mM disodium glycerol-2-P retained the allosteric sensitivity characteristic of the *ab* form shown in Fig. 1. These findings argue for the rate-limiting role of conformational changes preceding the removal of PLP by cysteine and exclude the reassociation into *b* or *a* dimers and a two-phase reaction of phosphorylated

Table 1. Ligand effects on the resolution of various phosphorylase forms. Relative efficiencies were calculated from the rate of inactivation by 0.2 M imidazole, 0.05 M citrate and 0.05 M cysteine at 30° C and pH 6.5 in the absence and presence of various ligands as k_0/k_{ligand} for phosphorylase *b* and *ab* or v_0/v_{ligand} for phosphorylase *a*. Rate constants for inactivation of phosphorylase *b* and *ab* and the rate of inactivation of phosphorylase *a* were determined as demonstrated in Fig. 4. Inactivation was measured as described in methods. Data represent the average of 3 independent determinations, S.D. values were within ± 10% of average.

Ligand		Phosphorylase *b*	*ab*	*a*
		relative efficiency k_0/k_{ligand}		v_0/v_{ligand}
AMP	10^{-5} M	1.0	1.30	1.53
	10^{-4} M	1.05	2.78	11.50
	10^{-3} M	1.79	31.0	∞
IMP	1×10^{-4} M	1.0	1.20	1.14
	5×10^{-4} M	1.10	1.77	3.70
Glucose-6-P	1×10^{-3} M	1.10	1.30	1.0
	5×10^{-3} M	5.74	3.85	1.0
Caffeine	1×10^{-4} M	1.95	2.04	1.23
	5×10^{-4} M	9.20	12.30	2.35
Glucose	25×10^{-3} M	6.5	7.4	3.0

and non-phosphorylated monomers during the resolution.

Discussion

In characterizing the ligand- and phosphorylation-induced conformational changes of skeletal muscle phosphorylase X-ray crystallography became especially powerful [23–27]. However, such investigations have not been performed on partially phosphorylated phosphorylase till now. Though cross-linking with bifunctional reagents proved useful in characterizing the movements along monomer and dimer contact surfaces elicited by partial phosphorylation [21], the participation of the two different subunits of the *ab* hybrid in conformational changes remained unclear. Besides the theoretical interest [3], asymmetry in the primary structure of *ab* hybrid might be a factor leading to destabilization of the dimeric enzyme as in the case of the phosphorylase hybrid consisting of heart and skeletal muscle type subunits [28].

To test the conformation of subunits in phosphorylase *ab* without multiple interactions, which might compensate for the structural asymmetry in activity measurements [11], we studied the accessibility of PLP to resolution. To our surprise, the negligible reactivity characterizing the phosphorylated forms at 0° C increased at 30° C or 37° C (Fig. 2), suggesting that the resistance of these forms in the absence of ligands at 0° C is due to a conformation related rather to their tetrameric state than to their phosphorylation.

Comparing the resolution of the *ab* hybrid to that of the *b* and *a* forms it resembled that of the *a* and differed from that of the *b*, occurring in a temperature-dependent way and showing non-first-order kinetics. The lack of two different reactivities characteristic of the *b* and *a* forms (Fig. 2) suggests an identical conformation of dephospho and phosphorylated subunits in dimeric *ab* hybrid. The inactivation in the presence of ligands reflects a uniform behaviour of the *ab* hybrid, too (Fig. 5).

Role of monomer-monomer interactions and the conformation of PLP region in the resolution

Since monomerization of phosphorylase seems to be a prerequisite for removal of PLP by aldehyde reagents the intersubunit interactions can influence the accessibility of PLP. Because of participation of Ser-14-phosphate(s) in the intersubunit interactions [23] different resistance of the *b*, *ab* and *a* forms can be expected in the absence of ligands. Indeed, the reactivity of the *ab* form proved to be intermediate between that of the *b* and *a* forms (Figs. 2, 3). The involvement of nucleotide site in the monomer-monomer contact [24, 25] can explain the protective effect of AMP, IMP and glucose-6-P, reflecting the sensitivities of the *b*, *ab* and *a* forms to those ligands (Table 1), which has been also found characteristic in activity measurements [11, 29]. The protective effect of glucose and caffeine can be related to their effect on the conformation of the PLP region. The proximity of the catalytic site to the PLP [30] makes possible direct interaction of glycosyl substrates with PLP [31], however, the binding of glucose does not affect the monomer contacts [21]. Similarities in the protective effect of glucose and caffeine (Table 1), moreover their common functional properties, de-

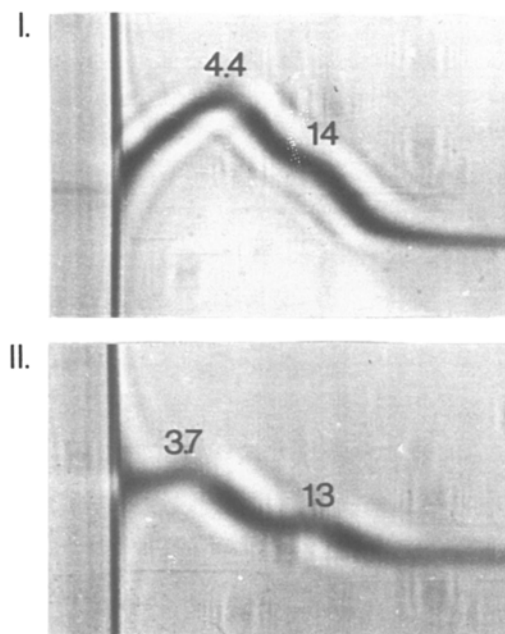

Fig. 6. Sedimentation pattern of phosphorylase *ab* and *a* in the presence of imidazole citrate. 2 mg/ml phosphorylase was incubated with 5 mM disodium glycerol-2-P and 5 mM 2-mercaptoethanol (pH 6.8) at 30° C, 30 min. After a second incubation (1 mg/ml phosphorylase *ab* or *a* in the presence of 0.2 M imidazole, 0.05 M citrate and 0.05 M 2-mercaptoethanol, 30° C, 30 min) samples were centrifuged (30° C, 50,000 rpm) as described in section methods. Pictures were taken 17 min after reaching maximum speed. Panel I: phosphorylase *ab*; panel II: phosphorylase *a*. The $s_{20,w}$ values are indicated on the picture.

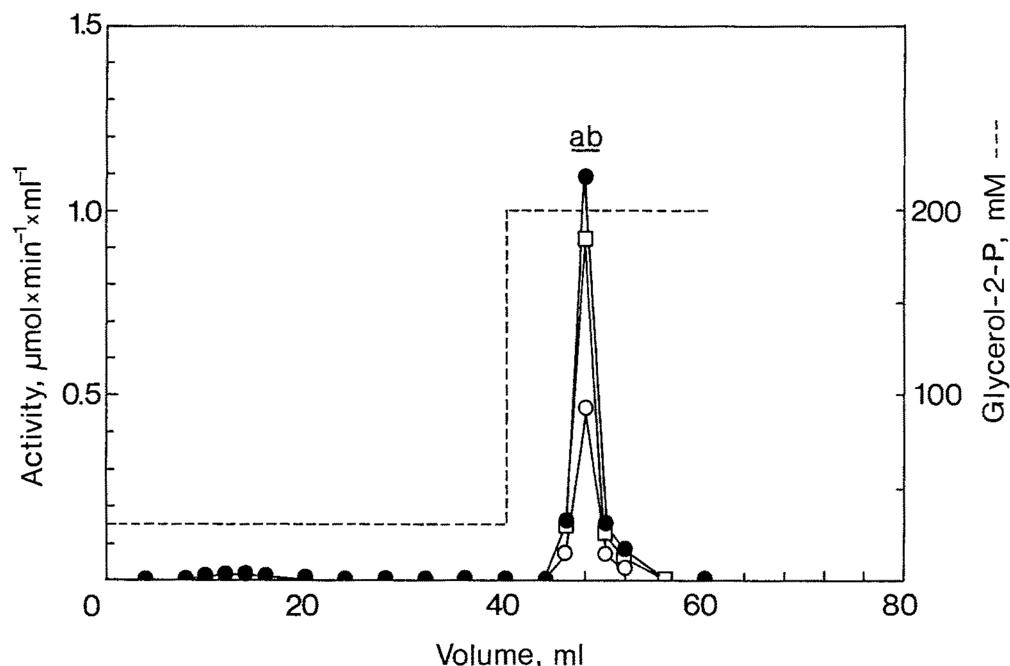

Fig. 7. Rechromatography of unreacted phosphorylase *ab* after imidazole citrate and cysteine treatment. 0.2 mg phosphorylase *ab* was treated with imidazole citrate and cysteine in 0.2 ml volume as described in methods until about 50% inactivation occurred. Then the reaction mixture was diluted with 20 volumes of 15 mM disodium glycerol-2-P, 0.5 mM EDTA, 25 mM 2-mercaptoethanol, 0.1 mg/ml bovine serum albumin pH 6.8 and loaded on a 4 ml DEAE-Sephacel column. For elution of phosphorylase *b* the column was washed with 10 volumes of 30 mM disodium glycerol-2-P, 1 mM EDTA and 10 mM 2-mercaptoethanol, pH 6.8, then the concentration of disodium glycerol-2-P was raised to 200 mM. 2 ml fractions were collected. Phosphorylase activity was measured without AMP (○), with 1 mM AMP (●) and with 1 mM AMP + 5 mM caffeine (□).

pending on the presence of PLP or its analogs [32, 33] suggest their similar conformational effects on the PLP region. The role of conformation of PLP region in the resolution reaction is further supported by the stereospecific effect of L-cysteine in removing the PLP [34]. Finally, there is a discrepancy between the easy formation of monomers from the phosphorylated forms in the presence of imidazole citrate and the poor reactivity with cysteine as compared to that of the *b* form (unpublished observations). Since during the resolution of PLP a ternary complex of apoenzyme-PLP-L-cysteine has been proposed [14, 34], it is reasonable to take into account also the conformation of PLP region during formation of such a complex. The absence of *a* dimers in the reaction mixture containing partially resolved phosphorylase *ab* (Fig. 7) suggests the participation of PLP regions in dimeric enzymes. In accordance with this a reversible binding of L-cysteine to dimeric phosphorylase was also demonstrated [34].

These considerations clearly indicate that the reaction of PLP by cysteine under our experimental conditions is more complex than a passive removal of exposed PLP's from preformed monomers. The participation of contact areas and PLP regions in the resolution may be variable in the presence of ligands. Considering the resolution of various phosphorylase forms in the unliganded and liganded state we can affirm a comparable stability of the *ab* hybrid to that of the other forms since a preferential monomerization tendency accelerating its resolution has not been observed.

Acknowledgements

This work was supported by OTKA grant (1501/1991-1994) from the Hungarian Academy of Sciences and ETT grant (T-10/T-460/1990) from the Ministry of Health. The authors wish to thank Dr. M. Szabolcs and Mr. B. Csaba (Central Research Laboratory, University School of Medicine, Debrecen) for their help in evaluating the sedimentation experiments. The skilful technical assistance of Mrs. Ágota Kelemen, the graphic works of Mrs. Ella Kovács and the secretarial assistance of Ms. Éva Kovács are gratefully appreciated.

References

1. Dombrádi V: Structural aspects of the catalytic and regulatory function of glycogen phosphorylase. Int J Biochem 13: 125–139, 1981
2. Madsen NB: Glycogen phosphorylase. In: PD Boyer, EG Krebs (eds.) The Enzymes, Academic Press, New York, 1986, Vol. 17, pp. 365–394
3. Walsh DA, Newsholme P, Cawley KC, Van Patten SM, Angelos KL: Motifs of protein phosphorylation and mechanisms of reversible covalent regulation. Physiol Rev 71: 285–304, 1991
4. Hurd SS, Teller D, Fischer EH: Probable formation of partially phosphorylated intermediates in interconversion of phosphorylase *a* and *b*. Biochem Biophys Res Commun 24: 79–84, 1966
5. Bot G, Kovács E, Gergely P: Partial phosphorylation of muscle phosphorylase I. Formation of a hybrid phosphorylase *in vitro*. Biochim Biophys Acta 370: 70–77, 1974
6. Gergely P, Bot G, Kovács E: Partial phosphorylation of muscle phosphorylase II. Formation of a hybrid phosphorylase *in vivo*. Biochim Biophys Acta 370: 78–84, 1974
7. Gergely P, Castle AG, Crawford N: Purification and characterization of phosphorylase *a* from human platelets. Int J Biochem 10: 807–814, 1979
8. Vereb G, Szücs K, Szabó J, Belanova M, Bot G: Formation of partially phosphorylated phosphorylase in isoproterenol stimulated rat hearts. Mol Cell Biochem 69: 139–146, 1986
9. Van Marrewijk WJA, Van den Broek ATHM, Beenakkers AMTH: Glycogen phosphorylase in the fat body of *Locusta migratoria*. Insect Biochem 15: 341–347, 1985
10. Schmidt H, Wegener G: Glycogen phosphorylase in fish muscle: demonstration of three interconvertible forms. Am J Physiol 258: C344–C351, 1990
11. Vereb G, Fodor A, Bot G: Kinetic characterization of rabbit skeletal muscle phosphorylase *ab* hybrid. Biochim Biophys Acta 915: 19–27, 1987
12. Fischer EH, Krebs EG: Relationship of structure to function of muscle phosphorylase. Fed Proc 25: 1511–1520, 1966
13. Shaltiel S, Hedrick JL, Fischer EH: On the role of pyridoxal 5'-phosphate in phosphorylase II. Resolution of rabbit muscle phosphorylase *b*. Biochemistry 5: 2108–2116, 1966
14. Hedrick JL, Shaltiel S, Fischer EH: Conformational changes and the mechanism of resolution of glycogen phosphorylase *b*. Biochemistry 8: 2422–2429, 1969
15. Harris WR, Miller JF, Graves DJ: Purification and characterization of a glycogen phosphorylase analog missing the amino-terminal segment. Arch Biochem Biophys 250: 446–455, 1986
16. Fischer EH, Krebs EG: The isolation and crystallization of rabbit skeletal muscle phosphorylase *b*. J Biol Chem 231: 65–71, 1958
17. Taussky HH, Shorr E: A microcolorimetric method for the determination of inorganic phosphorus. J Biol Chem 202: 675–695, 1953
18. Wang JH, Graves DJ: The relationship of the dissociation to the catalytic activity of glycogen phosphorylase *a*. Biochemistry 3: 1437–1445, 1964
19. Helmreich E, Michaelides MC, Cori CF: Effects of substrates and a substrate analog on the binding of 5'-adenylic acid to muscle phosphorylase *a*. Biochemistry 6: 3695–3710, 1967
20. Kastenschmidt LL, Kastenschmidt J, Helmreich E: The effect of temperature on the allosteric transitions of rabbit skeletal muscle phosphorylase *b*. Biochemistry 7: 4543–4556, 1968
21. Dombrádi V, Hajdú J, Bot G, Friedrich P: Structural changes in glycogen phosphorylase as revealed by cross-linking with bifunctional diimidates: phospho-dephospho hybrid and phosphorylase *a*. Biochemistry 19: 2295–2299, 1980
22. Wang JH, Shonka ML, Graves DJ: The effect of glucose on the sedimentation and catalytic activity of glycogen phosphorylase. Biochem Biophys Res Commun 18: 131–135, 1965
23. Sprang S, Fletterick RJ: Subunit interactions and the allosteric response in phosphorylase. Biophys J 32: 175–192, 1980
24. Sprang SR, Acharya KR, Goldsmith EJ, Stuart DJ, Varvill K, Fletterick RJ, Madsen NB, Johnson LN: Structural changes in glycogen phosphorylase induced by phosphorylation. Nature 336: 215–221, 1988
25. Barford D, Johnson LN: The allosteric transition of glycogen phosphorylase. Nature 340: 609–616, 1989
26. Goldsmith EJ, Sprang SR, Hamlin R, Xuong NH, Fletterick RJ: Domain separation in the activation of glycogen phosphorylase *a*. Science 245: 528–532, 1989
27. Johnson LN, Barford D: The structural basis of the allosteric response and comparison with other allosteric proteins. J Biol Chem 265: 2409–2412, 1990
28. Guenard D, Morange M, Buc H: Comparative study of the effect of 5'AMP and its analogs on rabbit glycogen phosphorylase *b* isoenzymes. Eur J Biochem 76: 447–452, 1977
29. Morgan HE, Parmeggiani A: Regulation of glycogenolysis in muscle. J Biol Chem 239: 2440–2445, 1964
30. Kasvinsky PJ, Madsen NB, Sygusch J, Fletterick RJ: The regulation of glycogen phosphorylase *a* by nucleotide derivatives. Kinetic and X-ray crystallographic studies. J Biol Chem 253: 3343–3351, 1978
31. McLaughlin PJ, Stuart DJ, Klein HW, Oikinomakos NG, Johnson LN: Substrate-cofactor interactions for glycogen phosphorylase *b*: a binding study in the crystal with heptenitol and heptulose 2-phosphate. Biochemistry 23: 5862–5873, 1984
32. Kasvinsky PJ, Shechosky S, Fletterick RJ: Synergistic regulation of phosphorylase *a* by glucose and caffeine. J Biol Chem 253: 9102–9106, 1978
33. Yan SCB, Uhing RJ, Parrish RF, Metzler DE, Graves DJ: A role for pyridoxal phosphate in the control of dephosphorylation of phosphorylase *a*. J Biol Chem 254: 8263–8269, 1979
34. Shaltiel S, Hedrick JL, Fischer EH: Stereospecific requirements for carbonyl reagents in the resolution and reconstitution of phosphorylase *b*. Biochemistry 8: 2429–2436, 1969