Biochimica et Biophysica Acta, 445 (1976) 610—621

BBA 67915

THE INTERACTION OF PHOSPHORYLASE B WITH AMP

ROBERT F. STEINER, LILLIAN GREER, CHRIS GUNTHER and CHARLENE HORAN

Department of Chemistry, University of Maryland Baltimore County, 5401 Wilkens Avenue, Catonsville, Md. 21228 (U.S.A.)

(Received May 28th, 1976)

Summary

Combined binding, calorimetry, fluorescence, and spin labeling measurements have been made upon the interaction of glycogen phosphorylase B (1,4-α-D-glucan:1,6-α-D-glucan 6-α-glucosyltransferase, EC 2.4.1.1) with AMP. The system exhibits positive cooperativity and appears to obey a model intermediate to the symmetry and sequential models.

Introduction

Glycogen phosphorylase B (1,4-α-D-glucan:1,6-α-D-glucan 6-α-glucosyltransferase, EC 2.4.1.1) is a classical example of an allosteric enzyme. It catalyzes in vitro both the synthesis and degradation of glycogen according to the equation:

glucose 1-phosphate + $(\text{glucose})_n \rightleftharpoons (\text{glucose})_{n+1}$ + phosphate

The catalytic activity depends upon AMP, both V and K being functions of the nucleotide concentration. The interaction between the nucleotide and the enzyme has been studied by several investigators, using both kinetic and equilibrium binding techniques [1—7]. Despite some differences in detail, all investigators appear to be in agreement that one strong binding site for AMP occurs on each of the two monomer units of molecular weight 92 500 that comprise the active dimer, which appears to be the functional form of the enzyme. Although values for the stepwise binding constants have not been reported, it is clear from the character of the Scatchard plots [2] and of the dependence of the reaction velocity upon AMP level that the binding process is cooperative [3,4] and that phosphorylase B must be classified as an allosteric enzyme.

The use of various conformational probes has provided indirect evidence that some kind of conformational change accompanies the binding of AMP [2,5,8]. The problem is complicated by the reported occurrence of a dimer-tetramer equilibrium in the presence of AMP at low temperature [9] and by the

existence of pronounced differences of behavior in different buffers [3,4].

The original studies of Buc and Buc [2] showed that the kinetic behavior of phosphorylase B, including the dependence of reaction velocity upon the levels of substrate and modifier, could be accounted for on the basis of the original two-state symmetry model of Monod et al. [10]. More recent studies have provided evidence for the existence of an intermediate allosteric state [3,4,6]. Griffiths et al. [11] have made combined binding and spin label studies upon the interaction of phosphorylase A with AMP, finding in this case results consistent with the two-state model. Ho and Wang [9] have determined the thermodynamic parameters characterizing the overall reaction of phosphorylase B with AMP.

It is the purpose of the present study to extend the classical work cited above by determining the stepwise thermodynamic parameters characterizing each stage of the combination of AMP with the two strong binding sites on the phosphorylase B dimer. A second objective is to combine binding and structural probe measurements under the same conditions in order to examine further the nature of the allosteric transition, including the question of whether it obeys the strict two-state model or whether additional states must be postulated.

The interpretation of data obtained using an extrinsic chemical label as a structural probe is considerably simplified if the probe has a specific and known location within the tertiary structure of the protein. Phosphorylase B which has been recrystallized from cysteine is known to contain at least one highly reactive sulfhydryl group per monomer unit, plus several other groups of lower reactivity [12].

Two kinds of structural probe were used in this study, both of which combine specifically with sulfhydryl groups. These were the fluorescent label, *N*-(iodoacetylaminoethyl)-5-naphthylamine-1-sulfonic acid, originally described by Hudson and Weber [13] and an ESR spin label, 3-(2-iodoacetamido)-2,2,5,5 tetramethyl-1-pyrrolidinyloxyl [14].

Experimental

Materials

Glycogen phosphorylase B was prepared from frozen rabbit muscle (Pel-Freeze) by the method of Fischer and Krebs [15] and was recrystallized three times from 0.03 M cysteine (pH 7.0), 1 mM AMP, and 10 ml magnesium acetate at 0°C. Solutions were prepared by dissolution of the crystals in 0.1 M glycylglycine, 0.01 M cysteine, pH 7.0, and freed from AMP by passage through a 1 × 100 cm Sephadex G-25 column eluted with 0.1 M glycylglycine, pH 7.0. A control experiment using [^{3}H]AMP established that quantitative removal of AMP was achieved by this procedure. The 260 : 280 absorbance ratio of the solutions employed here was normally in the range 0.51—0.53.

Unless the solutions were to be treated with a sulfhydryl-specific reagent, they were subsequently made 1 mM in Cleland's reagent (dithiothreitol).

The presence of cysteine in the dissolving buffer proved to be important for obtaining reproducible preparations, even though the enzyme was initially crystallized from cysteine solution. Solutions prepared as described above possessed 0.9—1 sulfhydryl groups per monomer unit which reacted with 10^{-3}

M Ellman's reagent (5,5′-dithio-bis-(2-nitrobenzoic acid) within 30 s [12]. If the reducing agent were omitted, the number of reactive sulfhydryls per monomer unit was variable and less than 1.

Glycylglycine, glucose 1-phosphate and α-glycerol phosphate were bought from Sigma. Unlabeled AMP was purchased from Sigma; tritiated AMP was obtained from New England Nuclear. *N*-(Iodoacetyl-aminoethyl)-5-napthylamine-1-sulfonic acid (AEDANS) was obtained as a gift through the courtesy of Dr. Joel Shaper. Two spin labels were used, both of which were purchased from Synvar. These were 3-(2-iodoacetamido)-2,2,5,5-tetramethyl-1-pyrrolidinyloxyl (I) and 3-[(2-iodoacetamido)acetamido]-2,2,5,5-tetramethyl-1-pyrrolidinyloxyl (II), which differs from I in possessing an additional -NH-CO-CH_2- group in the linear portion of the molecule. All other chemicals were reagent or analytical grade. Glass-redistilled water was used for the preparation of all solutions.

Methods

The binding at 23°C by phosphorylase B of AMP labeled with tritiated AMP was measured using the gel filtration technique of Hummel and Dreyer [16]. A Sephadex G-25 column (0.5 × 40 cm) was equilibrated and eluted with buffer containing the desired level of labeled AMP. The effluent solution was monitored by the absorbance at 260 nm, using a Gilford spectrophotometer equipped with a flow cell. When equilibration was complete, as indicated by a constant base line, 1 ml of protein solution, containing the same concentration of labeled AMP, was added to the top of the column and then eluted with the original AMP-containing buffer. Radioactivities were measured for pooled fractions before the protein peak (as monitored from absorbance), for the peak itself, and for pooled fractions following the trough in absorbance subsequent to the peak. The concentration of protein was determined from the absorbance at 280 nm of a 1 : 3 dilution, blanked against the same dilution of AMP-containing buffer. The radioactivities of the effluent solution preceding and following the protein peak were averaged and subtracted from that of the peak, yielding the concentration of bound AMP. This quantity, divided by the molar concentration of enzyme, yields the average number of AMP molecules combined with one molecule of phosphorylase B.

Radioactivities, as counts per minute, were determined using a Packard scintillation counter. One ml aliquots of solution were added to 15 ml of counting solution, which had the following composition per three liter batch: 2 liters toluene (scintillation grade), 1 liter triton-X (scintillation grade), 375 mg dimethyl-POPOP (1,4-bis-(4-methyl-5-phenyloxazolyl-2)-benzene), and 16.5 g PPO (2,5-diphenyloxazole).

The heats of interaction of AMP with phosphorylase B were determined using an LKB batch microcalorimeter, equipped with two bicompartmented gold cells. Each cell was partially separated by a partition into 2- and 4 ml compartments. In a typical run 2 ml of AMP solution was placed in one compartment of each cell, while the second contained 4 ml protein solution for one cell and 4 ml buffer for the other. Rotation of the calorimeter drum produced mixing of the cell components and a net evolution of heat, registered as a peak on the recording of the differential thermopile output. The area under the peak was proportional to the heat of interaction of AMP with the protein, corrected

for the heat of dilution of AMP. In a second run, AMP was replaced by buffer, in order to obtain the heat of dilution of the protein. This was subtracted from the total heat observed in the first run. Calibration of the microcalorimeter was routinely checked, using the electrical system supplied with the instrument.

Covalent conjugates of AEDANS with phosphorylase B were prepared by adding 1—2 mg of AEDANS dissolved in 0.5 ml 0.1 M glycylglycine, pH 7.0, to 5 ml of a 1% solution of the enzyme in the same buffer prepared as described above. After 40 min the conjugate was freed from excess label by passage through a 1 × 40 cm Sephadex G-25 column.

The degree of labeling was estimated spectrophotometrically, assuming a value of $6.0 \cdot 10^3$ for the molar extinction of an AEDANS group at 350 nm [13]. The conjugate described here had 0.2 groups per monomer unit.

Conjugation of phosphorylase B with the spin labels was carried out as follows: 1—2 mg of the spin label (I or II) was dissolved in 200 μl absolute ethanol and added to 300 μl buffer (0.1 M glycylglycine, pH 7.0). 100 μl of the above solution was added to 2 ml of 1% enzyme in the same buffer. After 30 min at 3°C, the mixture was passed through a 40 × 1 cm column containing Sephadex G-25, equilibrated with the above buffer.

ESR spectra were measured using a Varian E-4 spectrometer. Typical settings were: field set: 3245 gauss, scan range: 100 gauss, microwave frequency: 9.156 GHz, time constant: 3.0 s, scan time: 30 min.

Measurements of the spectral distribution and intensity of fluorescence were made using an Aminco spectrofluorometer.

Concentrations of protein were determined from the absorbance at 280 nm. An absorbance of 1.32 was assumed for a 1 mg/ml solution [3,4]. This value has been used by Birkett et al. [5], Buc and Buc [2], Kastenschmidt et al. [3,4] and Buc-Caron and Buc [7]. A somewhat lower value (1.17) was earlier reported by Appleman et al. [17]. A molecular weight of 92 500 was used in computing molar concentrations of phosphorylase monomer [18].

Results

AMP-binding

Fig. 1 shows the AMP binding data in 0.1 M glycylglycine, pH 7.0, 10^{-3} M dithiothreitol at 23°C. The data were analyzed in terms of the consecutive binding constants by the following procedure [19].

The equilibria involved in the consecutive binding of ligand, A, by enzyme, E, are as follows:

$$\begin{aligned} &E + A \rightleftharpoons EA; \quad K_1 = \frac{[EA]}{[E][A]} \\ &EA + A \rightleftharpoons EA_2; \quad K_2 = \frac{[EA_2]}{[E][A]^2} \\ &EA_{i-1} + A \rightleftharpoons EA_i; \quad K_i = \frac{[EA_i]}{[E][A]^i} \end{aligned} \tag{1}$$

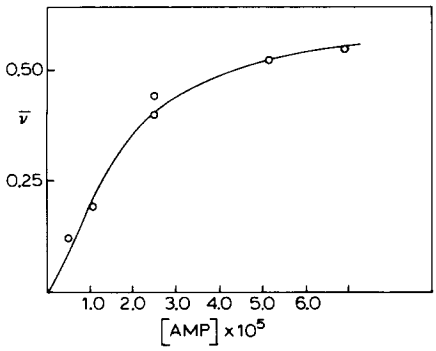

Fig. 1. The binding of AMP by phosphorylase B in 0.1 M glycylglycine, pH 7.0, 23°C. The ordinate is the number of moles of AMP bound per monomer unit.

For each complex species there is a corresponding macroscopic association constant which relates its molar concentration to those of uncombined enzyme, [E], and uncombined ligand, [A]. One may also define a set of stepwise equilibrium constants:

$$k_1 = K_1 = \frac{[EA]}{[E][A]}$$

$$k_2 = K_2/K_1 = \frac{[EA_2]}{[EA][A]} \tag{2}$$

$$k_i = K_i/K_{i-1} = \frac{[EA_i]}{[EA_{i-1}][A]}$$

Since the two sets of equilibrium constants are related by eqns. 2 the determination of the macroscopic constants automatically specifies the values of the stepwise constants.

If the sites are equivalent and cooperative effects are absent, then, from purely statistical considerations [21], we have for n sites:

$$k_i\left(\frac{i}{n-i+1}\right) = k^* = \text{intrinsic binding constant} \tag{3}$$

The existence of cooperativity may be tested for by comparing values of $k_i^* = k_i\ \{i/(n-i+1)\}$. An increase in k_i^* with increasing i indicates the presence of positive cooperativity.

The average number of ligand molecules bound per molecule of enzyme is given by:

$$\bar{\gamma} = \frac{\sum_{i\geq 1} iK_i[A]^i}{1 + \sum_{i\geq 1} K_i[A]^i} \tag{4}$$

If a quantity λ is defined by [19]:

$$\lambda \equiv 1 + K_1[A] + K_2[A]^2 + \ldots + K_i[A]^i + \ldots \quad (5)$$

then it has been shown that [19]

$$\ln \lambda = \int_0^{[A]} \frac{\bar{\gamma}}{[A]} d[A] \quad (6)$$

If λ is known as a function of [A], then by least squares polynomial fitting of Eqn. 5, the set of constants K_i may be determined. The quantity $1-1/\lambda$ is equal to the fraction of protein molecules which have combined with one or more molecules of ligand.

The results are shown in Fig. 2 and Table I. The ratio of k_2* to k_1* is 2—3, where k_2* (=$2k_2$) and k_1* (=$K_1/2$) are the values of k_2 and k_1 corrected for the statistical factor arising in binding by two equivalent sites [21]. The elevation of this ratio, which would be equal to unity for independent, non-interacting sites, indicates the presence of significant cooperativity of binding. The Hill coefficient [21] computed for the binding data is 1.3. The failure of the plot of $(\lambda-1)/[AMP]$ versus [AMP] to show significant upward curvature indicates that any binding by secondary sites [9] is not detectible at these AMP levels.

It is not easy to compare these results with the earlier data in the literature, since these have not been analyzed in terms of stepwise constants and the experimental conditions in no case duplicate those of this study. In the studies of Buc and Buc [2] the buffer is unspecified, but has been alleged to be glycylglycine [1,3,4]. Interpolation of their curves of $\bar{\gamma}$ versus [AMP] to 23° C yields a binding isotherm similar to ours. On the other hand, Kastenschmidt et al. [3,4] cite a value for the average dissociation constant of AMP in glycylglycine buffer greater

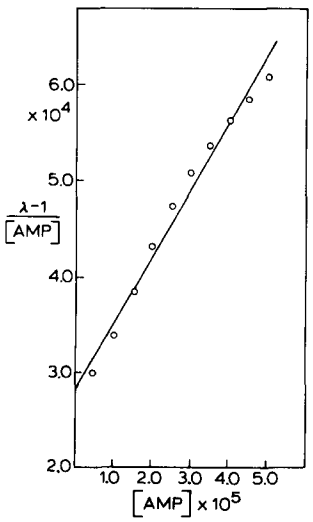

Fig. 2. Plot of (λ-1)/[AMP] versus [AMP], the molar concentration of free AMP, for the data of Fig. 1.

by orders of magnitude than that predicted from these data. This appears to be based upon one determination at a single AMP concentration.

Calorimetry

Fig. 3 summarizes the calorimetric data for the same conditions. The heat evolved per mol of monomer unit attains a plateau at concentrations of free AMP greater than about $3 \cdot 10^{-4}$ M. If the heat per mol is plotted as a function of $\bar{\gamma}$, it is seen that there is a perceptible degree of upward curvature. The heats corresponding to the two consecutive steps of binding, ΔH_1 and ΔH_2, may be computed from the limiting values of $\Delta H/\bar{\gamma}$ at low and at high levels of AMP and are cited in Table I.

The values of $\overline{\Delta H}$ obtained in glycylglycine buffer are slightly smaller than those cited by Ho and Wang [9] for glycerol phosphate. These measurements were therefore repeated using 0.1 M β-glycerol phosphate buffer. The results, cited in Table I, indicate a minor increase in heat evolved under these conditions and suggest a possible change in the properties of the enzyme in this buffer, as has been inferred by other workers [3,4]. The heats obtained in β-glycerol phosphate are in reasonable agreement with those reported by Ho and Wang [9].

Sedimentation velocity measurements for phorphorylase B at 23°C in either buffer showed only a single symmetrical peak with $s_{20,w} = 8.0$. No change in sedimentation coefficient or peak shape was observed in the presence of 1 mM AMP. There is thus no indication of any significant self-association of the phosphorylase dimer under these conditions.

Transmitted conformational change

A semi-speculative argument may be developed as follows with respect to the free energy change of the induced conformational transition. The possible changes accompanying the consecutive binding of two molecules of AMP by a

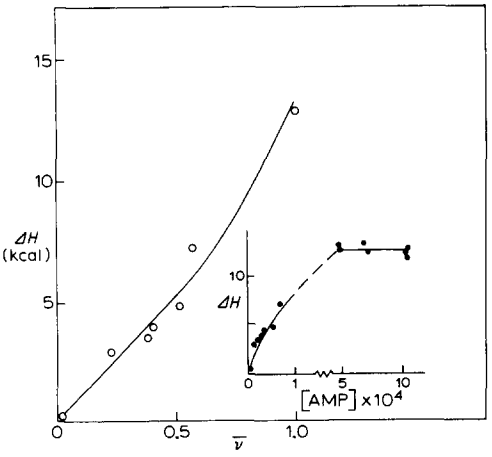

Fig. 3. Enthalpy of binding of AMP as a function of the number of moles of AMP bound per monomer unit for phosphorylase B in 0.1 M glycylglycine, pH 7.0, 23°C. Inset: Heat involved per mole of monomer units as a function of the concentration of free AMP.

TABLE I

Buffer	k_1^* ($\times 10^{-4}$)	k_2^* ($\times 10^{-4}$)	$\Delta F^\circ_{1,c}$	$\Delta F^\circ_{2,c}$	ΔH_1 (kcal)	ΔH_2 (kcal)	ΔS_1 (e.u.)	ΔS_2 (e.u.)	ΔH (kcal)
0.1 M glycylglycine, 10^{-3} M dithiothreitol, pH 7.0	1.4 ± 0.4	4.0 ± 1.5	−5.62 ± 0.16	−6.37 ± 0.21	−11.0 ± 2.0	−14.6 ± 2.0	−16.5 ± 6	−25.8 ± 6	12.8 ± 0.5
0.1 M β-glycerol phosphate, 10^{-3} M dithiothreitol, pH 7.0									13.4 ± 0.5

phosphorylase B dimer may be depicted as follows:

$$A{-}A \xrightarrow[\Delta F^\circ_1]{AMP} B^*{-}C \xrightarrow[\Delta F^\circ_2]{AMP} D^*{-}D^* \tag{7}$$

where A, B, C and D designate different possible conformational states of the monomer and an asterisk denotes the presence of a molecule of bound AMP. If $\Delta F^\circ{}_{1,c}$ and $\Delta F^\circ{}_{2,c}$ represent the free energy changes for the two steps of binding after correction for the purely statistical factor ($\Delta F^\circ{}_{1,c} = \Delta F^\circ{}_1 + RT\ln 2; \Delta F^\circ{}_{2,c} = \Delta F^\circ{}_2 - RT\ln 2$), then

$$\Delta(\Delta F) = \Delta F^\circ_{2,c} - \Delta F^\circ_{1,c} = \Delta F^\circ_{B^*\to D^*} + \Delta F^\circ_{C\to D^*} - \Delta F^\circ_{A\to B^*} - \Delta F^\circ_{A\to C} + \sigma_{DD}$$

$$-2\sigma_{BC} + \sigma_{AA} \tag{8}$$

where σ_{DD}, σ_{BC}, and σ_{AA} represent the nearest neighbor interaction energies for the corresponding subunit pairs. If it is assumed that states B * and D * are equivalent, then

$$\Delta(\Delta F) = \Delta F^\circ_{C\to B^*} - \Delta F^\circ_{A\to B^*} - \Delta F^\circ_{A\to C} + \Sigma\sigma = \Delta F^\circ_{C\to B^*} - (\Delta F^\circ_{A\to C} + \Delta F^\circ_{C\to B^*})$$

$$- \Delta F^\circ_{A\to C} + \Sigma\sigma = -2\Delta F^\circ_{A\to C} + \Sigma\sigma \tag{9}$$

where $\Sigma\sigma = \sigma_{BB} - 2\sigma_{BC} + \sigma_{AA} = 2\{\frac{1}{2}(\sigma_{BB} + \sigma_{AA}) - \sigma_{BC}\}$

The quantity $\Delta F^\circ{}_{A\to C}$ is the standard free energy for the conversion of an isolated monomer unit from the A to the C conformation. Unfortunately, it is not possible to evaluate $\Sigma\sigma$ independently. However the state BC corresponds to a "strained" configuration which is not energetically favored, in view of the observed cooperative effects. Hence σ_{BC} is in all probability less negative than $1/2(\sigma_{BB} + \sigma_{AA})$, so that $\Sigma\sigma < 0$. Its magnitude is uncertain, but is probably small in comparison with $\Delta F^\circ{}_{A\to C}$. Thus, $\Delta(\Delta F)$ probably represents an upper limit to the magnitude of $\Delta F^\circ{}_{A\to C}$.

For the case of 0.1 M glycylglycine at pH 7.0, 23°C, we have (in kcal) $\Delta F^\circ_{1,c} = -6.62$, $\Delta F^\circ_{2,c} = -6.37$, $\Delta(\Delta F) = -0.75$, and $\Delta F^\circ_{A\to C} \leqslant 0.37$ kcal.

The low magnitude of $\Delta F^\circ{}_{A\to C}$ is noteworthy and suggests that the conformational change induced in the second subunit by the binding of a molecule of AMP by the first may be of a relatively limited and localized character.

Fluorescence

AEDANS conjugates of phosphorylase B displayed a single emission band with a maximum close to 500 nm (Fig. 4). No significant change in the peak shape or in the position of the emission maximum occurred in the presence of AMP. However, there was a significant decrease in the intensity of fluorescence at 500 nm upon AMP addition. The effect attained its limiting extent at AMP levels above 0.2 mM. The maximum change was about 12% for activation at 300 nm. No further change was observed for AMP levels between 0.2 mM and 1 mM. The estimated uncertainty in the fractional change was ±3%. Birkett et al. [5] have observed a similar dependence of intensity upon AMP level for another sulfhydryl-specific fluorescent label. The behavior described here was

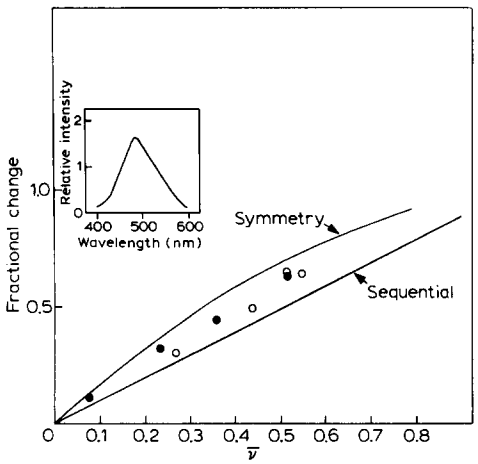

Fig. 4. Fractional change in fluorescence intensity of an AEDANS (3 mg/ml) conjugate of phosphorylase B (○) and of ESR signal for a conjugate of phosphorylase B (3 mg/ml) with spin label I (●) as a function of AMP bound per monomer unit. For the fluorescence measurements the excitation and emission wavelengths are 300 nm and 500 nm, respectively. For the ESR measurements the peak-to-trough separation of the downfield band was taken as a measure of the extent of change. The buffer was 0.1 M glycylglycine, pH 7.0, 23°C. ———, computed variation for sequential model; ———, computed variation for symmetry model. Inset: Emission spectrum for AEDANS conjugate of phosphorylase B. The excitation wavelength was 300 nm.

characteristic of lightly labeled conjugates (<0.3 AEDANS groups per monomer unit).

Fig. 4. shows the fractional change as a function of $\bar{\nu}$ for 0.1 M glycylglycine, 10^{-3} M dithiothreitol, pH 7.0, 23°C. A model of the sequential type [21] would predict that only those monomer units which have combined with AMP would be altered in conformation, reflected by a change in quantum yield of the label. In this case the fractional change would be equal to $\bar{\nu}$, the average number of AMP molecules bound per monomer unit. As Fig. 4 shows the observed points fall consistently above the curve predicted for this model.

Alternatively, if the mechanism were of the symmetry type, then each phosphorylase B dimer which had combined with at least one molecule of AMP would be transformed into the final, active state with both monomer units in equivalent conformations. According to this model the fractional change would be equal to $1-(1/\lambda)$, the fraction of phosphorylase dimers which have bound one or more molecules of AMP. As Fig. 4 shows, the observed data points fall distinctly below the curve predicted for the symmetry model and intermediate to this curve and that expected for a model of the sequential type, although the difference is not in either case greatly in excess of experimental uncertainty.

It is of course difficult to rule out with finality the possibility that reaction with AEDANS has altered the allosteric properties of the enzyme significantly. However, the enzymic activity of the conjugate as determined by the method of Cori et al. [23] was equivalent to that of the native enzyme at saturating (1 mM) and lower (0.05 and 0.1 mM) levels of AMP.

One can only speculate as to the origin of the fall in quantum yield of the label. The microenvironmental changes which it reflects are probably of a limited and localized character (see Discussion), with the implication that the AMP binding site is fairly close to the reactive sulfhydryl [8].

Spin labeled phosphorelase

The ESR spectrum of a phosphorylase B preparation coupled with spin label I showed a pronounced asymmetry, reflected by a broadening and flattening of the upfield and downfield bands. This is indicative of partial immobilization of the label, as was found for a similar nitroxide label by Campbell et al. [6] and Bennick et al. [8]. The observed spectra were similar to those reported earlier [6,8].

The addition of AMP to the spin labeled enzyme in 0.1 M glycylglycine, pH 7.0, results in a progressive change in the shape of the ESR spectrum, corresponding to increased asymmetry. Qualitatively, the direction of the change is that expected for increased immobilization of the label in the presence of AMP. The major change occurs in the downfield band, which is further broadened and flattened.

As in the case of the fluorescent conjugate, a question arises as to the possible alteration of the allosteric properties by sulfhydryl substitution. However, Bennick et al. [8] have found no change in enzymic activity upon conjugation with a similar spin label.

If the peak-to-trough separation of the downfield band is chosen as an index of the extent of change, the fractional change may be tabulated as a function of $\bar{\gamma}$. As Fig. 4 shows the results are consistent with a model intermediate to the symmetry and sequential cases, although the experimental points fall closer to the curve computed for the symmetry model than in the case of the fluorescent AEDANS label.

Discussion

The driving force for the binding of AMP by phosphorylase B appears to be predominantly enthalpic, at least for the conditions cited at 23° C. Moreover the difference in the intrinsic free energies of binding for the two consecutive steps of binding can be largely accounted for in terms of the observed differences in the corresponding enthalpies. The data presented here are suggestive of a significant degree of cooperativity in the binding of AMP by phosphorylase B, although the magnitude of the cooperativity is less than for several other allosteric systems, including hemoglobin.

The physical measurements which respond to the state of all, or a major portion of, the phosphorylase molecule, including hydrodynamic properties and circular dichroism have, in general, failed to give any indication of a conformational change upon the binding of AMP by phosphorylase B [18]. The direct evidence for such a change has been obtained by means of techniques which monitor selectively a restricted portion of the molecule, in particular that in proximity to the reactive sulfhydryl. The existing and earlier data suggest that any conformational change may be of a limited and localized character. The degree of cooperativity observed and the approximate estimate of the free energy change for the transition induced in the second subunit by the binding of a molecule of AMP by the first are consistent with a structural transition of this kind.

The observed cooperative effects may be reconciled with this picture if the two AMP binding sites on the two subunits are in proximity to the zone of

contact and to each other, so that a relatively limited change may be transmitted between subunits.

The results obtained in this study using both the fluorescent label and the spin label appear to fit precisely neither the two-state symmetry model [10] nor the sequential model of Koshland et al. [21], but rather to correspond formally more to an intermediate case, for which both subunits are altered in conformation by the binding of a single molecule of AMP, but are not converted to identical conformations equivalent to that attained at saturating levels of AMP. This picture is in harmony with some earlier suggestions that a third molecular state is involved in the allosteric equilibria of the phosphorylase B-AMP system [2,3,4], although uncertainties in the reported values of extinction coefficient [3,4,17] and molecular weight [18,20], which affect the computed values of γ, render this result tentative.

This observation suggests some difference in behavior between phosphorylase B and phosphorylase A, for which Griffiths et al. [11], using a similar approach, have found adherence to the two-state model. However, a significant structural change, as reflected by changes in the ESR spectrum of an attached spin label [8], accompanies the B → A conversion and it is quite possible that the character of the interaction with AMP may also be modified.

References

1 Madsen, N.B. and Shechosky, S. (1967) J. Biol. Chem. 242, 3301–3307
2 Buc, M.H. and Buc, H. (1968) in Regulation of Enzyme Activity and Allosteric Interactions (Kramme, E. and Pihl, A., ed.), pp. 109–130, Academic Press, New York
3 Kastenschmidt, L.L., Kastenschmidt, J. and Helmreich, E. (1968) Biochemistry 7, 3590–3608
4 Kastenschmidt, L.L., Kastenschmidt, J. and Helmreich, E. (1968) Biochemistry 7, 4543–4556
5 Birkett, D.J., Dwek, R.A., Radda, G.K., Richards, R.E. and Salmon, A.G. (1971) Eur. J. Biochem. 20, 494–508
6 Campbell, I.D., Dwek, R.A., Price, N.C.P. and Radda, G.K. (1972) Eur. J. Biochem. 30, 339–347
7 Buc-Caron, M.H. and Buc, H. (1975) Eur. J. Biochem. 52, 575–588
8 Bennick, A., Dwek, R.A., Griffiths, J.R. and Radda, G.K. (1973) Ann. N.Y. Acad. Sci. 222, 175–190
9 Ho, H.C. and Wang, J.H. (1973) Biochemistry 12, 4750–4755
10 Monod, J., Wyman, J. and Changeux, J.P. (1975) J. Mol. Biol. 12, 88–118
11 Griffiths, J.R., Price, N.C. and Radda, G.K. (1974) Biochim. Biophys. Acta 358, 275–280
12 Sanner, T. and Tron, L. (1975) Biochemistry 14, 230–235
13 Hudson, E.H. and Weber, G. (1973) Biochemistry 12, 4154–4161
14 McConnell, H.M. and McFarland, B.G. (1970) Q. Rev. Biophys. 3, 91–136
15 Fischer, E.H. and Krebs, E.G. (1962) Methods Enzymol. 5, 369–373
16 Hummel, J.P. and Dreyer, W.J. (1962) Biochim. Biophys. Acta 63, 529–532
17 Appleman, M.M., Yunis, A.A., Krebs, E.G. and Fisher, E.H. (1963) J. Biol. Chem. 238, 1358–1361
18 Fischer, E.H., Pocker, A. and Saari, J.C. (1971) Essays in Biochemistry, Vol. 6, pp. 23–40, Academic Press, New York
19 Magar, M.E., Steiner, R.F. and Fletcher, J.E. (1971) J. Theor. Biol. 32, 59–74
20 Cohen, P., Duswer, T. and Fischer, E.H. (1971) Biochemistry 10, 2683–2694
21 Van Holde, K.E. (1970) Physical Biochemistry, Prentice-Hall, Englewood Cliffs, New Yersey
22 Koshland, D.E., Nemethy, G. and Filmer, D. (1966) Biochemistry 5, 365–385
23 Cori, G.T., Illingworth, B. and Keller, P.J. (1955) in Methods in Enzymology, (Colowick, S. and Kaplan, N.O., eds.), Vol. 1, pp. 200–203, Academic Press, New York