Volume 247, number 2, 480–482 FEB 07089 April 1989

Conformational changes of type II regulatory subunit of cAMP-dependent protein kinase on cAMP binding

Dennis R. Johnson* and Shan S. Wong

The Biochemistry Program, Department of Chemistry, University of Lowell, Lowell, MA 01854, USA

Received 26 December 1988; revised version received 6 February 1989

The effect of cAMP on the conformation of the regulatory subunit of type II cAMP-dependent protein kinase (RII) from bovine heart was investigated by UV-difference and circular dichroism (CD) spectroscopy. The UV-difference spectrum of RII with and without cAMP showed a positive band around 278 nm and a negative band at 256 nm. Similarly, cAMP enhanced the ellipticity of RII in the region between 280 and 300 nm and decreased that between 250 and 280 nm. In addition, cAMP transformed the far-UV CD spectrum of RII from that of a negative band minimally at 209 nm with a shoulder at 223 nm to one with two minima at 222 and 211 nm. These data show that cAMP induces conformational changes of RII upon binding. Such structural changes may be the basis of activation of cAMP-dependent protein kinases by cAMP.

cyclic AMP-dependent protein kinase; cyclic AMP-binding protein; Conformation; CD

1. INTRODUCTION

Cyclic AMP (cAMP), a second messenger, manifests its hormonal activity in eukaryotes through the activation of protein kinases. There are at least two forms of cAMP-dependent protein kinases (EC 2.7.1.37, ATP:protein phosphotransferase) in the mammalian tissues: type I, the predominant form in skeletal muscle and type II, the predominant form in bovine cardiac muscle [1]. Both forms are composed of two catalytic (C) and two regulatory (R) subunits [2]. The differences between these types are attributed primarily to the structures of the R subunits, which are also classified as type I and II [3,4]. As a tetramer, the holoenzyme is catalytically inactive. cAMP activates the enzyme by binding to the R subunit, causing the dissociation of the free active C subunits from the holeenzyme [5,6].

The cAMP-binding sites on the R subunits, two per monomer, are not equivalent [7,8]. Studies with cAMP analogues have distinguished these sites as fast and slow dissociating [9,10]. They are synergistic in the activation of the protein kinases [11]. However, it remains unknown how the binding of cAMP causes the holoenzyme to dissociate. In this report we demonstrate the effect of cAMP on the conformation of RII from bovine heart in an effort to ellucidate the activation mechanism of cAMP.

Correspondence address: S.S. Wang, The Biochemistry Program, Department of Chemistry, University of Lowell, Lowell, MA 01854, USA

*Present address: Ohio State University College of Medicine, Columbus, OH 43210, USA

2. EXPERIMENTAL

The RII subunit of cAMP-dependent protein kinase was prepared from bovine heart according to Rannels and Corbin [12] using urea elution from a cAMP-agarose column (Sigma) followed immediately by dialysis. The isolated RII was shown to bind 1.5–2.0 mol cAMP per mol subunit as reported [12]. It was homogeneous as assessed by SDS-polyacrylamide electrophoresis and inhibited the C subunit, which was reversible by cAMP.

CD spectra of RII in 0.1 M potassium phosphate buffer, pH 7.0, were measured at ambient temperature on a Jasco J-500A spectropolarimeter at the Ohio State University (Columbus, OH). For the near-UV CD, 0.9 mg/ml RII was used whereas 0.04 mg/ml was used for the far-UV region. The solution was made 0.31 and 0.24 mM in cAMP, respectively. The protein concentration was determined by absorption on a Cary model 118C spectrophotometer using $A^{1\%,1\ cm} = 6.0$ at 280 nm [5]. The mean ellipticity, θ, was expressed in degree $\cdot$ cm^2 $\cdot$ dmol^{-1} of

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

amino acid residues, using the mean residue weight of 130.4 calculated from the amino acid information published by Takio et al. [13].

The UV-difference spectrum was measured on a Cary model 2200 spectrophotometer at ambient temperature. Two-compartment cuvettes with light path of 0.878 cm were used. A baseline was recorded with separated RII (0.76 mg/ml) and cAMP (50 μM) in each compartment of both sample and reference cells. The difference spectrum was obtained after mixing RII and cAMP in the sample cuvette.

3. RESULTS AND DISCUSSION

The regulation of cAMP-dependent protein kinase lies in the fact that the catalytic activity of the C subunit is inhibited by the R subunit and that cAMP can reverse this inhibition by binding to the R subunit, which leads to the dissociation of the holoenzyme. Granot et al. [14] reported that cAMP increased the dissociation constant of the holoenzyme about 10^4-fold. According to Weber et al. [15], there are specific cAMP binding domains in the RII molecule. The effect of cAMP binding must be transmitted to the R and C subunit interaction sites to diminish their affinity. Evidence for such conformational changes induced by cAMP is presented here.

Fig.1 depicts a UV-difference spectrum of RII with bound and unbound cAMP. The spectrum reveals a positive difference peak at 278 nm with shoulders at 288 and 272 nm and a negative band at 256 nm. The positive peak at 278 nm probably arises from perturbations of Tyr 381 and Tyr 196 located in the cAMP-binding domains [8,15]. A single tryptophan, Trp 226 in RII which is 16 Å away from the cAMP-binding sites [15], may give rise to the small difference band at 288 nm. The origin of the difference absorbance at 272 nm could be contributed by any of the 15 phenylalanine residues in the RII subunit.

The negative band at 256 nm is most likely due to the hypochromaticity of cAMP as a result on binding. Weber et al. [15] have predicted possible stacking interactions between the cAMP base and the aromatic rings of tyrosine and phenylalanine. In addition, LaPonte et al. [16] have shown that the fluorescence of cAMP was quenched on binding, which may be reflected as a decrease in absorbance shown in fig.1.

Our CD studies further support the above observations. The near-UV CD spectrum of RII in the presence of cAMP is drastically enhanced in the

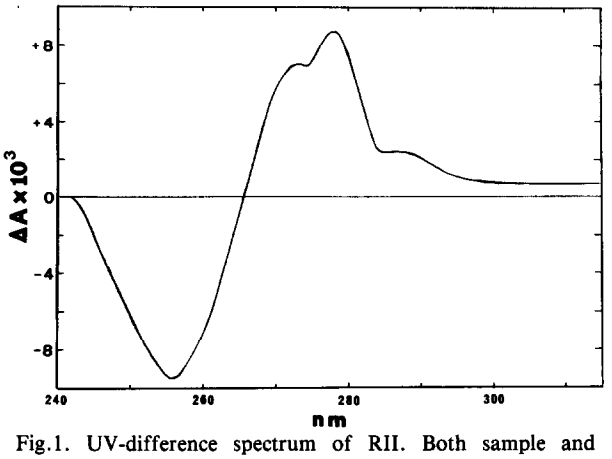

Fig.1. UV-difference spectrum of RII. Both sample and reference cuvettes contained 0.76 mg RII and 50 nmol cAMP in separate compartments of the cell. The spectrum was recorded after mixing RII and cAMP in the sample cuvette.

aromatic amino acid region but diminished in the nucleotide absorption band (fig.2A). There are major peaks at around 295, 290 and 285 nm, which may arise from the ellipticities of tyrosine, tryptophan, phenylalanine and disulfide [17]. Since there are 12 tyrosine, 15 phenylalanine, 6 cysteine and one tryptophan residue in the RII subunit [13], the degree of contribution of each of the amino acids to the change in ellipticity in this region is not clear.

That the binding of cAMP causes secondary structural changes is documented by the far-UV CD spectrum shown in fig.2B. The spectrum of the R subunit shows a negative band at about 209 nm with a shoulder at about 223 nm. In the presence of cAMP, the spectrum was transformed to one with two minima at about 222 and 211 nm. It should be noted that although free cAMP absorbs strongly below 220 nm, it has little or no observable CD in this region at the concentration used in the experiment. The possibility cannot be ruled out, however, that the bound cAMP may contribute to some extent to the observed ellipticity of the secondary protein structure. The significant increase in ellipticity in the 209 nm region most likely reflects an increase in β-structure and unordered form at the expense of the α-helix structure [18]. This speculation is supported by the slight blue shift of the 223 nm shoulder to 222 nm nd the increase in ellipticity at 216 nm [19].

These data clearly establish structural differences between the free and cAMP-bound RII

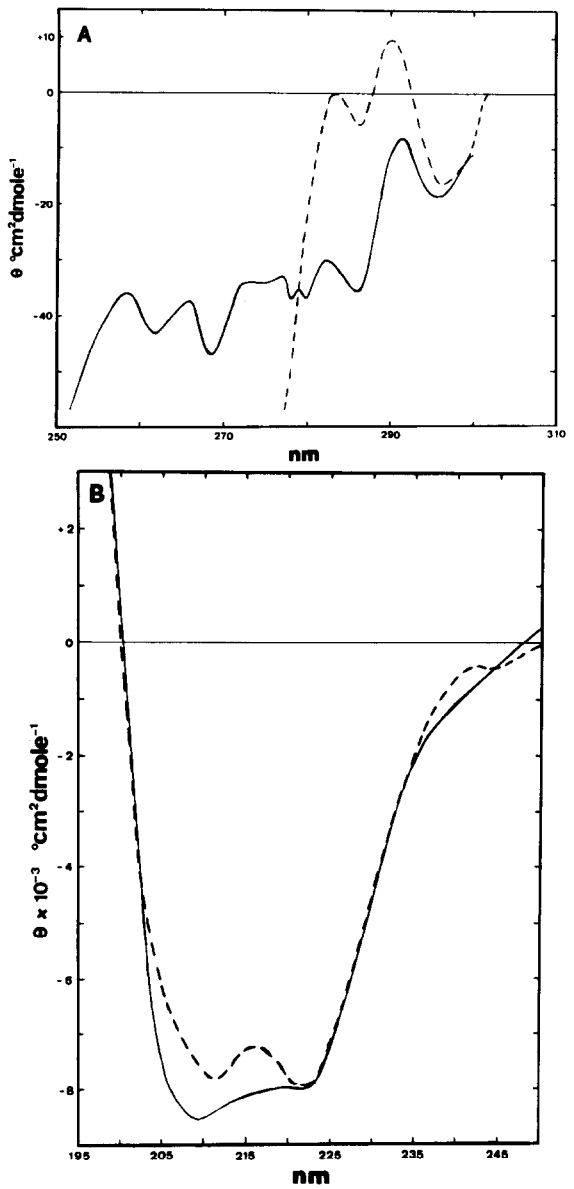

Fig.2. CD spectra of RII. (A) Near-UV CD. The concentration of RII used was 0.9 mg/ml. (————) In the absence of cAMP, (-----) in the presence of 0.23 mM cAMP. (B) Far-UV CD. The RII concentration was 0.04 mg/ml. (————) In the absence of cAMP, (------) in the presence of 0.23 mM cAMP.

subunit. Although the observed effect may be speculated as being a consequence of renaturation of the protein in the presence of cAMP, since RII was prepared by urea detachment from an affinity column, we have demonstrated that the isolated RII is fully active in the inhibition of the C subunit. The detected changes most likely reflect the differences in conformation of the native RII on cAMP binding. These induced structural alterations presumably affect the amino acids at the protein-protein interface such that the C subunit is no longer in a favorable position to bind, which results in an increase in the dissociation constant of the holoenzyme. This molecular mechanism of activation by cAMP may also operate in the type I protein kinase.

Acknowledgements: The research was supported in part by a Seed Money Grant administered by Dr Leon Beghian, Associate Vice President for Research, University of Lowell. The authors wish to thank Dr J. Y. Cassim at the Ohio State University for the use of the CD spectropolarimeter and Dr M.J. McDonald and Ms Linda A. Michalski for technical assistance and the use of their Cary 2200 spectrophotometer.

REFERENCES

[1] Corbin, J.D., Keely, F.L. and Park, C.R. (1975) J. Biol. Chem. 250, 218–225.
[2] Krebs, E.G. (1972) Curr. Top. Cell. Regul. 5, 99–133.
[3] Hofmann, F., Beavo, J.A., Bechtel, P.J. and Krebs, E.G. (1975) J. Biol. Chem. 250, 7795–7801.
[4] Zoller, M.J., Kerlavage, A.R. and Taylor, S.S. (1979) J. Biol. Chem. 245, 12427–12432.
[5] Corbin, J.D., Sugden, P.H., West, L., Flockhart, D.A., Lincoln, T.M. and McCarthy, D. (1978) J. Biol. Chem. 253, 3997–4003.
[6] Builder, S.E., Beavo, J.A. and Krebs, E.G. (1980) J. Biol. Chem. 255, 2350–2354.
[7] Bubis, J. and Taylor, S.S. (1987) Biochemistry 26, 3478–3486.
[8] Bubis, J. and Taylor, S.S. (1987) Biochemistry 26, 5997–6004.
[9] Rannels, S.R. and Corbin, J.D. (1980) J. Biol. Chem. 255, 7085–7088.
[10] Corbin, J.D. and Rannels, S.R. (1981) J. Biol. Chem. 256, 11671–11676.
[11] Robinson-Steiner, A.M. and Corbin, J.D. (1983) J. Biol. Chem. 258, 1032–1040.
[12] Rannels, S.R. and Corbin, J.D. (1981) J. Biol. Chem. 256, 7871–7876.
[13] Takio, K., Smith, S.B., Krebs, E.G., Walsh, K.A. and Titani, K. (1982) Proc. Natl. Acad. Sci. USA 79, 2544–2548.
[14] Granot, J., Mildvan, A.S., Hiyama, K., Kondo, H. and Kaiser, E.T. (1980) J. Biol. Chem. 255, 4569–4573.
[15] Weber, I.T., Steitz, T.A., Dubis, J. and Taylor, S.S. (1987) Biochemistry 26, 343–351.
[16] LaPonte, D.C., Builder, S.E. and Storm, D.E. (1980) J. Biol. Chem. 255, 2343–2349.
[17] Kerlavage, A.R. and Taylor, S.S. (1980) J. Biol. Chem. 255, 8483–8488.
[18] Chen, Y.-H., Yang, J.T. and Martinez, H.M. (1972) Biochemistry 11, 4120–4131.
[19] Greenfield, N. and Fasman, G.D. (1969) Biochemistry 8, 4108–4116.