Structural Mapping of Rabbit Muscle Phosphofructokinase. Distance between the Adenosine Cyclic 3′,5′-Phosphate Binding Site and a Reactive Sulfhydryl Group†

David W. Craig‡ and Gordon G. Hammes*

ABSTRACT: The cAMP binding site of rabbit muscle phosphofructokinase has been labeled with the fluorescent molecule 5′-(*p*-fluorosulfonylbenzoyl)-2-aza-1,N^6-ethenoadenosine. The most reactive sulfhydryl group of this modified enzyme, which is catalytically active, has been labeled with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole and with *N*-[4-(dimethylamino)-3,5-dinitrophenyl]maleimide. The calculated distances between the cAMP binding site and the most reactive sulfhydryl group, as determined by resonance energy transfer measurements, are 31 and 26 Å, respectively, for the two sulfhydryl group labels. Both steady-state and fluorescent lifetime techniques were used to measure the energy transfer efficiencies in 50 mM potassium phosphate (pH 8.0) and 1 mM ethylenediaminetetraacetic acid, and a value of 2/3 was assumed for the donor–acceptor orientation factor. If the difference in calculated distances is attributed to a difference in the orientation factor for the two donor–acceptor pairs, the actual distance between the cAMP ligand binding site and the most reactive sulfhydryl group on phosphofructokinase is shown to be 28 ± 6 Å.

Rabbit skeletal muscle phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) plays an important role in regulating the metabolic flux through the glycolytic and pentose phosphate pathways (Cori, 1942; Lowry et al., 1964). The catalytic and regulatory properties of the enzyme are dependent upon pH, concentration, and the presence or absence of regulatory ligands such as cAMP, ATP, and citrate (Passoneau & Lowry, 1962; Aaronson & Frieden, 1972; Pavelich & Hammes, 1973; Lad et al., 1973). Phosphofructokinase contains identical polypeptide chains of molecular weight 80 000 and exists in multiple aggregation states; the dimer and tetramer probably predominate under physiological conditions, the former being catalytically inactive and the latter being active (Pavelich & Hammes, 1973; Coffee et al., 1973). Several models have been proposed to explain the regulatory properties of the enzyme in terms of a dimer–tetramer aggregation and a concerted conformational change (Kemp et al., 1976; Bock & Frieden, 1976; Goldhammer & Hammes, 1978; Pettigrew & Frieden, 1979).

In this investigation, fluorescence resonance energy transfer has been used to measure the distance between the cAMP binding site and the most reactive sulfhydryl group of the enzyme (Kemp & Forest, 1968). The cAMP site has been covalently labeled with the fluorescent probe FSBaεA[1] and has been used as an energy donor to two different energy acceptors, NBD and DDPM, located at a reactive sulfhydryl group. The intramolecular distance between donor and acceptors was found to be 28 ± 6 Å.

Experimental Section

Materials. ATP, fructose 6-phosphate, dithiothreitol, aldolase, α-glycerophosphate dehydrogenase, triosephosphate isomerase, bovine serum albumin, and NBD-Cl were purchased from Sigma Chemical Co.; DDPM and *N*-ethylmaleimide were from Eastman Kodak; quinine bisulfate and *p*-(fluorosulfonyl)benzoyl chloride were from Aldrich Chemical Co.; 2-aza-1,N^6-ethenoadenosine was from Molecular Probes; the Ludox HS-30 colloidal silica was from E. I. du Pont de Nemours. Other chemicals were the best available commercial grades, and all solutions were prepared with doubly deionized water.

Preparation of FSBaεA. The FSBaεA was synthesized by the procedure of Wyatt & Coleman (1977). The hexamethylphosphoric triamide was dried with calcium hydride by refluxing and distilling in vacuo. 2-Aza-1,N^6-ethenoadenosine (50 mg, 0.17 mmol) was dissolved in 0.41 mL of hexamethylphosphoric triamide by warming to 50 °C in a water bath. After the solution was cooled to room temperature, *p*-(fluorosulfonyl)benzoyl chloride (55 mg, 0.25 mmol) was added. The reaction mixture was allowed to stand at room temperature for 18 h and then was extracted 3 times with 2-mL portions of petroleum ether. The reaction product was precipitated by slow addition of ~10 mL of ethyl acetate–diethyl ether (1:1 v/v). If an oil formed, it was converted to a solid by trituration. This procedure yielded 50–70 mg of air-dried product. The product exhibited a single fluorescent spot on Eastman Kodak silica gel plates (13179 silica gel) when developed in a solvent system of 2-butanone–acetone–water (65:20:15 v/v/v). Relative mobilities were 0.66 for FSBaεA and 0.41 for 2-aza-1,N^6-ethenoadenosine.

Phosphofructokinase. Rabbit skeletal muscle phosphofructokinase was purified by the method of Ling et al. (1966). The final ammonium sulfate precipitate was dissolved in 0.1 M potassium phosphate, 1 mM EDTA, and 0.5 mM dithiothreitol at pH 8.0 and dialyzed against the same buffer to give a stock solution of 10–15 mg/mL that was stable for up to 4 weeks at 4 °C. The enzyme concentration was determined by using an extinction coefficient of 1.02 mL mg^{-1} cm^{-1} (Parmeggiani et al., 1966). The specific activity of the enzyme was determined as previously described (Lad et al., 1977). The assay mixture consisted of 33 mM Tris-HCl, 2 mM ATP, 5 mM $MgCl_2$, 2 mM fructose 6-phosphate, 0.1 mM NADH,

† From the Department of Chemistry, Cornell University, Ithaca, New York 14853. *Received August 8, 1979.* This work was supported by a grant from the National Institutes of Health (GM 13292).

‡ National Institutes of Health Postdoctoral Fellow (AM05855).

[1] Abbreviations used: FSBaεA, 5′-[*p*-(fluorosulfonyl)benzoyl]-2-aza-1,N^6-ethenoadenosine; NBD-Cl, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; DDPM, *N*-[4-(dimethylamino)-3,5-dinitrophenyl]maleimide; Tris, tris(hydroxymethyl)aminoethane; EDTA, ethylenediaminetetraacetic acid.

0006-2960/80/0419-0330$01.00/0 © 1980 American Chemical Society

2 mM dithiothreitol, 0.20 unit/mL aldolase, 35 units/mL triosephosphate isomerase, 3.2 units/mL α-glycerophosphate dehydrogenase, and 0.03–0.13 μg/mL phosphofructokinase at pH 8.0 in a total volume of 3.0 mL. The reaction was initiated by the addition of phosphofructokinase, and the production of NADH was monitored at 340 nm in a Cary 118C spectrophotometer thermostated at 25 °C. The phosphofructokinase used in the experiments described here had a specific activity of 100–130 units/mg at 25 °C (1 unit = 1 μmol of fructose 1,6-bisphosphate produced per min).

Chemical Modification of Phosphofructokinase. Phosphofructokinase was labeled with FSBaεA by using the following procedure. Enzyme was dialyzed for 24 h against 50 mM potassium phosphate and 10 mM EDTA at pH 8.0 to remove dithiothreitol and to lower the phosphate concentration. The reaction was initiated by diluting the enzyme to 0.1 mg/mL into the same buffer containing 10% dimethylformamide and 100 μM FSBaεA at 25 °C. The reaction was quenched by chilling the reaction mixture to 0 °C on ice, adding 0.5 mM dithiothreitol and 100 μM cAMP, and dialyzing against 50 mM potassium phosphate (pH 8.0), 1 mM EDTA, and 1 mM dithiothreitol for 5–8 h. The labeled enzyme was concentrated to 1–2 mg/mL by ultrafiltration with a PM30 Amicon membrane, and the enzyme was further dialyzed against the same buffer. As a control, an enzyme solution was treated in parallel except the FSBaεA was omitted. The extent of reaction was determined by measuring the increase in absorbance relative to the control at 350 nm with an extinction coefficient of 2.01×10^3 M^{-1} cm^{-1} (Tsou et al., 1974).

Modification of the reactive sulfhydryl on FSBaεA-modified phosphofructokinase was accomplished by using conditions previously described (Lad et al., 1977): FSBaεA-modified enzyme was dialyzed against 25 mM diglycine, 25 mM potassium phosphate, 1 mM EDTA, 5 mM ATP, and 5 mM fructose 6-phosphate at pH 7.0; the enzyme concentration was adjusted to 1.0–1.5 mg/mL and the reaction was initiated by adding either a 1.5 molar excess of DDPM or *N*-ethylmaleimide or a 2.5 molar excess of NBD-Cl. The 10 mM NBD-Cl, *N*-ethylmaleimide, and DDPM stock solutions were in dimethyl sulfoxide. The time course for the reaction of enzyme with NBD-Cl was monitored by measuring the increase in absorbance at 420 nm in a Cary 118C spectrophotometer thermostated at 25 °C. The course of the reaction of enzyme with DDPM was monitored by measuring the quenching of enzyme fluorescence at 490 nm (350-nm excitation) in a Hitachi Perkin-Elmer MPF-3 fluorescence spectrophotometer thermostated at 25 °C. The extent of modification of the enzyme with *N*-ethylmaleimide was monitored by measuring the decrease in catalytic activity. The reactions were quenched by cooling to 0 °C on ice, adding dithiothreitol to 0.5 mM when DDPM or *N*-ethylmaleimide was a reactant, and applying the mixture to a PD-10 column (Pharmacia Fine Chemicals). The doubly modified enzyme was eluted with 50 mM potassium phosphate and 1 mM EDTA at pH 8.0. The number of modified sulfhydryl groups was determined spectrophotometrically with a molar extinction coefficient of 1.3×10^4 M^{-1} cm^{-1} at 420 nm for the NBD product (Birkett et al., 1971) and 3.0×10^3 M^{-1} cm^{-1} at 440 nm for the DDPM product (Gold & Segal, 1964). Prior to spectroscopic measurements, the enzyme was either centrifuged at 15000*g* for 20 min or filtered through 0.45-μm pore size Millipore filters to remove particulate material. The concentrations of the modified enzymes were determined by the procedure of Bensadoun & Weinstein (1976).

Table I: Properties of Modified Phosphofructokinase

modification	sp act. (units/mg)	% control	quantum yield	fluorescence lifetime (ns)
control	63.4	100		
FSBaεA	48.8	77	0.18	44.5
FSBaεA-NBD	11.1	17.5	0.12	24.0
FSBaεA-NBD plus dithiothreitol	36.1	57	0.18	35.3
FSBaεA-DDPM	10.9	17.2	0.090	22.4

Steady-State Fluorescence Measurements. Steady-state fluorescence measurements were made with a Hitachi Perkin-Elmer MPF-3 fluorescence spectrophotometer equipped with a polarization accessory. An excitation wavelength of 350 nm was used for all measurements. The quantum yield of enzyme-bound FSBaεA, Q_2, was determined at 25 °C in 50 mM potassium phosphate (pH 8.0) and 1 mM EDTA by using the relationship (Parker & Rees, 1966)

$$\frac{Q_2}{Q_1} = \frac{F_2 A_1}{F_1 A_2} \tag{1}$$

where Q is the quantum yield of quinine bisulfate in 0.1 N H_2SO_4, 0.7 (Scott et al., 1970), the F_i's are the areas of the corrected emission spectra, and the A_i's are the absorbances at the excitation wavelength.

The fluorescence emission polarization was determined as previously described (Azumi & McGlynn, 1962). The limiting polarization, P_0, was determined by plotting the reciprocal of polarization vs. T/η for a series of solutions containing 50 μM FSBaεA and 10–60% (w/v) sucrose in the same buffer as above. (T is the absolute temperature and η is the solvent viscosity; Perrin, 1926.)

Fluorescence Lifetime Measurements. Fluorescence decay times were measured in a 3-mm square cuvette with an Ortec 9200 nanosecond fluorescence spectrophotometer (Matsumoto & Hammes, 1975). The excitation and emission wavelengths, 350 and 490 nm, were obtained with interference filters (Ditric Optics, Inc.). A colloidal silica (Du Pont Ludox HS-30) solution was used to determine the shape of the lamp pulse. All fluorescence decay data were collected for the same length of time at 25 °C in 50 mM potassium phosphate (pH 8.0) and 1 mM EDTA, and the protein-scattering component of the decay curve was eliminated by subtraction of the decay curve of a sample containing only unmodified control enzyme. Data were analyzed on a PDP 11/20 computer with the convolution and deconvolution programs supplied by Ortec.

Results

Phosphofructokinase can be modified at pH 8.0 with the fluorescent reagent FSBaεA. The modification appears to be covalent since it is stable to extensive dialysis for up to 5 days. The increase in absorbance at 350 nm of modified phosphofructokinase relative to native phosphofructokinase indicates that only a single site per 80000 molecular weight is modified. The FSBaεA modification results in a small loss of catalytic activity at pH 8.0 (Table I).

The specificity of reaction was demonstrated by the experiments shown in Figure 1. The extent of phosphofructokinase modification at pH 8.0 is plotted vs. time for the reaction with FSBaεA in the presence of 5 mM MgATP, in the presence of 5 mM cAMP, and in the absence of these ligands. Incorporation of FSBaεA into phosphofructokinase is almost completely inhibited by cAMP and only slightly retarded by MgATP. Furthermore, Pettigrew & Frieden (1978) have shown that modification of phosphofructokinase by 5′-[*p*-(fluorosulfonyl)benzoyl]adenosine relieves the ATP inhibition

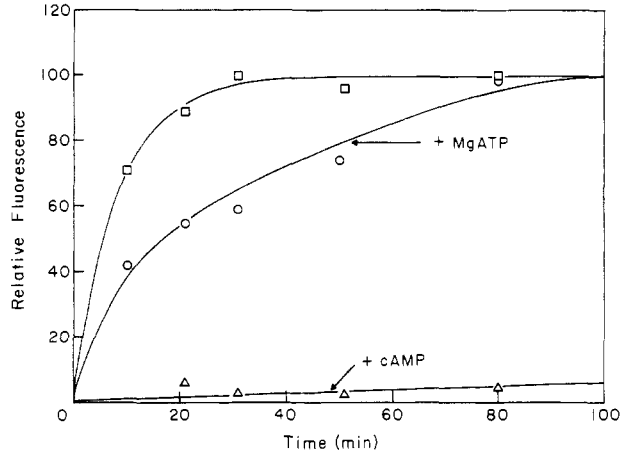

FIGURE 1: Time course of the incorporation of FSBaεA (50 μM) into phosphofructokinase (1.4 mg/mL) in 50 mM potassium phosphate and 1 mM EDTA, pH 8.0, at 25 °C. (□) No additions; (○) 1 mM ATP and 3 mM $MgCl_2$; (△) 1 mM cAMP. Fluorescence emission was measured at 490 nm with excitation at 356 nm.

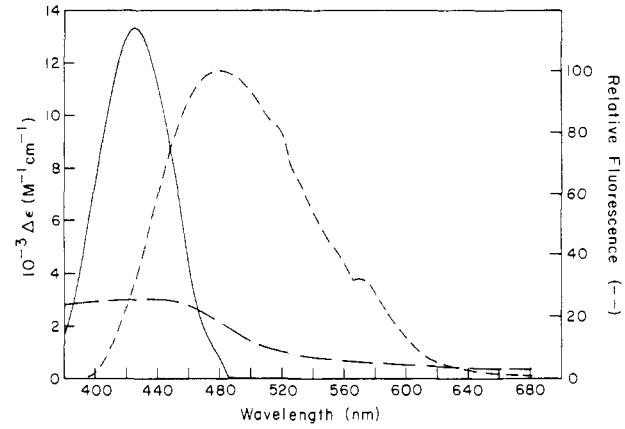

FIGURE 2: Overlap of the corrected fluorescence emission spectrum (356-nm excitation) of enzyme-bound FSBaεA (---) with the extinction coefficient of enzyme-bound NBD (—) and the extinction coefficient of enzyme-bound DDPM (– – –) in 50 mM potassium phosphate (pH 8.0) and 1 mM EDTA at 25 °C.

at pH 7.0 which is characteristic of the native enzyme with cAMP bound. These results suggest that only the cAMP site is modified by FSBaεA.

Phosphofructokinase modified with FSBaεA has a quantum yield of 0.18 at 25 °C (356-nm excitation, Figure 2). The excitation maximum is at 356 nm and the polarization at 25 °C is 0.24 with a limiting polarization of 0.31 (356-nm excitation; 490-nm emission). The unreacted FSBaεA has excitation and emission maxima at the same frequencies but with a quantum yield of 0.089 and a polarization of 0.0.

Previous workers have shown that a single sulfhydryl group can be modified on each subunit of phosphofructokinase (Kemp & Forest, 1968; Schwartz et al., 1976; Lad et al., 1977). Enzyme labeled with FSBaεA has been further modified with two sulfhydryl reagents. As shown in Figure 3, a single sulfhydryl group is modified by NBD-Cl and DDPM. The time course of reaction also can be measured by the quenching of fluorescence of the FSBaεA moiety on the enzyme: the time course for labeling of the enzyme with DDPM determined in this manner is included in Figure 3. When the sulfhydryl group is labeled with *N*-ethylmaleimide, virtually no fluorescence quenching is detectable during the course of reaction, as shown in Figure 3. This indicates that the quenching observed with DDPM and NBD can be attributed to energy transfer.

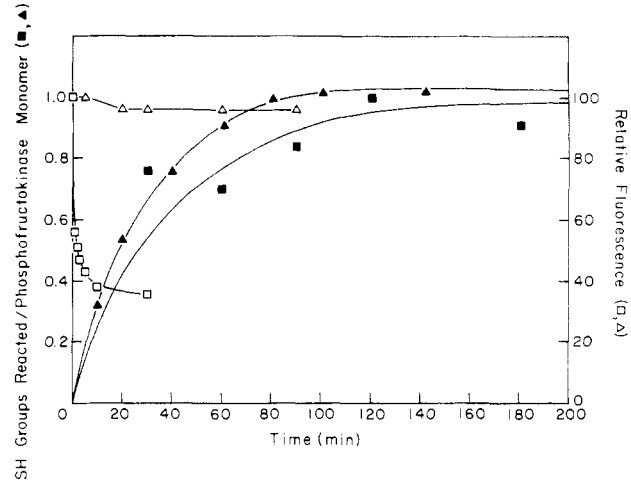

FIGURE 3: Time course of the sulfhydryl group modification of FSBaεA-phosphofructokinase (1 mg/mL) with NBD-Cl, DDPM, and *N*-ethylmaleimide. The number of modified sulfhydryl groups was determined by measuring the increase in absorbance at 420 nm for NBD-Cl (▲) and at 440 nm for DDPM (■). The fluorescence quenching (356-nm excitation; 490-nm emission) upon modification with DDPM (□) and *N*-ethylmaleimide (△) also is shown. The experimental conditions are described under Experimental Section.

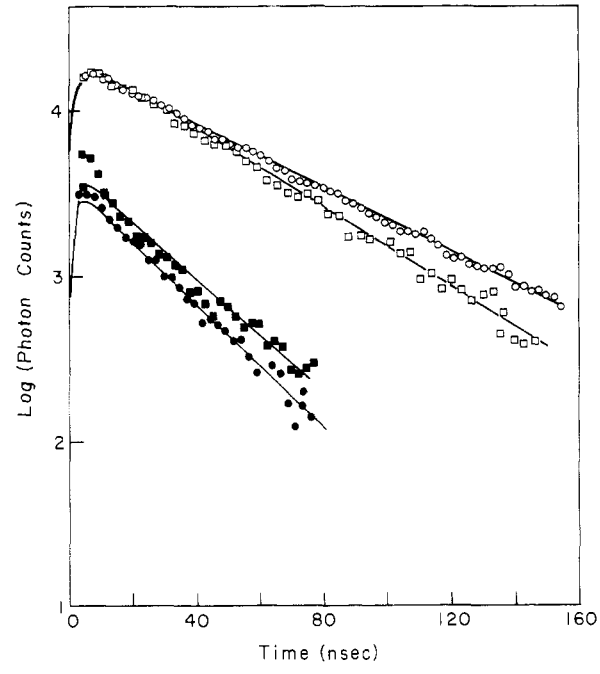

FIGURE 4: Fluorescence decay curves of FSBaεA-modified enzyme (0.8 mg/mL) in 50 mM potassium phosphate (pH 8.0) and 1 mM EDTA at 25 °C (350-nm excitation; 490-nm emission). FSBaεA–enzyme (○); FSBaεA–DDPM–enzyme (●); FSBaεA–NBD–enzyme (■); FSBaεA–NBD–enzyme plus dithiothreitol (□). The lines have been calculated assuming the fluorescence lifetimes in Table I.

Table I summarizes the spectral and catalytic properties of the modified enzyme products.

The fluorescence decay curve of FSBaεA-modified enzyme can be fit to a single exponential with a lifetime of 44.5 ns at 25 °C. A typical decay curve is presented in Figure 4. The fluorescence decay of doubly labeled phosphofructokinase also can be described by a single exponential although the quenching of fluorescence was so great that light scattering caused some deviation from the theoretical curves in the initial portion of the decay curve. Typical decay curves of the doubly modified enzymes are shown in Figure 4, and the lifetimes are included in Table I.

Table II: Energy Transfer Parameters[a]

energy transfer pair	R_0 (Å)	steady state		lifetime	
		E	R (Å)	E	R (Å)
FSBaεA-NBD	27.7	0.33[b]	31	0.46[b]	28
		0.33[c]	31	0.32[c]	31
FSBaεA-DDPM	25.6	0.49	26	0.47	26

[a] 50 mM potassium phosphate (pH 8.0) and 1 mM EDTA, 25 °C. [b] Calculated by using FSBaεA-modified enzyme as the reference. [c] Calculated by using FSBaεA-NBD-modified enzyme plus dithiothreitol as the reference.

The FSBaεA is an energy donor for resonance energy transfer to NBD or DDPM. The efficiency of energy transfer is defined as (Förster, 1959)

$$E = 1 - Q_{DA}/Q_D = 1 - \tau_{DA}/\tau_D \quad (2)$$

where Q_{DA} and Q_D are the quantum yields of the donor in the presence and absence of the energy acceptor, respectively, and τ_{DA} and τ_D are the corresponding fluorescence lifetimes. For an isolated donor–acceptor pair, the efficiency is related to the distance between the energy donor and acceptor, R, according to the relationship (Förster, 1959)

$$E = \frac{R^{-6}}{R^{-6} + R_0^{-6}} \quad (3)$$

where

$$R_0 = (9.79 \times 10^3)(Q_D J \kappa^2 n^{-4})^{1/6} \quad (4)$$

In eq 4 J is the normalized spectral overlap integral, n is the refractive index of the medium, and κ^2 is a dipole–dipole orientation factor. The spectral overlap integral is a measure of how well the fluorescence emission of the donor overlaps the absorption spectrum of the acceptor and is calculated from the equation

$$J = \frac{\int F(\lambda)\epsilon(\lambda)\lambda^4 \, d\lambda}{\int F(\lambda) \, d\lambda} \quad (5)$$

where F is the donor fluorescence, ϵ is the molar extinction coefficient of the acceptor, and λ is the wavelength. The overlap of the emission spectrum of FSBaεA-modified enzyme with the absorption spectrum of the energy acceptors is shown in Figure 2. The orientation factor, κ^2, can range from 0 to 4.0. If the acceptor and donor rotate rapidly relative to the donor excited-state lifetime, κ^2 is 2/3. The calculated values of R_0, assuming κ^2 to be 2/3 and n = 1.4, are summarized in Table II for the two donor–acceptor pairs. The energy transfer efficiencies and calculated distances between donor and acceptor also are presented in Table II.

When NBD was used as an energy acceptor, the quantum yield and lifetimes of the donor in the presence and absence of the acceptor could be measured easily by displacing NBD with 1 mM dithiothreitol. This eliminates complications due to errors in determination of concentrations and in differences in light scattering from sample to sample. Such a procedure is not possible when DDPM is the label; therefore, energy transfer efficiencies determined with this acceptor are subject to more uncertainty. The efficiencies and distances have been calculated by using both the FSBaεA-modified enzyme and the FSBaεA–NBD-modified enzyme plus dithiothreitol as reference substances (Table II). Steady-state energy transfer measurements with the doubly modified enzymes in 50 mM phosphate and 1 mM EDTA (pH 7.0) give the same energy transfer efficiencies as those at pH 8.0.

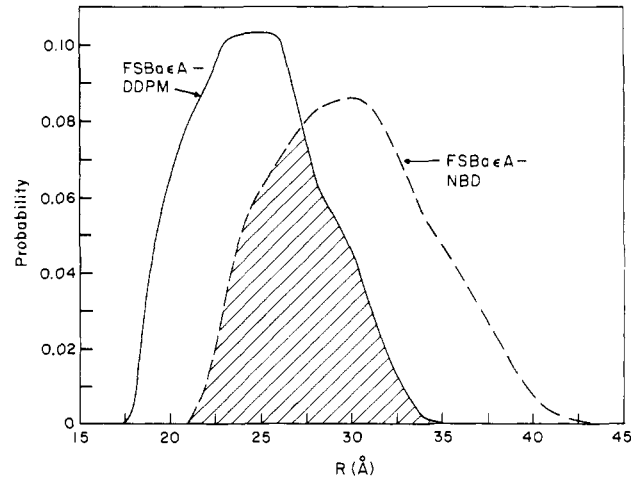

FIGURE 5: Probability distribution of R for the two donor–acceptor pairs. FSBaεA–DDPM (—); FSBaεA–NBD (---).

Discussion

The results reported here indicate that the cAMP regulatory site on rabbit muscle phosphofructokinase (one per protomer of molecular weight 80 000) has been covalently labeled with the fluorescent probe FSBaεA. The nonfluorescent molecule 5′-[*p*-(fluorosulfonyl)benzoyl]adenosine reacts with rabbit muscle and sheep heart phosphofructokinase in a similar manner (Pettigrew & Frieden, 1978; Mansour & Martensen, 1978). In addition, a single sulfhydryl group of FSBaεA–phosphofructokinase has been modified with NBD-Cl and DDPM. The stoichiometry and kinetics of modification are similar to those of the native enzyme (Kemp & Forest, 1968; Schwartz et al., 1976; Lad et al., 1977). The enzymatic activity of FSBaεA–phosphofructokinase is similar to that of the control enzyme although dimethylformamide causes a significant loss in activity of the control. However, the specificity of the sulfhydryl group modification indicates the native structure has not been appreciably altered. The extent of loss of activity after sulfhydryl group modification is similar to that found with the native enzyme (Lad et al., 1977).

Since modification of the reactive sulfhydryl group by *N*-ethylmaleimide does not quench or shorten the fluorescence lifetime of enzyme-bound FSBaεA, the quenching and shortening can be attributed to energy transfer when the sulfhydryl group is modified with NBD-Cl or DDPM. The efficiencies of energy transfer determined from quantum yield and fluorescence lifetime measurements are in good agreement, especially if FSBaεA–NBD–enzyme plus dithiothreitol is used as the reference (Table II). When NBD is displaced by dithiothreitol, the activity and fluorescence lifetime do not return quantitatively to their original values. This slight irreversibility has been noted previously (Wolfman & Hammes, 1977), but its basis is unknown.

The major uncertainty in calculating the distance between enzyme-bound FSBaεA and the modified sulfhydryl group is in assigning a value to κ^2. A probability distribution of R due to the uncertainties in κ^2 and the energy transfer efficiency can be calculated from the fluorescence polarization of enzyme-bound FSBaεA (Hillel & Wu, 1976; Dale & Eisinger, 1974). The measured polarization indicates the transition moment can be described by a precession of 26° on the surface of a cone (Dale & Eisinger, 1974). The probability distributions calculated for the two donor–acceptor pairs assuming this angle and an uncertainty of ±10% in the energy transfer

efficiency are shown in Figure 5. This calculation assumes the energy acceptor is rigidly bound to the enzyme; however, the fluorescence polarization of *N*-[*p*-(2-benzoxazolyl)-phenyl]maleimide and 4-(dimethylamino)-4′-maleimidostilbene covalently bound to the sulfhydryl, 0.35 and 0.36, respectively (N. M. Wolfman, unpublished results), suggests that the energy acceptor has some mobility; this would reduce the width of the probability distributions. If the difference in R calculated with the two donor–acceptor pairs is attributed to a difference in κ^2, the actual distance must be within the area of overlap of the two probability distributions. This region is shaded in Figure 5 and shows the most probable distance is 28 Å. The minimum and maximum values of R are 22 and 34 Å. This may represent the first known case where significant deviations from $\kappa^2 = 2/3$ have been experimentally demonstrated. The possibility exists that intersubunit energy transfer may occur since the enzyme is predominantly tetrameric under the experimental conditions employed. Because this possibility cannot be excluded, the calculated distance must be strictly regarded as a lower bound. Although phosphofructokinase displays regulatory properties at pH 7 and not at pH 8, the energy transfer efficiencies are the same at both pH values. This suggests the enzyme conformation is quite similar at both pH 7 and pH 8.

Resonance energy transfer now has been used to establish that the distance between the most reactive sulfhydryl group and the cAMP binding site is 28 Å and that the distance between this same sulfhydryl and the citrate binding site is 40 Å (Wolfman & Hammes, 1977). Electron spin resonance studies have suggested that the sulfhydryl group is near a MnATP binding site, which may be catalytic or regulatory (Jones et al., 1973). These results indicate the ligand binding sites on phosphofructokinase are separated by relatively large distances, which is consistent with the regulatory mechanism being allosteric in nature.

References

Aaronson, R. P., & Frieden, C. (1972) *J. Biol. Chem. 247*, 7502.

Azumi, T., & McGlynn, S. P. (1962) *J. Chem. Phys. 37*, 2413.

Bensadoun, A., & Weinstein, D. (1976) *Anal. Biochem. 70*, 241.

Birkett, D. J., Dwek, R. A., Radda, G. K., Richards, R. E., & Salmon, A. G. (1971) *Eur. J. Biochem. 20*, 494.

Bock, P. E., & Frieden, C. (1976) *J. Biol. Chem. 251*, 5630.

Coffee, C. J., Aaronson, R. P., & Frieden, C. (1973) *J. Biol. Chem. 248*, 1381.

Cori, C. F. (1942) in *A Symposium on Respiratory Enzymes*, p 175, University of Wisconsin Press, Madison, WI.

Dale, R. E., & Eisinger, J. E. (1974) *Biopolymers 13*, 1573.

Förster, T. (1959) *Discuss. Faraday Soc. 27*, 7.

Gold, A. H., & Segal, H. L. (1964) *Biochemistry 3*, 778.

Goldhammer, A. R., & Hammes, G. G. (1978) *Biochemistry 17*, 1818.

Hillel, Z., & Wu, C.-W. (1976) *Biochemistry 15*, 2105.

Jones, R., Dwek, R. A., & Walker, I. O. (1973) *Eur. J. Biochem. 34*, 28.

Kemp, R. G., & Forest, P. (1968) *Biochemistry 7*, 2596.

Kemp, R. G., Tsai, M. Y., & Colombo, G. (1976) *Biochem. Biophys. Res. Commun. 68*, 942.

Lad, P. M., Hill, D. R., & Hammes, G. G. (1973) *Biochemistry 12*, 4303.

Lad, P. M., Wolfman, N. M., & Hammes, G. G. (1977) *Biochemistry 16*, 4802.

Ling, K. H., Paetkau, V., Marcus, F., & Lardy, H. A. (1966) *Methods Enzymol. 9*, 425.

Lowry, O. H., Passoneau, J. V., Hasselberger, F. X., & Schulz, D. W. (1964) *J. Biol. Chem. 239*, 18.

Mansour, T., & Martensen, T. (1978) *J. Biol. Chem. 253*, 3628.

Matsumoto, S., & Hammes, G. G. (1975) *Biochemistry 14*, 214.

Parker, C. A., & Rees, W. T. (1966) *Analyst (London) 85*, 587.

Parmeggiani, A., Luft, J. M., Love, D. S., & Krebs, E. G. (1966) *J. Biol. Chem. 241*, 4625.

Passoneau, J. V., & Lowry, O. H. (1962) *Biochem. Biophys. Res. Commun. 7*, 10.

Pavelich, M. J., & Hammes, G. G. (1973) *Biochemistry 12*, 1408.

Perrin, F. (1926) *J. Physiol. (London) 7 (6)*, 390.

Pettigrew, D. W., & Frieden, C. (1978) *J. Biol. Chem. 253*, 3623.

Pettigrew, D. W., & Frieden, C. (1979) *J. Biol. Chem. 254*, 1896.

Schwartz, K. L., Nakagawa, Y., & Kaiser, E. T. (1976) *J. Am. Chem. Soc. 98*, 6369.

Scott, T. G., Spencer, R. D., Leonard, N. J., & Weber, G. (1970) *J. Am. Chem. Soc. 92*, 687.

Tsou, K. C., Yip, K. F., Miller, E. E., & Lo, K. W. (1974) *Nucleic Acids Res. 1*, 531.

Wolfman, N. M., & Hammes, G. G. (1977) *Biochemistry 16*, 4806.

Wyatt, J. L., & Coleman, R. (1977) *Biochemistry 16*, 1333.