DIRECT INVOLVEMENT OF THE CYCLIC NUCLEOTIDE BINDING SITES IN THE CYCLIC-NUCLEOTIDE-INDUCED CHARGE SHIFT OF PROTEIN KINASES

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SUMMARY

The holoenzymes of cAMP- and cGMP-dependent protein kinases (cAK and cGK) exist as cyclic nucleotide-free and -bound forms (C.E. Cobb, A.H. Beth, and J.D. Corbin (1987) J. Biol. Chem. 262, 16566-74; L. Wolfe, S.H. Francis, L.R. Landiss, and J.D. Corbin (1987) J. Biol. Chem. 262, 16906-13), which can be resolved by DEAE chromatography due to a cyclic nucleotide-induced increase in surface electronegativity. In the present study, the isolated type I and type II regulatory subunits (R_I and R_II) of cAK also exhibited an electronegative charge-shift in the presence of cAMP, implying that the catalytic subunit was not involved in the apparent change in conformation resulting in the charge-shift of the holoenzyme. A mutant bovine R_I that contained the cAMP binding sites, but was missing regions involved in dimerization and interaction with the catalytic subunit, was expressed in E. coli and purified. This truncated R_I also demonstrated an electronegative charge-shift following cAMP binding, indicating that the binding sites themselves were directly involved in the shift. The increase in surface electronegativity of the cyclic nucleotide binding sites may either be a reflection of an overall conformational change due to cyclic nucleotide binding or it could be directly involved in regulating function.

The abbreviations used are: cAK, cAMP-dependent protein kinase; cGK, cGMP-dependent protein kinase; HPLC, high performance liquid chromatography; R_I and R_II, types I and II regulatory subunits of cAMP-dependent protein kinase; C, catalytic subunit of cAMP-dependent protein kinase.
INTRODUCTION

The cAMP-dependent protein kinase (cAK) and cGMP-dependent protein kinase (cGK) are thought to be homologous proteins, with overall similarities in molecular structure and mechanism of activation (Lincoln and Corbin, 1977; Gill, 1977; Takio et al., 1984). The cAK is a tetramer composed of two catalytic (C) and two regulatory (R) subunits. The two main isozymic forms of cAK, types I and II, have the same C subunits, but different R subunits (RI and RII) (Corbin, Keely, Park, 1975; Nimmo, Cohen, 1977; Carlson, Bechtel, Graves, 1979; Doskeland, Ogred, 1981). The cGK also has two R and two C domains, but since R and C are joined by peptide bond, this enzyme is a dimer (Gill et al., 1976; Lincoln et al., 1976; Lincoln, Dills, Corbin, 1977). There are also at least two isozymic forms of this enzyme (Wolfe, Corbin, Francis, 1989). The cAK and cGK dimerize in the amino-terminal region of the R components. The cAK and cGK R components each have two cyclic nucleotide binding sites which differ in cyclic nucleotide affinity and analog selectivity (Rannels, Corbin 1980; Rannels, Corbin, 1981; Corbin et al., 1982; Mackenzie, 1982; Corbin et al., 1986; Doskeland et al., 1987). The slowly-exchanging binding site is designated site 1 for each of these enzymes, and the rapidly-exchanging site is designated site 2. The cAK and cGK each has an inhibitory domain that is believed to lie between the dimerization domain and the two cyclic nucleotide binding domains in the primary sequence (Takio et al., 1984; Corbin et al., 1978; Flockhart, Corbin, 1982; Weldon, Taylor, 1985; Hofmann, Gensheimer, Gobel, 1985). This region interacts with the respective C component to suppress C activity. However, the inhibitory function is blocked when the cyclic nucleotide binding domains are occupied.

The two intrasubunit cyclic nucleotide binding sites of the regulatory components of cAK and cGK are relatively specific for the respective cyclic nucleotide (Corbin et al., 1986). The binding sites have apparently

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2The two intrasubunit binding sites of cyclic nucleotide-dependent protein kinase are denoted as the slowly exchanging site and the rapidly exchanging site or as site 1 and site 2, respectively. These sites have also been referred to as sites I and II (Mackenzie, 1982) and sites B and A (Doskeland et al., 1987), respectively.