ISSN 0006-2979, Biochemistry (Moscow), 2009, Vol. 74, No. 5, pp. 562-568. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © N. A. Chebotareva, A. V. Meremyanin, V. F. Makeeva, T. B. Eronina, B. I. Kurganov, 2009, published in Biokhimiya, 2009, Vol. 74, No. 5, pp. 691-698.

Glycogen Phosphorylase *b* and Phosphorylase Kinase Binding to Glycogen under Molecular Crowding Conditions. Inhibitory Effect of FAD

N. A. Chebotareva*, A. V. Meremyanin, V. F. Makeeva, T. B. Eronina, and B. I. Kurganov

Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky pr. 33, 119071 Moscow, Russia; fax: (495) 954-2732; E-mail: chebotareva@inbi.ras.ru

Received April 22, 2008
Revision received June 19, 2008

Abstract—Dynamic light scattering was used to study the interaction of phosphorylase kinase (PhK) and glycogen phosphorylase *b* (Ph*b*) from rabbit skeletal muscle with glycogen under molecular crowding conditions arising from the presence of 1 M trimethylamine N-oxide and at physiological ionic strength. The mean value of hydrodynamic radius of the initial glycogen particles was 52 nm. Crowding stimulated Ph*b* and PhK combined binding on glycogen particles. Two-stage character of PhK binding to glycogen particles containing adsorbed Ph*b* was found in the presence of the crowding agent. At the initial stage, limited size particles with hydrodynamic radius of ~220 nm are formed, whereas the second stage is accompanied by linear growth of hydrodynamic radius. Flavin adenine dinucleotide (FAD) selectively inhibited PhK binding at the second stage. The data indicate that in the first stage Ph*b* is involved in PhK binding by glycogen particles containing adsorbed Ph*b*, whereas PhK binding in the second stage does not involve Ph*b*.

DOI: 10.1134/S0006297909050125

Key words: phosphorylase kinase, glycogen phosphorylase *b*, crowding, trimethylamine N-oxide, glycogen, FAD, dynamic light scattering

According to current concepts, from 5 to 40% of the total volume of a living cell are occupied by macromolecules, which creates conditions of so-called crowding [1]. Molecular crowding noticeably influences interactions between macromolecules as well as the rate and equilibrium of intracellular biochemical processes and stimulates formation of compact structures [1-7]. Among such processes are protein–protein interactions, conformational transitions of biomacromolecules, protein folding and aggregation, as well as formation of multienzyme complexes and supramolecular structures. It is supposed that crowding plays a noticeable role in maintenance of constant cell volume [8].

Glycogen phosphorylase (EC 2.4.1.1) is one of the key enzymes of glycogenolysis and catalyzes glycogen phosphorolysis. The enzyme molecule is a dimer of two identical 97.4 kDa subunits [9]. In a resting muscle, the enzyme exists as an unphosphorylated form *b* (Ph*b*), inactive in the absence of AMP. The Ph*b* activity is regulated both by phosphorylation and by means of the enzyme conformational changes upon interaction with allosteric effectors.

Phosphorylase kinase (PhK) (EC 2.7.1.38) plays a key role in nervous and hormonal regulation of glycogenolysis in skeletal muscle. PhK catalyzes the Ca^{2+}- and cAMP-dependent Ph*b* phosphorylation and activation. The 1320 kDa enzyme molecule has complex molecular organization and is a hexadecamer [10-12] of four different subunits characterized by stoichiometry $(\alpha\beta\gamma\delta)_4$, the γ-subunit being catalytic while the α-, β-, and δ-subunits are regulatory [13-16]. Ca^{2+} and Mg^{2+} ions stimulate PhK activity by induction of tertiary and quaternary structural changes in its molecule [14, 16-18] and by stimulation of association of the hexadecameric enzyme [19-22].

We showed earlier with the example of Ph*b* and PhK that crowding influences such important biochemical reactions of key enzymes of glycogenolysis as association, conformational transitions, interaction with allosteric ligands, denaturation, aggregation, and formation of the enzyme supramolecular structures [7, 19-21, 23-27].

Abbreviations: FAD, flavin adenine dinucleotide; Ph*b*, glycogen phosphorylase *b*; PhK, phosphorylase kinase; TMAO, trimethylamine N-oxide.

* To whom correspondence should be addressed.

Since many glycogenolysis enzymes are associated in the muscle cell cytoplasm with glycogen granules [28, 29], investigation of interaction of these enzymes with glycogen under crowding conditions imitating the intracellular state should be important for understanding the mechanism of protein–glycogen particle formation and the mechanism of functioning of glycogenolysis enzymes. Sedimentation analysis and turbidimetry have shown that the interaction of PhK with glycogen is enhanced under conditions of crowding created by trimethylamine N-oxide (TMAO) and betaine in high concentrations [20, 30]. However the effect of crowding on the interaction of PhK with Ph*b* was unexpected. As shown by sedimentation analysis, crowding prevents formation of the PhK–Ph*b* complex [29, 30]. At first sight, the fact that crowding conditions prevent PhK complex formation with its substrate Ph*b* seems physiologically inexpedient. As far as it was shown earlier that Ph*b* in the absence of crowding enhances the interaction of PhK with glycogen [31, 32], it was supposed [30] that crowding conditions will be favorable for interactions in the ternary system PhK–Ph*b*–glycogen. This hypothesis is confirmed in this work. As shown by dynamic light scattering, crowding significantly enhances interactions in the ternary system containing PhK, Ph*b*, and glycogen. Crowding was imposed by 1 M TMAO. If it is considered that the TMAO specific partial volume is 0.96 cm^3/g [33], then for 1 M TMAO solution the volume occupied by its molecules should be ~10% of the total volume. The effect of FAD (flavin adenine dinucleotide) on the ternary complex formation was also studied under conditions of molecular crowding and at ionic strength close to physiological.

MATERIALS AND METHODS

Hepes, FAD, and TMAO of Sigma (USA), pig liver glycogen (Olaine, Latvia), and NaCl (Reakhim, Russia) were used.

Phosphorylase kinase was isolated from rabbit skeletal muscle according to Cohen [13] using at the final stage of purification ion-exchange chromatography on a column of DEAE-Toyopearl 650M (Tosoh, Japan) [34]. The enzyme purity was checked by electrophoresis according to Laemmli [35]. The phosphorylase kinase preparations were practically homogeneous.

Ph*b* was isolated from rabbit skeletal muscle according to Fischer and Krebs [36] but with dithiothreitol instead of cysteine and with fourfold enzyme crystallization. The Ph*b* and PhK concentrations were determined spectrophotometrically using absorption coefficients 13.2 and 12.4 for 1% solutions of Ph*b* and PhK [13, 37] at 280 nm. Before experiments, Ph*b* and PhK were dialyzed for 2 h at 4°C against 40 mM Hepes buffer, pH 6.9, 0.1 M NaCl.

The kinetics of the interaction of PhK with glycogen in the absence and presence of Ph*b* was studied by dynamic light scattering, which makes it possible to determine dimensions of formed particles in real time. The kinetics of interaction of the protein with glycogen was studied using a Photocor Complex of Photocor Instruments (USA) as described in [38]. An HeNe laser was used as the light source (wavelength 632.8 nm, maximal power 15 mW). Measurements were carried out at the fixed scattering angle 90°. The polydispersity analysis program DynaLS (Alango, Israel) was used for analysis of autocorrelation functions.

All solutions (0.04 M Hepes buffer, pH 6.9, 0.1 M NaCl, 0.1 mM Ca^{2+}, and 10 mM Mg^{2+}) were prepared in deionized water obtained using an Easy-Pure II RF system (Barnstead, USA). The reaction was initiated by addition of Ca^{2+} and Mg^{2+} solution (10 μl) to reaction mixture containing all the other components. Total volume of reaction mixture was 0.5 ml.

RESULTS AND DISCUSSION

Interaction of PhK with glycogen in the presence of Ph*b*. To study the kinetics of PhK binding to glycogen, the dynamic light scattering technique was used. Figure 1 shows the enhancement of the light scattering intensity upon addition of PhK to glycogen solution in the presence of 0.1 mM $CaCl_2$, 10 mM $MgCl_2$, and 0.1 M NaCl (curve *1*). The glycogen and PhK concentrations were 0.5 and 0.1 mg/ml, respectively. In the presence of Ph*b* (0.2 mg/ml), the increase in the light scattering intensity for the PhK + glycogen system becomes much more pronounced (curve *2*). For the Ph*b* + glycogen system, the

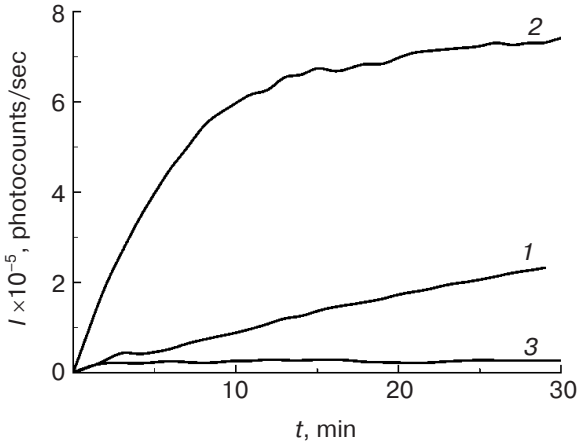


Fig. 1. Kinetics of interaction of PhK (0.1 mg/ml) with glycogen (0.5 mg/ml) in the absence (curve *1*) and presence of 0.2 mg/ml Ph*b* (curve *2*). *I*, intensity of light scattering at λ = 632.8 nm. Curve *3* corresponds to change in glycogen light scattering after Ph*b* addition. Experimental conditions: 40 mM Hepes, pH 6.9, 0.1 M NaCl, 0.1 mM Ca^{2+}, 10 mM Mg^{2+}, 20°C.

kinetics of interaction is very rapid (as we showed previously, values of half-transformation time are within the millisecond interval [39, 40]). Owing to this, at the initial moment a jump in intensity is observed, and then just slight growth is seen (curve *3*).

The dynamic light scattering technique can be used to estimate the dimensions of glycogen particles and of those formed upon the interaction of glycogen with PhK and Ph*b*. The mean hydrodynamic radius (R_h) of the glycogen particles was 52 ± 1 nm (Fig. 2a). The binding of PhK to glycogen under the studied conditions is a relatively slow process. The increase in R_h with time was observed; the R_h value obtained by extrapolation to the zero moment of time (R_h = 52 nm) coincided with R_h value for the glycogen particles (Fig. 2b). Since the interaction of Ph*b* with glycogen is a very rapid process, the mean radius of glycogen particles containing bound Ph*b* did not change with time. The mean value of R_h was 62 ± 1 nm (Fig. 2c). When Ph*b* was present in the system simultaneously with PhK and glycogen, the rate of hydrodynamic radius growth noticeably increased with time and the R_h value obtained by extrapolation to the zero time moment was 62 nm (Fig. 2d), i.e. it coincided with the hydrodynamic radius value for the Ph*b*–glycogen complex (Fig. 2c).

Effect of crowding on the interaction of PhK and Ph*b* with glycogen. Under conditions of crowding created by 1 M TMAO, the rate of PhK interaction with glycogen in the presence of Ph*b* increased significantly (Fig. 3a). This is seen in comparison of curves *1* and *2* showing the increase in light scattering intensity in the PhK + Ph*b* + glycogen system in the absence and presence of the crowding agent (Fig. 3a). Note that control experiments were carried out by measuring the light scattering intensity in the glycogen + PhK, glycogen + Ph*b*, and Ph*b* + PhK systems both in the absence and presence of 1 M TMAO (0.04 M Hepes, pH 6.9, 0.1 M NaCl, 0.1 mM Ca^{2+}, and 10 mM Mg^{2+}, 20°C). The effect of crowding on

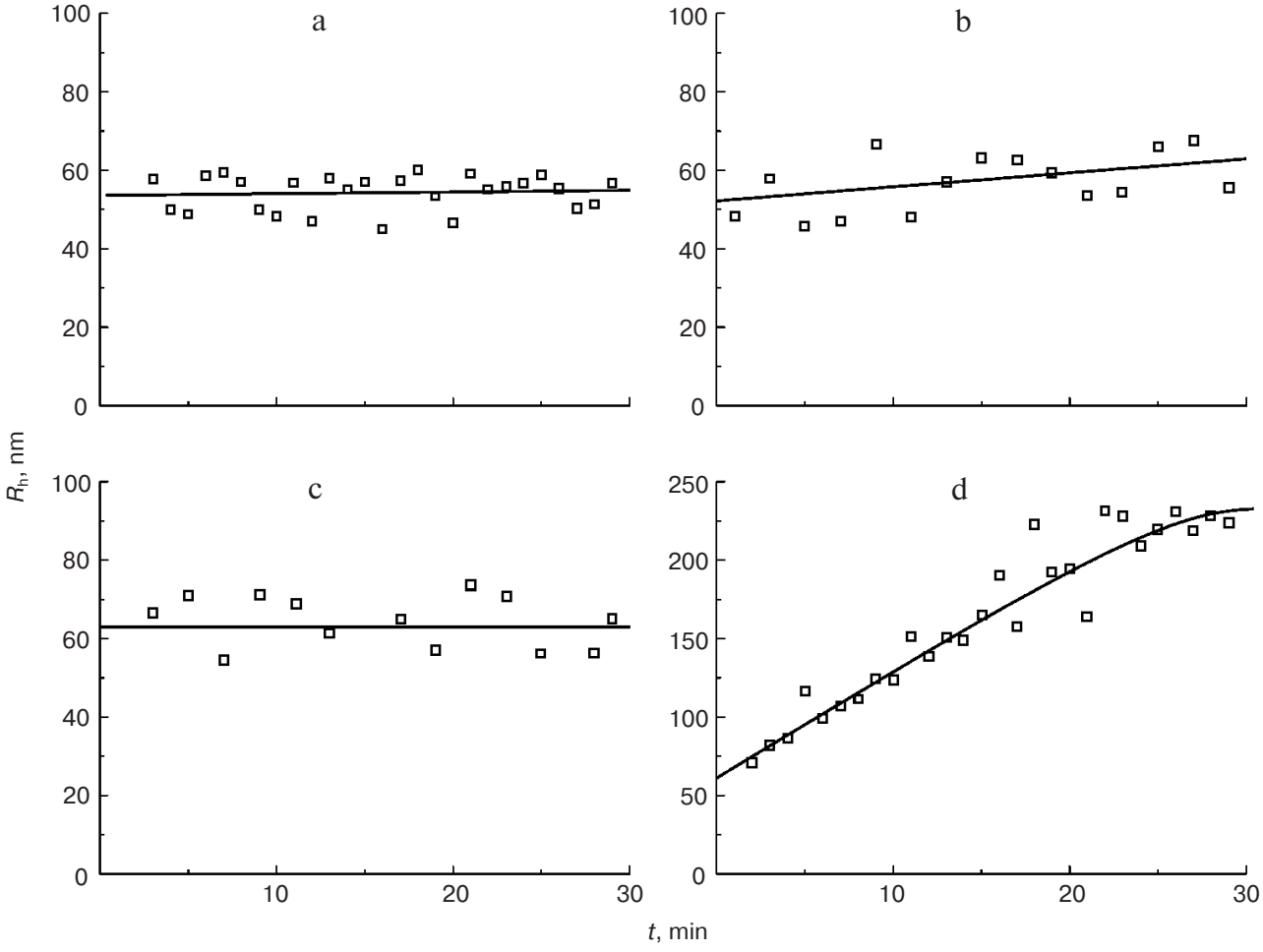


Fig. 2. a) Estimation of hydrodynamic radius (R_h) of glycogen particles; b-d) changes in R_h for glycogen particles in systems: glycogen + PhK, glycogen + Ph*b*, and glycogen + Ph*b* + PhK, respectively. Concentrations of glycogen, Ph*b*, and PhK were 0.5, 0.2, and 0.1 mg/ml, respectively. The reactions of glycogen interaction with PhK in the absence and presence of Ph*b* were initiated by addition of Ca^{2+} and Mg^{2+} to final concentrations 0.1 and 10 mM, respectively.

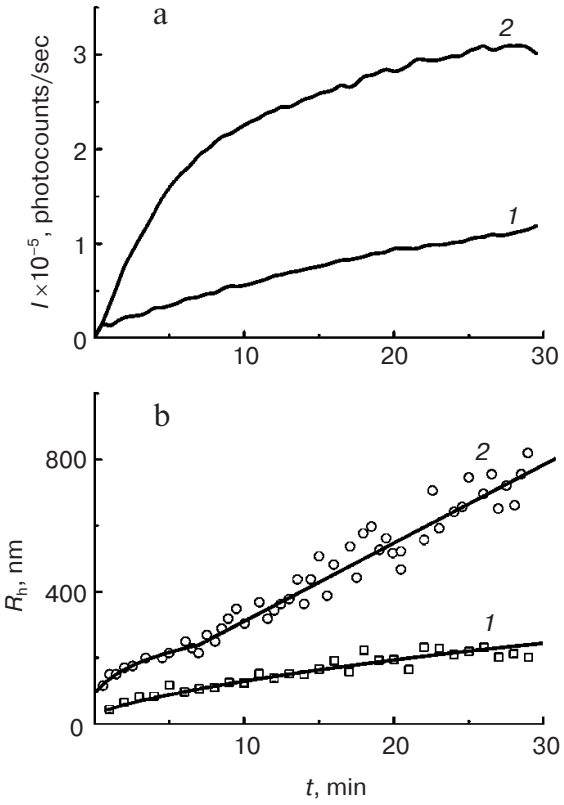


Fig. 3. Effect of crowding created by 1 M TMAO on interaction of glycogen with Ph*b* and PhK. Time dependences of light scattering intensity (a) and hydrodynamic radius (b) for the glycogen + Ph*b* + PhK system in the absence and presence of 1 M TMAO (curves *1* and *2*, respectively). Concentrations of glycogen, Ph*b*, and PhK were 0.5, 0.2, and 0.1 mg/ml, respectively.

the increase in the light scattering intensity in the glycogen + PhK and glycogen + Ph*b* systems was significantly lower than that in the complete system (PhK + Ph*b* + glycogen), whereas no interaction was observed in Ph*b* + PhK systems under crowding conditions.

Additional information concerning the mechanism of enzyme binding on glycogen particles was obtained from measurement of hydrodynamic radius of formed particles. It is clearly seen that under crowding conditions the PhK binding to glycogen particles having adsorbed Ph*b* is a two-stage process. During the first 7 min, R_h increases and reaches a limiting value of ~220 nm (Fig. 3b, curve *2*). At long time, a monotonous increase in R_h is observed. In the absence of crowding agent, only the first stage of R_h increase is observed during 30 min (curve *1*). Comparison of curves *1* and *2* in Fig. 3b shows that under crowding conditions the first stage of the glycogen–PhK–Ph*b* complex formation accelerates.

Effect of FAD on PhK and Ph*b* binding to glycogen under crowding conditions. We showed earlier that FAD binds to PhK and prevents the interaction of PhK with glycogen [27]. It seemed interesting to determine how FAD influences the binding of PhK to the glycogen–Ph*b* complex under crowding conditions. As seen in Fig. 4a, in the presence of FAD the increase in light scattering intensity in the glycogen + PhK + Ph*b* system is lowered under conditions of crowding created by 1 M TMAO. Note that the inhibitory effect of FAD is not complete and reaches a maximal value at FAD concentrations ~30 μM. The factor responsible for incomplete inhibitory effect of FAD becomes apparent on plotting time dependences of the hydrodynamic radius. Figure 4b shows that FAD at high concentrations completely inhibits the second stage of PhK binding to glycogen particles containing Ph*b*. In this case, FAD does not influence the first stage of complex formation resulting in emergence of particles with R_h ~220 nm.

The data show two types of interaction of PhK with glycogen particles containing bound Ph*b*. One type of interaction is completely resistant to FAD, while the other is practically completely inhibited at sufficiently high FAD concentrations. To explain two types of inter-

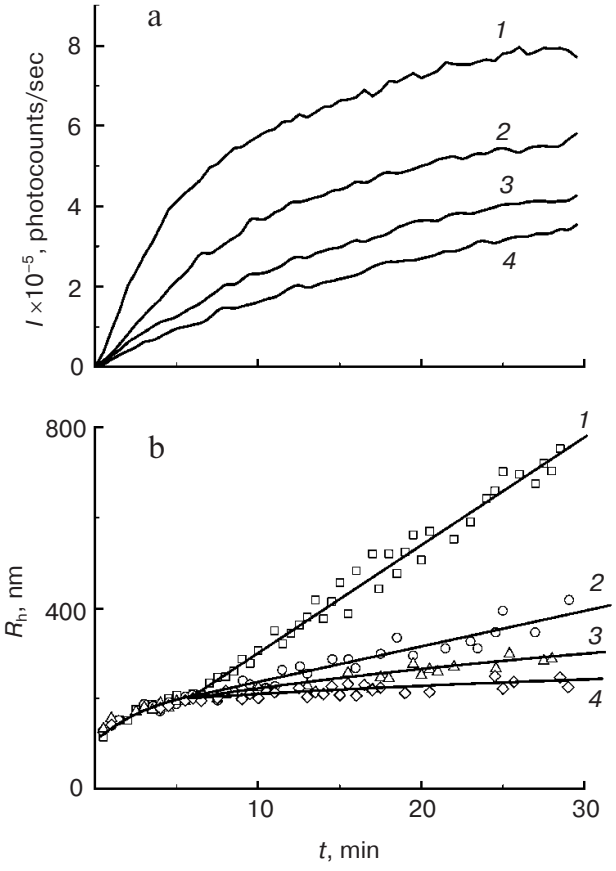
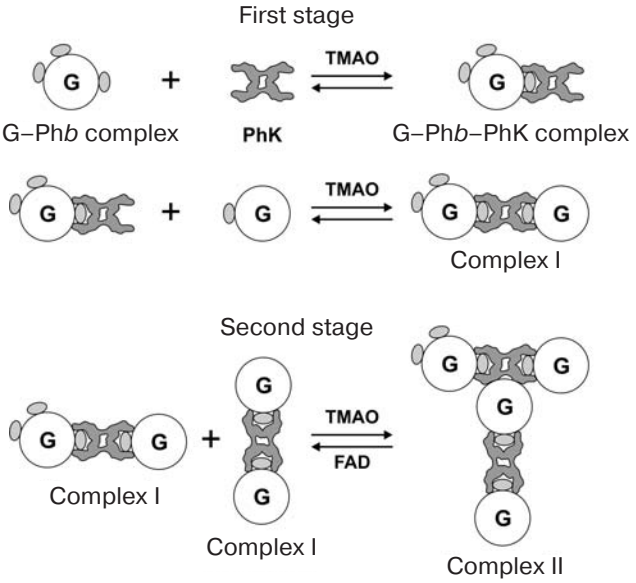


Fig. 4. Effect of FAD on the interaction of Ph*b* and PhK with glycogen under conditions of crowding created by 1 M TMAO. Time dependences of light scattering intensity (a) and hydrodynamic radius (b) for the glycogen + PhK + Ph*b* system in the presence of 0 (*1*), 5 (*2*), 15 (*3*), 30, and 60 μM FAD (*4*). Glycogen, Ph*b*, and PhK concentrations were 0.5, 0.2, and 0.1 mg/ml, respectively.



Proposed stages of interaction of PhK with Ph*b*–glycogen complex under crowding conditions. G, glycogen particle. The binding of PhK to glycogen particles is possible with involvement of Ph*b* (first stage) or without it (second stage). Complexes I and II correspond to the types of contacts between components of the complexes. The real number of glycogen particles in these complexes can reach several tens

action of PhK with glycogen particles, we propose a two-stage scheme of PhK binding to glycogen–Ph*b* complexes. It is reasonable to estimate in advance relative molar concentrations of Ph*b*, PhK, and Ph*b*-binding centers on glycogen particles. The adsorption capacity of pig liver glycogen for Ph*b* is $3.64 \cdot 10^{-6}$ mol Ph*b* dimer per gram glycogen [41]. At glycogen concentration of 0.5 g/liter, the concentration of Ph*b*-binding centers was $1.82 \cdot 10^{-6}$ M. The Ph*b* molar concentration in our experiments was $1.03 \cdot 10^{-6}$ M (per dimer). If it is considered that the microscopic dissociation constant for the glycogen–Ph*b* complex is $2.1 \cdot 10^{-6}$ M (0.05 M glycyl-glycine buffer, pH 6.8, 0.1 M KCl, 20°C) [42], then, as shown by appropriate calculations, a significant part of added Ph*b* binds to glycogen particles. Note that the molar concentration of PhK (per hexadecamer) was $0.077 \cdot 10^{-6}$ M, i.e. it was 13.4 times lower than the molar concentration of Ph*b*. Since the affinity of PhK to glycogen increases in the presence of Ph*b* (data from [31] and those obtained in this work), we believe that the first stage of PhK binding to the Ph*b*–glycogen complex is adsorption of PhK on glycogen particles during which PhK simultaneously interacts with glycogen and Ph*b* (Scheme).

We discussed previously formation of a similar ternary complex [31, 32]. Since the hydrodynamic radius of particles formed in the first stage reaches ~220 nm, we think that the first stage is not limited by adsorption of PhK molecules on Ph*b*–glycogen complexes but includes formation of more complicated units containing several glycogen particles. In these complexes, the PhK molecules interacting with Ph*b* molecules adsorbed on different glycogen particles act as bridges between glycogen particles. Taking into account that the PhK binding is studied in the presence of at least tenfold excess of Ph*b*, we believe that complete PhK binding takes place in the first stage of interaction of PhK with Ph*b*–glycogen complexes. We have designated formed complexes as type-I complexes (Scheme). In type-I complexes, Ph*b* is involved in PhK binding on glycogen particles. The hydrodynamic radius of type-I complexes reaches with time ~220 nm. Under these conditions type-I complexes contain tens of glycogen particles with bound Ph*b* and PhK molecules. Our data on the effect of FAD on the PhK + Ph*b* + glycogen system show that the PhK contacts with Ph*b*–glycogen complexes formed at this stage are FAD-resistant.

The existence of a break on the R_h–time dependence curve for PhK binding to glycogen in the presence of Ph*b* and under crowding conditions (Fig. 3b, curve *2*) suggests that the second stage of PhK binding corresponds to the complex I sticking stage. To justify this supposition, we can use data that characterize the kinetics of protein heat aggregation. We have shown [43] that the initial stage of protein aggregation is the stage of formation of starting aggregates that include hundreds of the denatured protein molecules. Up to a certain moment, the size of starting aggregates does not change, but their concentration grows, which is revealed by increased intensity of light scattering. The moment when the increase in the protein aggregate dimensions is observed was interpreted in [43] as the beginning of sticking of starting aggregates. Thus, it is most logical to explain the appearance of the second stage of PhK binding by glycogen particles in the presence of Ph*b* under crowding conditions by adhesion of already formed glycogen–Ph*b*–PhK complexes, i.e. type-I complex. Complex formed in such way is designated in the Scheme as type-II complex.

The following facts should be taken into account in explaining the nature of contacts established at the second stage: FAD completely inhibits PhK binding to glycogen in the absence of Ph*b* and crowding agents [27], and FAD stimulates complete inhibition of the second stage of PhK binding to glycogen complexes under crowding conditions (this work). The dependence of the rate of complex formation between PhK and glycogen (v) on FAD concentration in the absence of Ph*b* and crowding agents was analyzed [27]. The 50% inhibition of the rate of PhK complex formation with glycogen was observed at FAD concentration 2.7 ± 0.2 μM. We have plotted the dependence of R_h rate increase with time (dR_h/dt) on FAD concentration for the second stage of PhK binding to Ph*b*–glycogen complexes under crowding conditions (Figs. 4b and 5). To analyze this dependence, the following type of equation was used:

$$dR_h/dt = (dR_h/dt)_0/(1 + [FAD]/[FAD]_{0.5}), \quad (1)$$

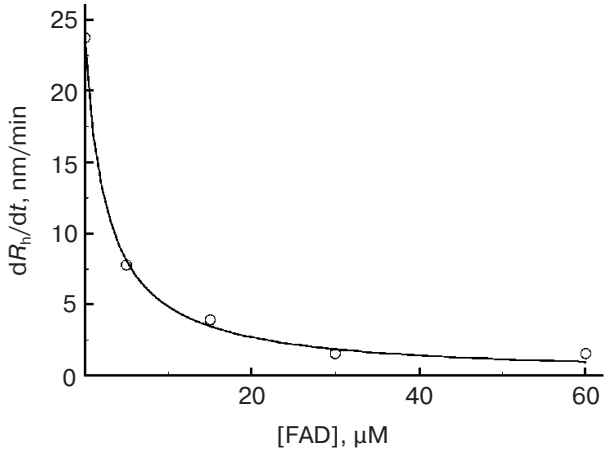


Fig. 5. Dependence on FAD concentration of dR_h/dt, characterizing the rate of hydrodynamic radius elongation with time, for the second stage of PhK binding to Ph*b*–glycogen complex. The curve was calculated using Eq. (1).

where $(dR_h/dt)_0$ is the dR_h/dt value at [FAD] = 0 and $[FAD]_{0.5}$ is the FAD concentration at which 50% decrease in dR_h/dt is observed. It was found that $[FAD]_{0.5} = 2.6 \pm 0.2$ μM. The similarity of $[FAD]_{0.5}$ values for v and dR_h/dt dependences on [FAD] indicates that at the second stage of interaction of PhK with Ph*b*–glycogen complexes under crowding conditions the PhK contacts with glycogen particles are formed without involvement of Ph*b* (the second stage in the Scheme).

We supposed earlier that the protein–glycogen complex might act as an intracellular depot of flavins [26, 27, 44, 45]. Flavins (riboflavin, FMN, and FAD) are allosteric inhibitors of muscle Ph*b* and exhibit high affinity to the enzyme [20, 44-49]. FAD is known as the dominating form among flavins in muscle tissue. We used the Ph*b* + FAD system to demonstrate the crowding effect on the protein–ligand type interaction. Under conditions of molecular crowding, a shift of the Ph*b* isomerization reaction towards the more compact state of dimer with the lowered affinity to FAD occurs [20, 24]. In the case of the interaction of Ph*b* with allosteric inhibitor FAD, under crowding conditions a conformational transition of dimeric molecule that results in appearance of cooperative interactions between flavin-binding centers of the enzyme occurs [20, 24]. We have also shown that FAD binds to phosphorylase kinase and thus inhibits enzyme association and PhK interaction with glycogen [27].

This investigation by dynamic light scattering of the glycogen + PhK + Ph*b* system under crowding conditions and at ionic strength close to physiological levels has shown that crowding significantly enhances interactions within protein–glycogen complex. Although crowding decreases the strength of the PhK–Ph*b* [30] complex, such crowding effect is relieved in the complete PhK–Ph*b*–glycogen system due to the presence of glycogen. Two-stage increase in the hydrodynamic radius of formed particles with time was detected under crowding conditions. The scheme of PhK binding to glycogen and to Ph*b*–glycogen complex with formation of the PhK–Ph*b*–glycogen ternary complex was proposed based on these data. It is supposed that in the PhK–Ph*b*–glycogen ternary complex there are two types of PhK contacts with glycogen: one type of interaction is realized via Ph*b* "anchored" on the glycogen particle, and the other is realized by the direct contact of PhK with glycogen. The data suggest that FAD inhibits only one type of contact, namely, the direct interaction between PhK and glycogen.

Since FAD does not influence catalytic activity of PhK [27], it can be assumed that owing to its high affinity FAD acts as a factor forming *in vivo* the structure of protein–glycogen particles rather than of a modulator of the enzymic activity of PhK. In the presence of FAD, the binding of PhK to glycogen particles via Ph*b* becomes more preferable, which should provide for functional connection between Ph*b* and PhK.

This work was supported by the Russian Foundation for Basic Research (grant 08-04-00666a) and by the "Molecular and Cell Biology" Program of the Presidium of the Russian Academy of Sciences.

REFERENCES

1. Ellis, R. J., and Minton, A. P. (2003) *Nature*, **425**, 27-28.
2. Zimmerman, S. B., and Minton, A. P. (1993) *Annu. Rev. Biophys. Biomol. Struct.*, **22**, 27-65.
3. Minton, A. P. (2001) *J. Biol. Chem.*, **276**, 10577-10580.
4. Ellis, R. J. (2001) *Trends Biochem. Sci.*, **26**, 597-604.
5. Chebotareva, N. A., Kurganov, B. I., and Livanova, N. B. (2004) *Biochemistry (Moscow)*, **69**, 1239-1251.
6. Hall, D., and Minton, A. P. (2003) *Biochim. Biophys. Acta*, **1649**, 127-139.
7. Chebotareva, N. A. (2007) *Biochemistry (Moscow)*, **72**, No. 13, 1478-1490.
8. Al-Habori, M. (2001) *Int. J. Biochem. Cell Biol.*, **33**, 844-864.
9. Barford, D., and Johnson, L. N. (1989) *Nature*, **340**, 606-616.
10. Krebs, E. G., Graves, D. J., and Fisher, E. H. (1959) *J. Biol. Chem.*, **23**, 2867-2873.
11. Brusharia, R. J., and Walsh, D. A. (1999) *Frontiers Biosci.*, **4**, 618-641.
12. Livanova, N. B. (1993) *Biochemistry (Moscow)*, **58**, 1234-1239.
13. Cohen, P. (1973) *Eur. J. Biochem.*, **34**, 1-14.
14. Nadeaw, O. W., Traxler, K. W., Fee, L. R., Baldwin, B. A., and Carlson, G. M. (1999) *Biochemistry*, **38**, 2551-2559.
15. Wilkinson, D. A., Fitzgerald, T. J., Marion, T. N., and Carlson, G. M. (1999) *J. Protein Chem.*, **18**, 157-164.
16. Priddy, T. S., Middaugh, C. R., and Carlson, G. M. (2007) *Protein Sci.*, **16**, 517-527.

17. Nadeaw, O. W., Carlson, G. M., and Gogol, E. P. (2002) *Structure*, **10**, 23-32.
18. Priddy, T. S., Price, E. S., Johnson, C. K., and Carlson, G. M. (2007) *Protein Sci.*, **16**, 1017-1023.
19. Chebotareva, N. A., Meremyanin, A. V., Makeeva, V. F., and Kurganov, B. I. (2006) *Progr. Colloid Polym. Sci.*, **131**, 83-92.
20. Chebotareva, N. A. (2006) *Interaction of Glycogenolysis Enzymes in Conditions of Molecular Crowding*: Doctoral dissertation [in Russian], Bach Institute of Biochemistry, RAN, Moscow.
21. Chebotareva, N. A., Andreeva, I. E., Makeeva, V. F., Kurganov, B. I., Livanova, N. B., and Harding, S. E. (2002) *Progr. Colloid Polym. Sci.*, **119**, 70-76.
22. Meremyanin, A. V., Chebotareva, N. A., Makeeva, V. F., and Kurganov, B. I. (2007) *Dokl. Biochem. Biophys.*, **415**, 183-185.
23. Chebotareva, N. A., Harding, S. E., and Winzor, D. J. (2001) *Eur. J. Biochem.*, **268**, 506-513.
24. Chebotareva, N. A., Kurganov, B. I., Harding, S. E., and Winzor, D. J. (2005) *Biophys. Chem.*, **113**, 61-66.
25. Eronina, T. B., Chebotareva, N. A., and Kurganov, B. I. (2005) *Biochemistry (Moscow)*, **70**, 1237-1244.
26. Chebotareva, N. A., Meremyanin, A. V., Makeeva, V. F., Livanova, N. B., and Kurganov, B. I. (2008) *Biophys. Chem.*, **133**, 45-53.
27. Makeeva, V. F., Chebotareva, N. A., Andreeva, I. E., Livanova, N. B., and Kurganov, B. I. (2006) *Biochemistry (Moscow)*, **71**, 652-657.
28. Polishchuk, S. V., Brandt, N. R., Meyer, H. E., Varsanyi, M., and Heilmeyer, L. M., Jr. (1995) *FEBS Lett.*, **362**, 271-275.
29. Meyer, H. E., Heilmeyer, L. M. G., Jr., and Haschke, R. H. (1970) *J. Biol. Chem.*, **245**, 6642-6648.
30. Chebotareva, N. A., Andreeva, I. E., Makeeva, V. F., Livanova, N. B., and Kurganov, B. I. (2004) *J. Mol. Recognit.*, **17**, 426-432.
31. Andreeva, I. E., Makeeva, V. F., Kurganov, B. I., Chebotareva, N. A., and Livanova, N. B. (1999) *Biochemistry (Moscow)*, **64**, 159-168.
32. Andreeva, I. E., Makeeva, V. F., Kurganov, B. I., Chebotareva, N. A., and Livanova, N. B. (1999) *FEBS Lett.*, **445**, 173-176.
33. Durchschlag, H. (1986) in *Thermodynamic Data for Biochemistry and Biotechnology* (Hinz, H.-J., ed.) Springer-Verlag, Berlin, pp. 45-182.
34. Morozov, V. E., Eronina, T. B., Andreeva, I. E., Silonova, G. V., Solov'eva, N. V., Shchors, E. I., Livanova, N. B., and Poglazov, B. F. (1989) *Biokhimiya*, **54**, 448-455.
35. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
36. Fischer, E. H., and Krebs, E. G. (1962) *Meth. Enzymol.*, **5**, 369-373.
37. Kastenschmidt, L. L., Kastenschmidt, J., and Helmreich, E. (1968) *Biochemistry*, **7**, 3590-3608.
38. Meremyanin, A. V., Eronina, T. B., Chebotareva, N. A., Kleimenov, S. Yu., Yudin, I. K., Muranov, K. O., Ostrovsky, M. A., and Kurganov, B. I. (2007) *Biochemistry (Moscow)*, **72**, 518-528.
39. Klinov, S. V., Davidov, D. R., Lissovskaya, N. P., Tarasov, O. S., and Kurganov, B. I. (1980) *Mol. Biol. (Moscow)*, **14**, 348-356.
40. Klinov, S. V., Chebotareva, N. A., Lissovskaya, N. P., Davidov, D. R., and Kurganov, B. I. (1982) *Biochim. Biophys. Acta*, **709**, 91-98.
41. Chebotareva, N. A., Lissovskaya, N. P., and Kurganov, B. I. (1979) *Mol. Biol. (Moscow)*, **13**, 228-236.
42. Chebotareva, N. A., Sugrobova, N. P., Bulanova, L. N., Poznanskaya, A. A., Kurganov, B. I., and Gunar, V. I. (1995) *Biochemistry (Moscow)*, **60**, 1551-1558.
43. Golub, N., Meremyanin, A., Markossian, K., Eronina, T., Chebotareva, N., Asryants, R., Muronets, V., and Kurganov, B. (2007) *FEBS Lett.*, **581**, 4223-4227.
44. Kurganov, B. I., Klinov, S. V., and Chebotareva, N. A. (1994) *Uspekhi Biol. Khim.*, **34**, 83-110.
45. Chebotareva, N. A., Klinov, S. V., and Kurganov, B. I. (2001) *Biotechnol. Genet. Eng. Rev.*, **18**, 265-297.
46. Chebotareva, N. A., Kurganov, B. I., Pekel, N. D., and Beresovskii, V. M. (1986) *Biochem. Int.*, **13**, 189-197.
47. Chebotareva, N. A., Kurganov, B. I., Lubarev, A. E., Davydov, D. R., and Pekel, N. D. (1991) *Biochimie*, **73**, 1339-1343.
48. Kurganov, B. I., Schors, E. I., Livanova, N. B., Chebotareva, N. A., Eronina, T. B., Andreeva, I. E., Makeeva, V. P., and Pekel, N. D. (1993) *Biochimie*, **75**, 481-485.
49. Livanova, N. B., Chebotareva, N. A., Eronina, T. B., and Kurganov, B. I. (2002) *Biochemistry (Moscow)*, **67**, 1089-1098.