phosphatidylcholine, 20559-16-4; lauroyllysophosphatidylcholine, 20559-18-6; capryllysophosphatidylcholine, 22248-63-1; caprylyllysophosphatidylcholine, 45287-18-1; NAD:arginine ADP-ribosyltransferase, 81457-93-4; agmatine, 306-60-5.

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Near- and Far-Ultraviolet Circular Dichroism of the Catalytic Subunit of Adenosine Cyclic 5′-Monophosphate Dependent Protein Kinase†

Jennifer Reed* and Volker Kinzel

ABSTRACT: The circular dichroism spectrum of the catalytic subunit of cAMP-dependent protein kinase was measured in the far-UV (190–240 nm) and near-UV (250–300 nm) region. Data from the far-UV spectra were processed with the CONTIN program for estimation of globular protein secondary structure [Provencher, S. W. (1982) *CONTIN (Version 2) User's Manual*, European Molecular Biology Laboratory, Heidelberg, West Germany]. The composition of the protein determined by this method was 49 ± 2% α-helix, 20 ± 4% β-sheet, and 31 ± 3% remainder. This composition changes when the protein is allowed to bind Kemptide, a synthetic peptide substrate, with more than half of the disordered portion of the protein taking the form of β-sheet. A certain portion of the α-helical structure also appears to move into a β-sheet form. The near-UV CD spectrum of catalytic subunit shows changes in aromatic amino acid dichroism associated with substrate binding. These changes can be ascribed with a fair degree of certainty to alterations in the orientation of a tyrosine residue at the surface of the protein. These findings are discussed in terms of previous work on induced dichroism in this enzyme with regard to control mechanisms operating at the active site.

The catalytic subunit of cAMP-dependent protein kinase (EC 2.7.1.37) phosphorylates serine or, less often, threonine residues on substrate proteins. The inactive holoenzyme is a tetramer of two regulatory and two catalytic subunits; the catalytic subunit is freed in active form on the binding of two cAMP molecules per regulatory subunit (Langan, 1967). The enzyme thus acts as the target for cAMP-mediated hormonal responses and might be expected to exhibit a high degree of specificity in its selection of protein substrate.

In isolated form in vitro, however, the catalytic subunit appears to phosphorylate any protein having an accessible serine or threonine in the specific primary sequence Arg-Arg-X-Ser (Daile et al., 1975; Kemp et al., 1977; Kemp, 1978; Feramisco et al., 1979; Meggio et al., 1981). A search for further control points is indicated. This search can be directed both toward looking for external controls such as the family of small, acid-stable proteins and toward understanding the normal function of this enzyme to show at what stages control factors might operate. With this latter goal in mind, we have recently used techniques of induced circular dichroism to observe specific conformational changes at the active site of the enzyme. Initial studies have shown that the binding of protein substrate induces a conformational change at the ATP-binding site that occurs in at least two discrete parts, one dependent on the basic subsite characteristic of the specific primary structure required and one dependent on the presence of a hydroxyl group on the target serine (Reed & Kinzel, 1984). There was some indication that this latter movement was triggered by interaction between the hydroxyl group and a tyrosine residue—one of which is known to be present at the surface of the ATP binding site (Witt & Roskoski, 1975). In an attempt to clarify this interaction, we decided to examine the ultraviolet circular dichroism (UV CD) spectrum of protein kinase catalytic subunit for any changes in intrinsic dichroism, especially that associated with tyrosine, which might be con-

† From the Institute of Experimental Pathology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany. *Received August 30, 1983.* This work was supported by the Deutsche Forschungsgemeinschaft.

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nected with substrate binding. In addition, the far-UV CD spectrum has enabled us to make an estimate of the secondary structure content of this enzyme prior to the availability of X-ray crystallographic data.

Materials and Methods

The catalytic subunit of cAMP-dependent protein kinase, isoenzyme type II, was prepared from rat skeletal muscle as previously described (Kübler et al., 1977) with minor modifications to obtain absolute electrophoretic homogeneity.

Circular dichroic spectra were obtained on a Jasco J-500 automatic recording spectropolarimeter coupled to a Jasco J-DPY data processor. Automatic slit width control was maintained with a dispersion of 1.0 nm. Digitally recorded curves were fed through the data processor for smoothing, signal averaging, and base-line subtraction.

Measurements were carried out in the spectral range 190–240 nm for secondary structure determinations and 250–300 nm for observations in the CD of aromatic amino acids. Enzyme at a concentration of 50–100 μg/mL was measured in a dichroically neutral quartz cuvette. For secondary structure determinations, the cuvette had a path length of 0.1 cm, and scanning conditions were as follows: sensitivity 2 mdeg/cm; time constant 1.0 s; scan speed 5 nm/min; wavelength expansion 5 nm/cm. Curves are presented as the signal average of four consecutive measurements with a similarly signal-averaged base line subtracted.

The near-UV region of the spectrum was measured in a 1.0-cm cuvette under the following conditions: sensitivity 0.5 mdeg/cm; time constant 2.0 s; scan speed 10 nm/min; wavelength expansion 5 nm/cm. Curves are presented as the signal average of 32 consecutive measurements, again with a similarly signal-averaged base line subtracted. All spectra were measured at room temperature.

For estimation of secondary structure, CD curves in the 190–240-nm range were processed with the Globular Protein Secondary Structure applications package of CONTIN, a general purpose program for solving linear integral equations developed by Stephen W. Provencher of the European Molecular Biology Laboratory. This program functions by analyzing a given CD spectrum directly as a linear combination of CD spectra from sixteen proteins whose secondary structures are already known from X-ray crystallography (Provencher & Glöckner, 1981). Because conformations of globular proteins deviate quite noticeably from those of ideal proteins, on the basis of model compounds such as poly(L-lysine), and can vary considerably in their CD contributions from a particular type of secondary structure depending on chain length or extent of β-sheet, the use of 16 reference spectra from actual proteins allows a much more accurate estimation of secondary structure from CD spectra than previous methods. Provencher achieves correlation coefficients of 0.96 for α-helix and 0.94 for β-sheet, an increase in accuracy of 12 and 74%, respectively, over previous methods.

For processing by this method, data points were taken at 0.5-nm intervals and expressed as mean residue ellipticity with the equation

$$(\theta)_\lambda = \frac{\theta_{obsd} \times MRW}{10dc''}$$

where λ = wavelength, θ_{obsd} = observed ellipticity in millidegrees, MRW = mean residue weight (taken here to be 115.81), d = path length in centimeters, and c'' = concentration in milligrams per milliliter. Measurements were carried out on five separate preparations of protein kinase, and standard deviation was calculated as σ_n.

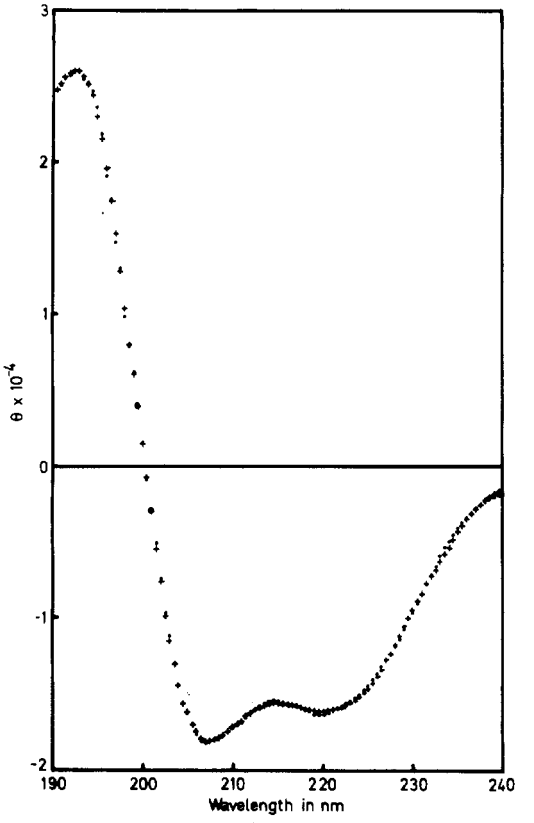

FIGURE 1: Far-UV circular dichroism spectrum of the catalytic subunit of cAMP-dependent protein kinase: (●) 50 μg/mL enzyme in 150 mM phosphate buffer, pH 6.8; (+) CONTIN-generated fit to the above data. θ = mean residue ellipticity in deg cm²/dmol.

Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly) was obtained from Penninsula Laboratories, Santa Clara, CA. All other chemicals used were of reagent grade.

Results

The far-UV circular dichroism spectrum (190–240 nm) of protein kinase catalytic subunit is shown in Figure 1. The CONTIN-generated fit to these data in terms of mean residue ellipticity is very good. The secondary structure composition estimated by this method is α-helix 49 ± 2%, β-sheet 20 ± 4%, and remainder 31 ± 3%. These values differ from those obtained for pig brain protein kinase catalytic subunit (Ul'masov et al., 1981; α-helix 25.3%, β-sheet 30.5%, remainder 45.5%) or for the catalytic subunit from beef liver protein kinase (Sugdon et al., 1976; α-helix 29%, β-sheet 18%, remainder 53%). They are in close agreement, however, with the secondary structure composition predicted (Shoji et al., 1981) from a Chou and Fassman treatment of the primary structure of beef heart catalytic subunit (α-helix 48%, β-sheet 19%, remainder 33%).[1] The discrepancy with the earlier CD-derived figures probably arises from the method used for secondary structure calculation in each case. K. A. Ul'masov et al. used reference spectra from model polypeptides, a method the drawbacks of which have been well documented (Adler et al., 1967). The method used by the second group does employ globular proteins as a standard but suffers from the bias inherent in using a particular reference protein for each type of structure. Since the method used here does not define reference spectra in terms of conformational classes, its accuracy and flexibility is much improved over those used pre-

[1] These figures were not published directly but can be derived from the secondary structure composition given for the three domains described.

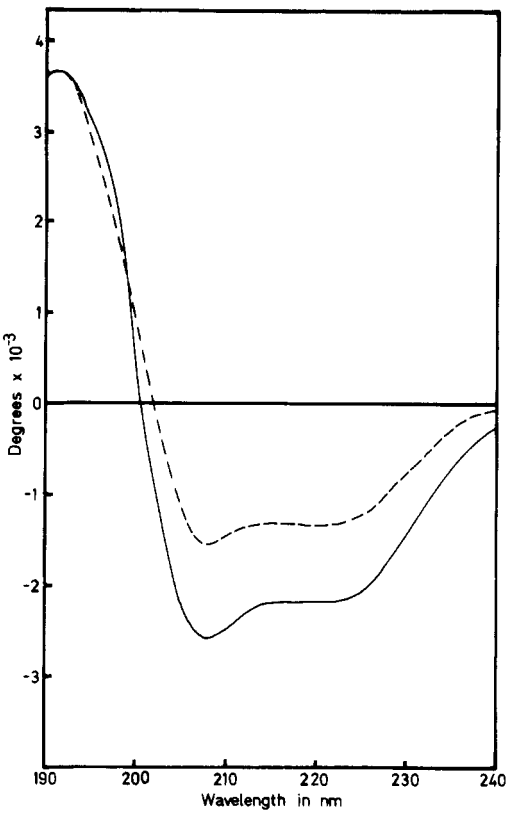

FIGURE 2: Far-UV CD spectrum of the catalytic subunit with buffer base line subtracted (—) compared with the far-UV CD spectrum of the enzyme plus bound Kemptide with a buffer plus Kemptide base line subtracted (--).

viously. The remarkable correspondence between the CONTIN-generated values and those obtained from a theoretical folding algorithm must eventually be checked against X-ray crystallographic data for this protein; this will prove a useful test of the method.

A synthetic substrate for cAMP-dependent protein kinase is the heptapeptide Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly), which mimics the primary structure around the phosphorylated serine in a natural substrate (Kemp et al., 1977; Kemp, 1978). Kemptide offers the advantages of being without either complex secondary structure to complicate spectra in the far-UV region or aromatic amino acids, which could interfere with near-UV dichroism. The CD spectrum of 250 mM Kemptide from 250 to 300 nm does not differ appreciably from the normal buffer base line.

As binding of substrate is known to induce a conformational change in the enzyme (Reed & Kinzel, 1984), the far-UV CD spectrum of the enzyme with bound substrate was taken to see if any change was observable at the level of secondary structure composition. Figure 2 shows that binding of Kemptide to the enzyme is accompanied by a definite change in secondary structure. When analyzed by the CONTIN program, the spectrum is fit by a composition of 31 ± 2% α-helix, 55 ± 6% β-sheet, and only 15 ± 7% remainder. Binding of Kemptide results in an increase in the secondary structure of the enzyme; most of this increase is in the form of β-sheet; and the increase occurs at the expense of the disordered portion of the molecule with some contribution from α-helix as well.

Figure 3 gives the near-UV circular dichroism spectrum of the catalytic subunit. Ellipticities in this region stem from circular dichroism associated with the aromatic amino acids and from disulfide bonds. The latter, however, have no fine structure and in complex curves are seen mainly through a shift of large segments of the spectrum in the same direction.

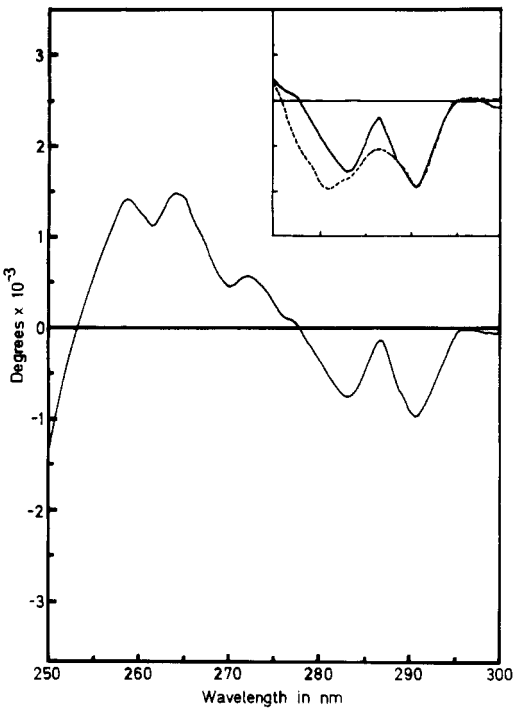

FIGURE 3: Near-UV circular dichroism spectrum of the catalytic subunit. Protein concentration was 45 μg/mL. (Inset) CD spectrum of the catalytic subunit from 275–300 nm: (—) enzyme alone; (--) enzyme plus 250 μM Kemptide (buffer plus Kemptide base line subtracted).

The catalytic subunit CD spectrum from 250 to 270 nm displays a series of complex ellipticity maxima characteristic of the CD bands of phenylalanine. The optically active near-UV absorption bands of phenylalanine compounds have been identified as coming from two main progressions, one based on a 0 – 0 transition and one from a 0 + 520 cm^{-1} transition (Horowitz et al., 1969). The first gives to bands at the longest wavelength (0 – 0 at ca. 268 nm) and at about 262 nm; the second gives rise to bands at about 265 and 259 nm. Resolution of shorter wavelength fine structure is not usually possible due to the high degree of overlap between bands. Phenylalanine bands are not observed at wavelengths longer than 268 nm.

In the CD spectrum of the catalytic subunit, positive peaks are resolved at 259 and 265 nm, with troughs at 261.5 and 268 nm. J. Horowitz et al. reported only positive bands for 5.8 mM phenylalanine in water at 298 K. In methanol–glycerol at room temperature, though, they observed a strong negative band at 268 nm, strong positive at 264.5 nm, another strong negative at 261.7 nm, and a strong positive at 258.6 nm. (Weaker bands at 266.7 and 260 nm are not resolved in our spectra.) The two negative bands they assigned to the 0 – 0 progression. Since the relative intensities of the progressions observed for phenylalanine in solution were dependent on factors such as temperature and solvent, the location of troughs in our spectrum at 261.5 and 268 nm and their resolution at comparatively low concentration (30–60 μM phenylalanine) at room temperature are most easily explained by the presence of negative ellipticities at these wavelengths. The CD of phenylalanine residues within the protein kinase molecule thus most closely resembles the spectrum of the pure amino acid in methanol–glycerol superimposed on a broad positive contribution typical of disulfide groups.

Contributions to the CD spectrum in the region from 275 to 300 nm can be expected from three sources, the aromatic amino acids tyrosine and tryptophan and disulfide bonds. Of

these, the disulfide contribution to the spectrum has little qualitative effect on ellipticities from other sources; the wide half-band width of disulfides (typically 25 nm) and their loss of intensity due to cancellation of oppositely signed spectra mean that their contribution to a complex spectrum is more in the nature of a shift in base line than in the addition of discrete peaks.

In proteins, the identification of particular bands as belonging to tryptophan or tyrosine can be difficult, as tyrosyl circular dichroism bands may overlap those tryptophanyl CD bands occurring below 289 nm. The situation is further complicated by the overlap of bands from the same residue type that occur at slightly different wavelength, sign, or intensity due to local environmental effects. Any peak seen in this region is likely to be complex, the sum of a number of different ellipticities. In some cases, however, careful analysis does allow one to ascribe a specific effect unambiguously to either tryptophan or tyrosine dichroism. This appears to be the case with certain changes in the near-UV CD spectrum of the catalytic subunit that occur as substrate is bound.

Protein kinase catalytic subunit has been shown to contain 6 tryptophan and 14 tyrosine residues (Shoji et al., 1981). The CD spectrum of catalytic subunit in the region from 275 to 300 nm exhibits negative ellipticity maxima at 283.5 and at 291.5 nm. Contributions in this region may be expected from the ^1L_b transitions of tyrosine and tryptophan and the ^1L_a transition of tryptophan, as well as disulfide bonds (Kahn, 1979). (The ^1L_a transition of tyrosine occurs in the far-UV and does not affect the spectrum in this region.)

The negative ellipticity maximum at 291.5 nm can be ascribed to the sum of the $0 - 0$ ^1L_b transitions in various tryptophan residues. This is because tyrosine has no bands at wavelengths longer than 289 nm, and if this peak were partially due to $0 + 400$ cm^{-1} ^1L_a transitions from tryptophan residues as well, one would expect to see a CD band from the $0 - 0$ ^1L_a transition at 298–300 nm (Strickland et al., 1969). The almost complete lack of dichroism at these wavelengths suggests that the contribution from tryptophanyl ^1L_a transition in this protein is so weak as to be negligible under these conditions. Therefore, only the ^1L_b transitions of the two aromatic amino acids need be considered here.

The ^1L_b CD bands of tryptophan may have either positive or negative signs, but not mixed (Strickland et al., 1969). Also, the $0 + 850$ cm^{-1} ^1L_b transition is found at a fairly constant 7 nm lower than the $0 - 0$ ^1L_b transition. This being the case, with the latter at 291.5 nm, we would expect to see a correspondingly negative maximum at around 284.5 nm. Thus at least part of the 283.5-nm negative peak is due to contributions from tryptophanyl residues, as might be expected. The asymmetry of the peak and its shift to a slightly lower wavelength is due to the presence of $0 - 0$ ^1L_b transitions of tyrosine at this position as well. Whereas the overlap of various tryptophan ^1L_b transitions results in a complex peak at 291.5 nm that is predominantly negative, the various $0 - 0$ ^1L_b tyrosine bands evidently present a much more complicated picture. The cumulative effect is seen in a slight blue shift of the total ellipticity down from the maximum expected from tryptophan contributions alone. This would indicate a somewhat diffuse negative ellipticity from $0 - 0$ ^1L_b tyrosine transitions at around 283 nm.

When saturating amounts of Kemptide are added to the catalytic subunit solution, the CD spectrum between 275 and 300 nm is altered in a specific fashion (Figure 3, insert). There is no change in the sign or intensity of the spectrum at longer wavelengths; the 291.5-nm negative maximum is unaltered. The negative peak at 283.5 nm, however, appears to shift sharply downward and increases somewhat in intensity. This is not due to any change in the contribution of the tryptophan residues—since the $0 - 0$ ^1L_b band is unaltered, the rest of the fine structure at shorter wavelengths will remain the same. The change can therefore be attributed to an alteration in at least one of the 14 tyrosine residues resulting from the binding of substrate. The appearance of a shift downward in such a complex peak could be caused either by a marked increase in intensity of a negative ^1L_b tyrosine band at around 281 nm or by a sign change from positive to negative in a tyrosine transition at that wavelength. While the former cannot be ruled out, such a clear effect in an area to which many residues contribute CD bands argues for the latter. Certainly, the sign of the ^1L_b tyrosine CD bands is known to be fairly sensitive to changes in the angle of the residue with respect to the main chain (Kahn, 1979). As the position of the $0 - 0$ ^1L_b transition in tyrosine is dependent on the local environment, occurring at longer wavelengths as the polarity of its surroundings decreases (Horowitz et al., 1970), the relatively low position of the altered tyrosine band suggests that the residue(s) involved occur(s) on the surface of the protein.

Discussion

Studies on the induced dichroism in this enzyme have revealed that binding of protein substrate is accompanied by a two-stage conformational change, one part of which is dependent on the presence of a hydroxyl group on the protein substrate in an appropriate position for phosphorylation. The most significant difference in the spectrum where this hydroxyl group is not present is a sign reversal in a prominant 375-nm ellipticity (Reed & Kinzel, 1984). A suggested interpretation of the origin of this signal was that it occurred through coupling of tyrosine transitions with phenyl ring B in the Blue Dextran used as a probe. The sign of ^1L_b tyrosine CD bands is extremely sensitive to the orientation of the residue with respect to the main chain. It was thought that the presence of a hydroxyl group on the substrate might affect the orientation of a tyrosine known to be present at the surface of the ATP binding site (and so in close proximity to the Blue Dextran). However, no direct evidence for alteration of a tyrosine residue was available at that time.

The near-UV circular dichroism spectrum of protein kinase catalytic subunit presents evidence that binding of a protein substrate to the enzyme is in fact accompanied by a change in the intensity and/or sign of dichroism bands associated with a tyrosine residue(s). The fact that the apparent position of the $0 - 0$ ^1L_b transition involved in this change is at a relatively short wavelength (ca. 281 nm) suggests that the residue(s) concerned is (are) in a polar environment, probably at the surface of the protein. The change in dichroic signal implies a change in the orientation of the residue(s) with respect to the rest of the molecule.

Although the three-dimensional structure of the catalytic subunit is not yet available from X-ray crystallographic work, an attempt can be made to determine the location of the dinucleotide fold that comprises the ATP binding site by analogy with the primary sequence of this structure in several known proteins. Figure 4 shows that a segment of the protein from residues 42 to 87 maps over the adenine nucleotide binding fragments of six known proteins (Rossman et al., 1974) with a high degree of correspondence among the residues most closely conserved. The latter part of this sequence is identical with the fragment identified (Hashimoto et al., 1982) through photolabeling techniques as being part of the ATP binding site in cAMP-dependent protein kinase. The initial 12 amino acid

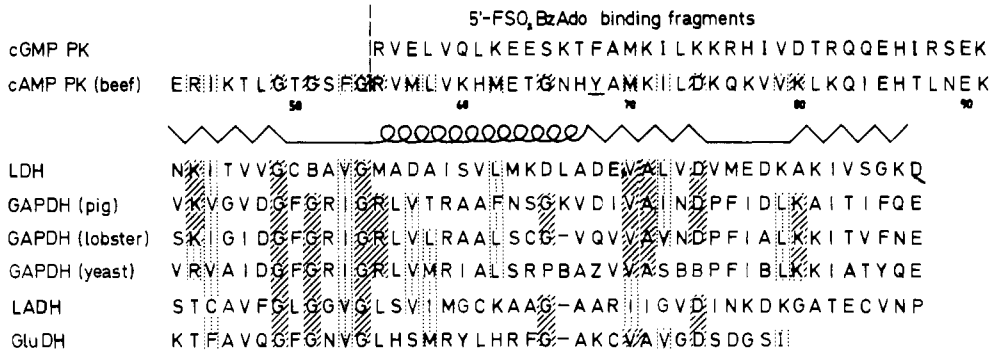

FIGURE 4: Mapping of residues 42–87 of protein kinase (Shoji et al., 1981) over the adenine nucleotide binding sequences of six proteins whose structure has been determined by X-ray crystallography (Rossman et al., 1974). The ATP binding fragments of cAMP- and cGMP-dependent protein kinases identified by photoaffinity labeling (Hashimoto et al., 1982) are shown above. Tyrosine-68 is underlined. (Hatched residues) Totally conserved residues; (stippled residues) functionally conserved residues. Note that residues 70 and 72, highly conserved in the various dehydrogenases, are completely different in the two kinases, suggesting that this subsite may be particularly concerned with phosphate transfer. Abbreviations: LDH, lactate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LADH, liver alcohol dehydrogenase; GluDH, glutamate dehydrogenase.

sequence not included in the Hashimoto fragment is certainly part of the fold as it contains the most highly conserved sequences in the whole structure and forms the first of the three parallel pleated sheets responsible for binding the adenine nucleotide.

Of the five or so tyrosines that can be judged from the primary structure of the enzyme to exist in a polar environment, only one, Tyr-68, is located in the region of the dinucleotide fold. This location is interesting from the mechanistic point of view as it appears on the end of the central β-pleated sheet at the point of transition between it and an α-helical segment, directed toward the adenine binding site. The structure of the ATP binding region might thus be expected to be sensitive to alterations in the orientation of this tyrosine.

The evidence presented from CD studies on the catalytic subunit suggests that the conformational change in the enzyme induced by the binding of protein substrate is mediated in part by the ability of the enzyme molecule to "sense" the presence of an appropriate hydroxyl group through its interaction with tyrosine at the surface of the ATP binding site. (The fact that this position is occupied by a phenylalanine in the cGMP-dependent protein kinase may mean that the interaction is more dependent on the bulk and/or apolar properties of the tyrosine than on its own hydroxyl group.) Substrate analogues containing an alanine substituted for the target serine or threonine act as inhibitors of the enzyme, so that the conformational change triggered by the serine hydroxyl group is probably central to enzyme activity.

The induced dichroism previously measured can reflect conformational change over a distance of at most 15 Å from the chromophore observed; the changes in tyrosine dichroism described above are also relatively small scale. The significant change in secondary structure observed as substrate binds, however, seems to indicate that the conformational changes induced by such binding are in fact on a much larger scale. Much of the unstructured and α-helical portion of the protein evidently moves into the organization of a β-pleated sheet. Such massive changes in secondary structure are unusual and may be connected with the apparently "floppy" nature of the catalytic subunit remarked on by other authors (Kupfer et al., 1980), at least as routinely isolated. The enzyme shows certain characteristics of an induced-fit mechanism in vitro (Reed & Kinzel, 1984); a ligand-induced substrate specificity could explain both the relatively major conformational change seen on substrate binding and the enzyme's lack of selectivity in vitro. The change from a disordered to a highly structured form on ligand binding is not without precedent. In trypsin, for example, segments of the protein in the activation domain that are disordered in trypsinogen are tightly ordered after conversion of the proenzyme by proteolytic cleavage. This folding of the activation domain can be imposed in the absence of proteolytic activation, however, by the binding of pancreatic trypsin inhibitor to trypsinogen (Bode et al., 1978; Huber, 1979). If protein kinase follows the example of trypsinogen, binding of small protein ligands such as the PKI could induce definite structural changes at the active site, offering an excellent basis for fine-level control of enzyme activity and even specificity.

Acknowledgments

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Registry No. Kemptide, 65189-71-1; protein kinase, 9026-43-1.

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^{1}H NMR and Circular Dichroism Studies of the B and Z Conformations of the Self-Complementary Deoxyhexanucleotide d(m^{5}C-G-C-G-m^{5}C-G): Mechanism of the Z–B–Coil Transitions†

Son Tran-Dinh,* Jean Taboury, Jean-Michel Neumann, Tam Huynh-Dinh, Bernadette Genissel, Beatrice Langlois d'Estaintot, and Jean Igolen*

ABSTRACT: The double-helical conformations of d(m^{5}C-G-C-G-m^{5}C-G) in aqueous solution were studied by circular dichroism and ^{1}H NMR spectroscopy. In 0.1 M NaCl, only the B form is detected whereas the Z form is strongly predominant in 3 M NaCl. In the presence of 2 M NaCl, two resonance signals corresponding to the B and Z duplexes were observed for each proton below 50 °C, indicating a slow exchange between B and Z. However, the B–Z exchange becomes intermediate or fast in the 55–80 °C temperature interval. By contrast the exchange between B helix and single-stranded (or coil) forms is much faster for the same temperature conditions. The Z form is only detectable when the coil form is practically absent. With decreasing temperature the B form decreases in favor of the Z form. From proton line-width measurements under various experimental conditions, it was also shown that Z exchanges only with B, while the latter also exchanges with the single-stranded form (S): $Z \rightleftarrows B \rightleftarrows S$. The enthalpy value is about 8 ± 1 kcal/mol for the B–Z transition and about 40 ± 2 kcal/mol for the B–S dissociation (2 M NaCl solution). The activation energy is about 47 ± 2 kcal/mol for the $Z \rightarrow B$ and 39 ± 2 kcal/mol for the $B \rightarrow Z$ reaction. Very good agreement between the experimental results and computed data (based on the above kinetic reaction model) was found for the B, Z, and coil proportions. The B–Z transition of methylated $d(C\text{-}G)_n$ oligomers is only possible when the Watson–Crick hydrogen bonds between the CG base pairs are firmly maintained; otherwise, the transformation from B to Z would not occur, and B–S dissociation would take place instead.

In human DNA, 4–5% of cytosine residues are methylated (Tolstoshev et al., 1981), and methylated cytosines are frequently found preceding a guanine residue. It now appears that DNA methylation plays an important part in gene expression during development. For example, DNA in the region of duplicated γ-globin genes ($^G\gamma$ and $^A\gamma$) is relatively undermethylated in cells where these genes are expressed, whereas methylated DNA is found in adult bone marrow cells where these genes are inactive (Van der Ploeg et al., 1980). Ley et al. (1982) have recently shown that administration of 5-azacytidine to a patient with β-thalassemia reduces the frequency of methylation of specific cytosine residues and causes a striking increase in the synthesis of both $^G\gamma$-globin and $^A\gamma$-globin. These authors suggested that hypomethylation of DNA may be necessary for high level gene expression.

On the other hand Behe & Felsenfeld (1981) have shown that methylation on the 5-position of the cytosine residue in poly[d(G-C)] has the effect of inducing the transition from right-handed B-DNA into left-handed Z-DNA under physiological salt and pH conditions. In B-DNA duplexes, all residues adopt the S(C_2-endo) conformation for the sugar ring and the anti orientation for the bases. Conversely, in Z-DNA duplexes the deoxyguanosines take the N(C_3-endo) and syn conformations (Wang et al., 1979).

About 10 years ago, direct evidence of syn–anti equilibria of guanosine and adenine monophosphates in solution was obtained by the use of NOE[1] techniques (Tran-Dinh et al., 1972a; Guéron et al., 1973); the population of the anti conformation is favored in 5′-nucleotides. The correlation between syn/anti and N(C_3-endo)/S(C_2-endo) has also been studied on GMPs: the syn and N(C_3-endo) proportions are predom-

† From the Service de Biophysique, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette Cedex, France (S.T.-D., J.T., and J.-M.N.), Unité de Chimie Organique, ERA/CNRS 927, Institut Pasteur, 75724 Paris Cedex 15, France (T.H.-D., B.G., B.L.E., and J.I.), and Laboratoire de Spectroscopie Biomoléculaire, U.E.R. de Médecine, 93012 Bobigny Cedex, France (J.T.). *Received May 5, 1983; revised manuscript received October 25, 1983.*

[1] Abbreviations: d(m^{5}C-G-C-G-m^{5}C-G), 2′-deoxy-5-methylcytidyl(3′–5′)deoxyguanylyl(3′–5′)deoxycytidyl(3′–5′)deoxyguanylyl(3′–5′)-deoxy-5-methylcytidyl(3′–5′)deoxyguanine; NOE, nuclear Overhauser effect; ORD, optical rotatory dispersion; CD, circular dichroism; HPLC, high-performance liquid chromatography; EDTA, ethylenediaminetetraacetic acid.

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