details of the computer calculation, see Vasavada et al. (1980). The theoretical spectrum is obtained by calculating $\mathcal{J}(\omega)$ for a number of closely spaced values of ω spanning the width of the observed spectrum.

Registry No. ATP, 56-65-5; ADP, 58-64-0; AMP, 61-19-8; ATP·Mg, 1476-84-2; ADP·Mg, 7384-99-8; adenylate kinase, 9013-02-9.

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Ligand Binding Site Interaction in Adenosine Cyclic 3′,5′-Monophosphate Dependent Protein Kinase Catalytic Subunit: Circular Dichroic Evidence for Intramolecular Transmission of Conformational Change†

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ABSTRACT: Blue Dextran has been shown to interact specifically with the nucleotide binding site of the catalytic subunit of cAMP-dependent protein kinase. By observing changes in the induced dichroism associated with the absorbance of the bound chromophore, one can monitor conformational changes in the immediate vicinity of the ATP binding site. With this technique, it has been possible to demonstrate that attachment of ligand at the protein substrate binding site of the enzyme results in a conformational change at the ATP binding site. This alteration takes place in at least two steps, one of which appears to be dependent on the presence of a phosphorylatable hydroxyl group on the substrate and the other being triggered by the "basic subsite" (usually one or more arginine residues) to the N-terminal side of the target serine or threonine. Competition experiments suggest that the change induced results in closure over the substrate protein after the initial electrostatic binding; the movement initiated by the presence of a serine hydroxyl group may also involve interaction with a tyrosine residue at the surface of the ATP binding site.

The precise method through which the phosphorylation of selected substrate proteins is carried out by cAMP-dependent protein kinase (EC 2.7.1.37) has been a surprisingly durable problem in research on this enzyme. It has been known for some time that the holoenzyme, consisting of two regulatory and two catalytic subunits, dissociates in the presence of cAMP to form a regulatory dimer and two active catalytic subunits (Langan, 1967). As the intracellular mediator of a number of hormonally regulated processes, the active catalytic subunit was expected to display a fair degree of substrate specificity. In vitro, however, it acts relatively nonspecifically, the only limiting factors at the molecular level being (a) the presence of a serine or threonine hydroxyl group in an exposed position on the protein and (b) the presence of one or more arginine residues to the N-terminal side of the phosphorylated site (Daile et al., 1975; Kemp et al., 1977; Yeaman et al., 1977; Kemp, 1978; Feramisco et al., 1979; Meggio et al., 1981). The search for further control mechanisms operating within the cell has implicated a number of possible processes. The most direct of these involves alteration of the tertiary structure of the enzyme itself: inhibition or modulation of activity by the binding of appropriate ligands, often small, acid-stable proteins (Beale et al., 1977; Demaille et al., 1977; Szmigielski et al., 1977; Ferraz et al., 1979; Hashizome & DeGroot, 1979). Recent work in this laboratory has shown that such modulators

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form a more complex system than was previously supposed (Gagelmann et al., 1980; Reed et al., 1983). It seemed important to learn more about the relation between the tertiary structure of the enzyme and its activity.

Circular dichroic spectra of proteins are a proven method of analyzing their conformation. Since all polypeptides exhibit an intrinsic dichroism in the ultraviolet region of the spectrum, in investigations with an enzyme such as protein kinase that binds other proteins both as substrate and as modifiers it is useful if one can arrange to work with enzyme-specific dichroism in the visible region. Protein kinase does not, like heme proteins, offer the convenience of a native chromophore absorbing at higher wavelengths.

Blue Dextran, a sulfonated polyaromatic blue dye, has been shown to interact with the nucleotide binding sites of a number of proteins (Thompson et al., 1975). The catalytic subunit of cAMP-dependent protein kinase also binds Blue Dextran at its ATP binding site (Witt & Roskoski, 1975; Reed et al., 1983). By observing the changes in the dichroism induced on the bound Blue Dextran chromophore, it is possible to monitor changes in the conformation of the protein in its immediate vicinity (Edwards & Woody, 1979). Blue Dextran is particularly suited to this purpose for several reasons. First, the circular dichroism (CD) of Blue Dextran bound to dinucleotide-fold enzymes is thought to have significant contributions from coupling to protein transitions as well as those from the inherent chirality of the dye in its bound form (Edwards & Woody, 1979). Second, Blue Dextran does not seem to have a unique conformation mimicing adenine or pyridine nucleotide, which fits the binding site, but rather shifts shape, assuming a conformation peculiar to the binding site involved. This means that changes in the geometry of the binding site will be reflected by changes in the dye probe. Finally, the low-energy transitions that give rise to Blue Dextran absorbance are localized; this means that even a small change in its conformation can drastically alter the induced CD (Edwards & Woody, 1979).

Using Blue Dextran covalently linked to Sepharose offers the advantage of permitting removal of unbound enzyme, ligand, etc. before the CD spectrum is measured. With this technique, it has been possible to observe specific and cooperative conformational changes in the protein kinase catalytic subunit that comprise a mechanism for recognition of appropriate substrate and appear to link such recognition inexorably with the functioning of the enzyme.

Materials and Methods

Catalytic subunit of cAMP-dependent protein kinase type II was prepared from Sprague-Dawley rat muscle as previously described (Kübler et al., 1979). Blue Dextran–Sepharose (BD–S) was prepared by washing 5.0 g of CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) for 15 min at room temperature with 10^{-3} M HCl. The washed beads were suspended in 5.0 mL of 0.4 M Na_2CO_3, pH 10, in which 0.1 g of Blue Dextran 2000 (Pharmacia) had been dissolved. The suspension was rocked gently overnight at 4 °C in a cell-culture shaker set at lowest speed. The resulting Blue Dextran–Sepharose was washed with 1.5 L of 1.0 M KCl and then equilibrated with 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.5.

Protein kinase catalytic subunit was dialyzed against 10 mM Tris-HCl, pH 7.5, and allowed to bind to Blue Dextran–Sepharose. At this ionic strength, the enzyme binds very firmly to Blue Dextran and remains attached to the beads throughout several washings. Usually, 0.2–0.3 mg of enzyme protein was bound to 4 g of wet-packed beads. Although the ratio of enzyme protein to beads was kept constant as far as possible, there is unavoidably some difference in the amount of active enzyme bound from one preparation to another; for this reason, experiments within a single series were always carried out on the same preparation.

Circular dichroism spectra were obtained on a Jasco J-500 spectral polarimeter coupled with a Jasco DP-500 data processor. Signal averaging, base-line subtraction, and curve smoothing were carried out on digitalized signals through the data processor. Spectra covered the region from 700 to 300 nm associated with the induced dichroism on the Blue Dextran chromophore (ϵ_{max} = 612 nm). Samples were measured at room temperature in a dichroically selected quartz cuvette with a 0.5-mm light path. The signal average of from 4 to 16 scans was corrected for background by subtraction of an equivalent signal average of the Blue Dextran–Sepharose curve alone.

Substrates etc. were dissolved in 10 mM Tris-HCl, pH 7.5, and added to the enzyme/bead system before placing it in the cuvette. Concentrations given are calculated on a total volume basis; as the Sepharose beads take up a finite volume, the effective concentration of additives to which the enzyme was exposed will be somewhat higher. Beads were allowed to pack in the cuvette, and the supernatant was withdrawn. Repeated checks of the background curves showed that packing anomalies either were not present or had no effect on the signal.

The use of Sepharose beads introduces the possibility of curve distortion through light scattering. This has been controlled through the following procedures: (a) Spectra have been scanned in the visible region where asymmetric, or Mi, scattering is not a significant factor. (b) The very short light path keeps scattering to a minimum. In addition, the cuvette is placed close to the phototube, so that any distortion is minimized as well. (c) Finally, such scattering distortion as does arise is corrected for by the subtraction of the Blue Dextran–Sepharose base line from all curves.

Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly) and the synthetic peptide substrate analogue Arg-Gly-Tyr-Ala-Leu-Gly were purchased from Peninsula Laboratories, Inc., San Carlos, CA. Glycogen synthase (UDPglucose:glycogen 4-α-glucosyltransferase, EC 2.4.1.11) from rabbit muscle and histone IIa from calf thymus were obtained from Sigma Chemical Co., St. Louis, MO.

Results

The circular dichroism spectrum of the protein kinase catalytic subunit bound to Blue Dextran–Sepharose (Figure 1) exhibits a complex positive peak in the 590–520-nm region (probably consisting of at least one maximum at 580 nm and another around 530 nm) and a broad, weak negative ellipticity at around 410 nm. Such a CD spectrum, with extrema of opposite sign at around 410–420 and (usually complex) 600 nm, is typical of the Cibacron blue/enzyme systems previously reported (Edwards & Woody, 1979). The spectrum of protein kinase is unusual in that the positive ellipticity at 580 nm is blue-shifted from the 612-nm absorbance maximum: it was typical of the kinases studied, however, to exhibit these extrema at somewhat shorter wavelengths than those of the dehydrogenases.

Another unusual feature of the protein kinase induced CD is the steady increase in amplitude of the positive signal extending into the near-UV. For the other Cibacron blue bound enzymes in the literature there is a reversal in ellipticity somewhere between 350 and 300 nm. While the lack of this may be due to unique features of the protein kinase ATP binding site, it is necessary to bear in mind that in a few enzymes containing the dinucleotide fold there are differences

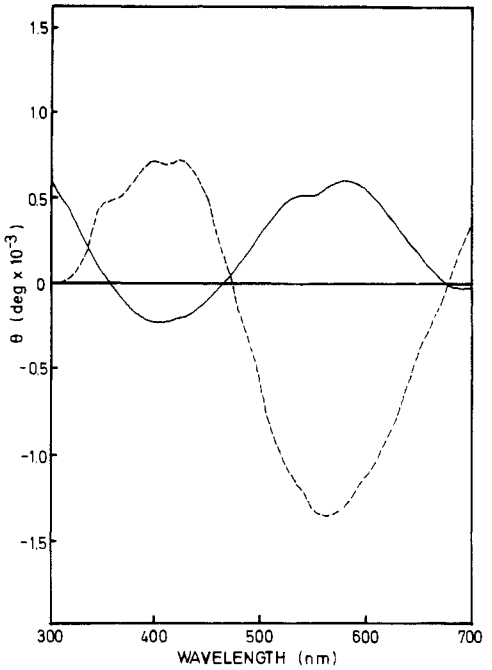

FIGURE 1: CD change on substrate binding. Circular dichroism spectra of protein kinase catalytic subunit bound to Blue Dextran–Sepharose. (—) Bound enzyme alone; (– –) bound enzyme in the presence of saturating amounts of histone IIa. Sensitivity = 0.5 mdeg/cm; time constant = 2 s; signal average of four scans.

in the conformation assumed by the chromophore on binding when it is conjugated with dextran. Aldolase, in particular, shows just such a continuous rise in the near-UV when bound to Blue Dextran although the more usual sign reversal occurs when Cibacron blue is bound (Edwards & Woody, 1979).

The induced dichroism signal of Blue Dextran bound to protein kinase changes when substrate is bound to the enzyme. When saturating amounts of histone IIa are added (Figure 1), the CD displays a strong complex negative maximum centering on 560 nm. At the same time, multiple positive bands appear between 370 and 430 nm. Since induced dichroic effects rarely occur at distances greater than 15 Å and are seldom intense at over 10 Å, the change in the induced CD means that ligand attachment at the substrate binding site causes a propagated conformational change at the ATP binding site.

The highly basic nature of histone, however, means that it is less than ideal as a probe of specifically substrate-induced effects. Ribonuclease and cytochrome *c* (p*I* = 9.6 and 10, respectively) are known to bind nonspecifically to Blue Dextran–Sepharose (Thompson et al., 1975). For this reason, CD spectra were taken of protein kinase bound Blue Dextran in the presence of three substrates with dissimilar physical properties, none of which itself bound adenine nucleotides. These were (a) histone IIa (M_r ca. 20 000; p*I* = 10.8), (b) glycogen synthase (M_r 400 000; p*I* = 5.0), and (c) Kemptide (M_r 772; neutral).

As the only quality the three proteins have in common is their ability to function as a substrate of cAMP-dependent protein kinase, induction of the same CD signal by all three would present a good case for substrate binding as the effective factor in altering the induced dichroism. CD spectra of protein kinase bound Blue Dextran in the presence of saturating amounts of these substrates are presented in Figure 2.

The binding of any one of these substrates to protein kinase causes a change in the CD spectrum, and the altered curves bear a strong family resemblence to one another. In all cases, the positive ellipticity around 590 nm is lost, and a series of

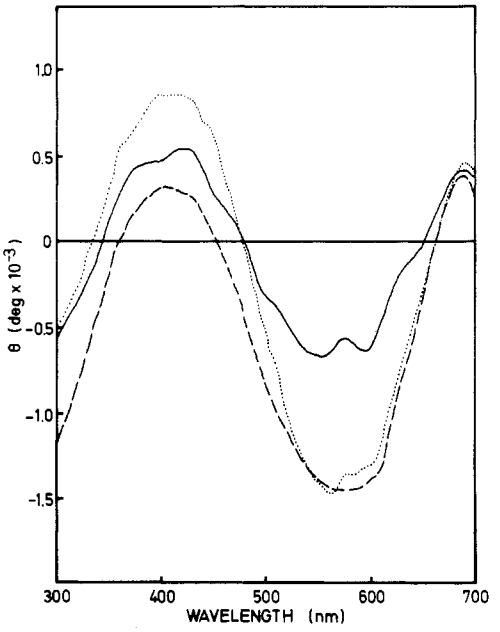

FIGURE 2: Similarity of substrate-induced changes. Circular dichroism spectra of bound protein kinase catalytic subunit in the presence of saturating amounts of (a) histone IIa (—), (b) glycogen synthase (– –), and (c) Kemptide (···). Other measuring conditions are as in Figure 1.

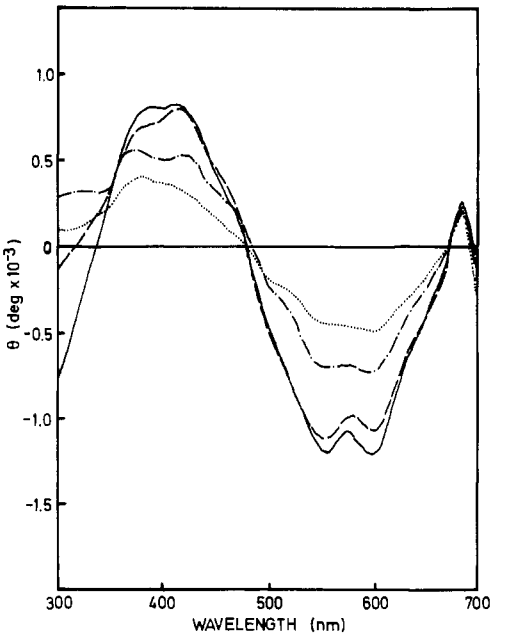

FIGURE 3: Dependence of signal on substrate bound. Circular dichroism spectra of bound protein kinase catalytic subunit titrated with histone IIa: (a) 0.5 μM (···); (b) 1.0 μM (–·–); (c) 2.5 μM (– –); (d) 10 μM (—). Other measuring conditions are as in Figure 1.

high-amplitude negative bands arises dominating the 540–590-nm region. The emergence of the set of positive extrema between 350 and 450 nm is emphasized by the absence and—at high concentrations—reversal of the positive ellipticity in the near-UV. Although there are slight differences in the apparent complexity of the major CD peaks,[1] it is clear that

[1] The curves in Figure 4 were signal averaged from 16 scans. By comparison of the saturated Kemptide curve with that in Figure 2, which comprised only four scans, it can be seen that the complex CD maxima are better resolved. This raises the possibility that it may eventually prove possible to determine what substrate-specific change occurs when the higher resolution data are used.

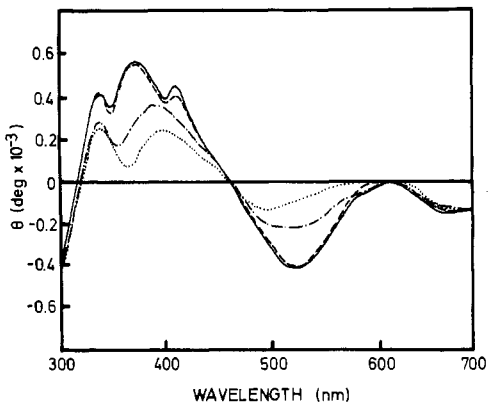

FIGURE 4: Dependence of signal on substrate bound. Circular dichroism spectra of bound protein kinase catalytic subunit titrated with Kemptide: (a) 0.1 μM (···); (b) 1.0 μM (-·-); (c) 10 μM (--); (d) 100 μM (—). Sensitivity = 0.2 mdeg/cm; time constant = 4 s; signal average of 16 scans.

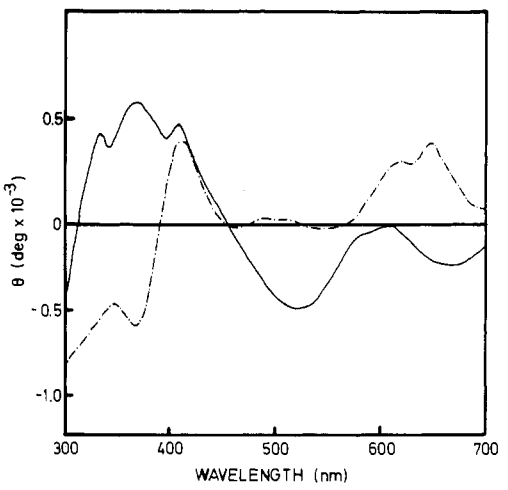

FIGURE 5: Comparison of Kemptide and the Ala-substituted analogue. Circular dichroism spectra of bound protein kinase catalytic subunit in the presence of saturating amounts of Kemptide or the Ala-substituted analogue. (a) Kemptide (—); (b) Ala-substituted analogue (-·-). Other measuring conditions are as in Figure 4.

the different substrates tested give rise to the same general phenomenon.

That the change in the induced dichroism is in fact provoked by substrate binding is further supported by the results of titration of protein kinase bound Blue Dextran with increasing amounts of histone and Kemptide (Figures 3 and 4). To avoid confusion, it should be emphasized that the titration experiments were not carried out with the aim of determining a K_m value for the substrate involved—no one would call CD the method of choice for determining simple kinetic constants—but in order to establish the substrate dependency of the relevant changes.

In the case of both histone and Kemptide, signal amplitude rises with increasing substrate concentration up to a certain point at which saturation occurs. The CD change is thus consistent with ligand/binding site interaction kinetics rather than some nonspecific change in the physical parameters of the system such as aggregation or shifts in enzyme–solvent equilibrium.

In addition, the titration curves are in agreement with the kinetic properties of the substrates concerned. If one takes the amplitude of the ellipticity maxima as proportional to the degree of saturation of the substrate binding site, then the concentration at which the signal reaches half this amplitude should correspond to an effective K_d. Since the phosphorylation reaction is not allowed to occur, one cannot speak in terms of a rate-limiting catalytic step. Thus, the K_d reflects a situation in which the initial binding plus the conformation step that precedes catalysis is being examined. In the case of histone, the half-maximal amplitude is at 1.0 μM; the K_m for histone IIa measured with protein kinase in this laboratory is 2.8 μM (unpublished results). For the Kemptide titration, the half-maximal amplitude is also at around 1.0 μM, and the K_m for Kemptide is 2.5 μM (Kemp et al., 1977). The agreement is even closer when one takes into account the higher effective concentration available to the enzyme due to the intrusion of bead volume.

The fact that the substrate K_d observed in this way is so close to the K_m determined under catalytic conditions is interesting and may indicate that the rate-controlling step in a free system is not the catalytic step itself but the conformational change that precedes catalysis. This would agree with the implication of a low K_m but high K_d measured by others (Feramisco & Krebs, 1978).

The hexapeptide Arg-Gly-Tyr-Ser-Leu-Gly corresponds to the amino acid sequence around a phosphorylated serine (Ser-24) in chick lysozyme. It, like Kemptide, serves as a substrate for cAMP-dependent protein kinase (Kemp et al., 1976). An analogue of this substrate with alanine substituted for serine was used to determine whether the basic residues on the N-terminal side of the serine in protein kinase substrates or the serine hydroxyl group itself is responsible for initiating the cooperative conformational change. The CD spectrum of Blue Dextran bound to protein kinase in the presence of saturating amounts of Ala-substituted analogue is given in Figure 5.

If recognition of the serine hydroxyl group is responsible for triggering the conformational change, the CD spectrum of the Ala-substituted analogue should resemble that of the Blue Dextran/enzyme system in the absence of substrate. If, on the other hand, recognition of the arginine residue(s) alone is sufficient, the Ala-substituted analogue should display a spectrum similar to that seen with bound substrate. In fact, although certain features typical of the substrate-induced CD spectrum are present in Figure 5, others are not. The positive ellipticity at 410–430 nm and the near-UV negative peak remain. The positive band at 340–350 nm resolved in the Kemptide spectrum also seems to be retained. The 375-nm positive extremum seen with all substrates, however, undergoes sign reversal and emerges as a negative extremum at the same position. In the longer wavelength region, the typical negative peak system from 540–590 nm is completely lost. A further positive couplet at 630–680 nm cannot be related to either the substrate curve or that of the enzyme alone. This retention of certain features of the substrate-induced spectrum while failing to retain others implies that the propagated conformational change at the ATP binding site proceeds in a minimum of two steps, one of which is dependent on the presence of an appropriate hydroxyl group in the substrate.

If the Ala-substituted analogue is added to an enzyme/Blue Dextran system that has already been allowed to bind histone, there is no change in the histone-induced CD curve. This is true even where the Ala-substituted analogue is added to 100-fold excess over the theoretical saturation values. This means that substrate that is bound (where phosphorylation cannot occur) is much more firmly attached to the enzyme than would be expected on the basis of the K_m value alone. When the Ala-substituted analogue is bound first, it can be driven off by very high concentrations of histone, although again the amount needed to achieve this is greater than one

would expect from the K_m values.

Discussion

The experiments discussed show that a conformational change at one part of the enzyme, the ATP binding site, is triggered by binding of the substrate protein at a different location. Intramolecular communication of this sort has been observed in certain other enzymes. Local conformational changes resulting from the binding of succinate to aspartate transcarbamylase are propagated to positions 20 Å away, a considerable distance in molecular terms (Yang & Schachman, 1980; Johnson & Schachman, 1980). Cooperativity in hemoglobin is also brought about by propagation of a change in quartenary structure from one subunit to another (Perutz, 1970; Baldwin & Chothia, 1979). In fact, conformational changes of this nature are thought to be the basis of allosteric mechanisms. The known cases, however, concern proteins consisting of two or more polypeptide chains. While there is no functional reason why a similar mechanism could not act between distant portions of a single-chain protein, such cases have received little attention.

The protein kinase catalytic subunit appears to recognize suitable protein substrates through two components of the local primary sequence: the serine or threonine residue to be phosphorylated and a basic region two to three residues on the N-terminal side of this consisting usually of at least one arginine. By using a substrate analogue that retained the arginine subsite but substituted alanine for the target serine, we hoped to establish which of the factors necessary for substrate recognition and binding was responsible for initiating the observed conformational change. The fact that the resulting CD spectrum retains some of the features typically induced by substrate binding but lacks others indicates that the alteration at the ATP binding site is not due to either subsite on the substrate alone. Part is apparently triggered simply by the electrostatic interaction between substrate guanido groups and negatively charged groups on the enzyme [probably the γ-carboxyl groups on glutamic acid (Matsuo et al., 1980)]. In the absence of the serine hydroxyl group, though, the Cotton effects in the longer wavelength region are substantially different. The substrate-induced signal thus seems to be the sum of at least two mechanistically independent movements.

Are any structural realignments known in this enzyme that might be related to the conformational changes implied by the CD signal? It has been suggested by Anderson that substrate-induced closure of the active site, with consequent trapping of the substrate molecule, will be a general feature of kinase enzymes (Anderson et al., 1979). He deduces this indirectly from crystallographic evidence although, as he mentions, experimental proof is difficult to obtain by this method. An induced fit at the substrate binding site has also been postulated for adenylate kinase (Pai et al., 1977) on the basis of X-ray crystallography. Changes in reactivity dependent on ionic strength have led Kupfer (Kupfer et al., 1980) to speak of an "intrinsic malleability" of protein kinase being the basis of substrate specificity. The consensus is that an enfoldment of the initially lightly bound substrate occurs in enzymes of this type.

Of the two separate movements reported here, we can be fairly sure that partial closure over the substrate protein is induced by the basic subsite alone. This is both strongly implied by the displacement experiments and logical in view of the behavior of the natural inhibitor and the regulatory subunit, both of which have the basic residue group as a prerequisite for binding (Demaille et al., 1977; Flockhart et al., 1980). Such relatively large-scale flexions would have attendant remote effects on the ATP binding site, producing changes in the induced CD. This leaves the question of what specific change could be associated with the difference in signal when a hydroxyl group is present.

Interestingly enough, there does seem to be a second category of conformational change discussed in connection with kinase enzymes; some sort of reorientation is probably necessary to the transphosphorylation reaction. Substrate-induced conformational changes are cited as the probable causative factor in excluding water from the active site and avoiding ATPase activity (Blake, 1978). Also, the distance along the reaction coordinate between the γ-phosphate of ATP and the serine hydroxyl groups should be reduced during catalysis if the reaction mechanism is to agree with the kinetic parameters (Granot et al., 1980, 1981). Yet, studies with peptide analogues have shown that the N-terminal guanido groups are required for binding but not for transphosphorylation, so that conformational changes associated with the latter are not necessarily part of the substrate enfoldment process.

One unequivocal difference in the induced CD where the hydroxyl group is not present is the sign reversal of the 375-nm ellipticity maximum. The 375-nm ellipticity stems from a Cibacron blue absorbance maximum at 257 nm with shoulders at 280 and 375 nm. Tyrosine absorbs at 275–280 nm due to a ^1L_b transition, which is exceedingly sensitive in both sign and magnitude of its associated CD signal to rotation around the C^β–C^γ bond connecting the aromatic ring to the rest of the molecule. Nondegenerate dipole–dipole coupling between near-UV tyrosine transitions and far-UV transitions in other groups is common. Since a tyrosine located on the ATP binding site of protein kinase is necessary to the activity of the enzyme (Witt & Roskoski, 1975)—a tyrosine in the ATP pocket occurs in both adenylate kinase and lactate dehydrogenase as well (Pai, 1977; Adams et al., 1973)—coupling between it and the phenyl B ring of bound Cibacron blue is feasible. In that case, rotation of the tyrosine with respect to the main chain resulting in a sign change in the associated dichroism might be expected to result in a sign change in the dichroism of the coupled Cibacron blue transition, of which only the 375-nm band occurs in the visible region. It is tempting to consider that the presence of a hydroxyl group at the appropriate position on the substrate is necessary for the correct orientation of this tyrosine during catalysis. A careful investigation of the ultraviolet CD of protein kinase should help to clarify this point.

The fact that the complete conformational change induced by substrate binding is dependent on at least two—possibly more—signals is encouraging in view of our interest in ligand-mediated control mechanisms. If the final conformation of the active site is reached through a multistage process, it is easy to see how specificity, reaction rate, and substrate affinity could be altered by ligands containing only some of the triggering groups needed. Continued studies on the CD of protein kinase in the presence of various inhibitors, modulators, etc. should help to solve the problem of protein kinase specificity in vivo.

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Registry No. Protein kinase, 9026-43-1; BD-S, 67701-59-1; Kemptide, 65189-71-1; Arg-Gly-Tyr-Ala-Leu-Gly, 59587-24-5; glycogen synthase, 9014-56-6.

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Activation of Bovine Brain Calmodulin-Dependent Protein Phosphatase by Limited Trypsinization†

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ABSTRACT: A calmodulin-dependent protein phosphatase isolated from bovine brain [Tallant, E. A., & Cheung, W. Y. (1983) *Biochemistry 22*, 3630–3635] is stimulated by limited trypsinization to the same activity level as that by calmodulin. Prolonged trypsinization caused gradual loss of phosphatase activity, a process retarded in the presence of Ca^{2+}, and even more in the presence of calmodulin. Trypsinized phosphatase, when fully activated, had a molecular weight of 60 000 and was composed of two protein species of 43 000 and 16 000 daltons. Trypsinization decreased the K_m of phosphatase for casein from 10.8 to 1.2 μM and increased the V_{max} from 4.9 to 30.9 nmol (mg of protein)$^{-1}$ min^{-1}. The proteolyzed enzyme was insensitive to calmodulin and did not bind to a calmodulin–Sepharose affinity column. It was, however, stimulated by Ca^{2+}, requiring 0.4 μM Ca^{2+} for half-maximal activation. Both native and trypsinized phosphatase were stimulated by Mn^{2+} to a level considerably higher than that by Ca^{2+}.

Calmodulin-dependent protein phosphatase, a major calmodulin-binding protein in bovine brain extracts, catalyzes the dephosphorylation of a variety of substrates, including the α subunit of phosphorylase kinase, inhibitor 1, casein, and histone (Stewart et al., 1982; Yang et al., 1982). The enzyme, referred to as CaM-BP_{80} (Wallace et al., 1980) or calcineurin (Klee et al., 1979) before its identification as a phosphatase, has a molecular weight (M_r) of 80 000 and is composed of two hetero subunits (Sharma et al., 1979; Wallace et al., 1979); subunit A (M_r 60 000) binds calmodulin (Richman & Klee, 1978), and subunit B (M_r 16 500) is itself a Ca^{2+}-binding protein (Klee et al., 1979). The enzyme is predominantly found in

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