Purification of a soluble casein kinase II from *Dictyostelium discoideum* lacking the β subunit: regulation during proliferation and differentiation

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**Abstract**

A type II casein kinase has been purified from the soluble fraction of *Dictyostelium discoideum* vegetative cells. The enzyme has been purified 370 fold and behaves catalytically as casein kinase type II, in the sense that it utilizes GTP as well as ATP as phosphoryl donors, it is inhibited by low heparin concentrations and phosphorylates a specific peptide for CK II. It is a tetramer of 38 kDa-subunits with catalytic activity and ability to autophosphorylate in vitro. The comparison of this activity with the nuclear enzyme previously purified from the same organism indicates that both have the same molecular structure. Both enzymes have antigenic determinants in common with casein kinase II from bovine thymus, suggesting a high degree of conservation during evolution. Studies on the activity of this enzyme during early differentiation, and in the transition from quiescence to proliferation shows an increase in specific activity suggesting a crucial role for the enzyme in this organism. (Mol Cell Biochem 118: 49-60, 1992)

**Key words**: protein phosphorylation, casein kinases, *Dictyostelium discoideum*

**Abbreviations**: CK – Casein Kinase; TLCK – N-α-p Tosyl Lysil-Chloromethylketone; SDS – Sodium Dodecyl Sulphate; PAGE – Polyacrylamide Gel Electrophoresis; R₃E₃TE₃ – Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu; TCA – Trichloroacetic Acid

**Introduction**

Casein kinase II is a cyclic AMP and calcium-independent protein kinase, which is ubiquitous in eukaryotic cells [1–4]. It is a unique protein kinase in the sense that it is able to use GATP as well as ATP as phosphoryl donor. It is a protein kinase which preferentially phosphorylates serine and threonine residues surrounded by a cluster of acidic amino acid residues (XSXXXEX) [5]. Eukaryotic CKII is a heterotetramer (Mr 150.000) composed of two α and two β subunits with a final
structure $\alpha\alpha\beta_2$ or $\alpha\beta_3$. The $\alpha$ subunit (Mr 37,000-45,000) is the catalytic subunit of the enzyme [6, 7]. The $\beta$ subunit (Mr 24,000-28,000) is required for optimal catalytic activity [8] and is rapidly autophosphorylated. The catalytic activity of CKII is inhibited by heparin ($IC_{50} = 0.3$ mg/ml) and stimulated by polycations such as spermine and polylysine [4].

Although the precise biological roles of CKII has not been determined, it has been proposed to play an important role in the regulation of metabolic pathways, replication, transcription and translation. It is of particular interest, therefore, that several recent studies point to a role of CKII in intracellular signal transduction during cell proliferation and differentiation [9]. The activity of the enzyme was reported to increase significantly in response to several mitogens and growth factors [10-13]. Besides, currently, special attention is being paid to CKII's ability to phosphorylate oncogene products as Myc [14], Myb [15] or the tumor suppressor protein p53 [16]. High levels of CKII activity were observed also during early development of the nematode Caenorhabditis elegans [17].

Autophosphorylation of the $\beta$ subunit could be one possible mechanism for the regulation of casein kinase II activity [18]. Autophosphorylation of other protein kinases has been associated with activation [19, 20].

cDNA from CKII $\alpha$ and $\beta$ subunits from various organisms have been isolated and sequenced [21-25]. The $\alpha$ subunit displays the expected homology to the catalytic domain of other protein kinases, confirming its identification as the catalytic subunit. The $\beta$ subunit has a promoter closely related to the promoter of the regulatory subunit of cAMP-dependent protein kinase [26].

Dicytostelium discoideum is a well known low eukaryote used as a differentiation model [27]. The developmental program of D. discoideum is a complex process, in which chemotaxis, differentiation and patterning play important roles. In all these processes, the cellular activities are regulated by extracellular molecules which act through intracellular signal transduction pathways. Since the chemotactic agent in this organism is cAMP [28] much attention has been paid to cAMP-dependent protein kinase. Casein kinases are the main cAMP-independent protein kinases in this organism. We have already described a nuclear casein kinase II [29] and several cytosolic casein kinases I [30]. At the moment no information is available on the possible role of these kinases in D. discoideum.

We have undertaken the purification and study of a soluble casein kinase II activity to investigate the possible presence of several isoenzymes with different subcellular locations. Using antibodies against bovine thymus casein kinase II we have studied the immunological relationship between the Dicytostelium and the bovine enzyme. With the aim of investigating the possible role of this enzyme, we have studied changes in its activity during the transition from quiescence to proliferation and the early differentiation period of this organism, using the casein kinase II specific peptide substrate Arg-Arg-Arg-Glu-Glu-Thr-Glu-Glu-Glu as the phosphate acceptor [31].

Materials and methods

**Chemicals**

Casein, phosvitin, heparin, polylysine, heparin-agarose, casein-agarose, amino acid standards and molecular weight markers were purchased from Sigma; DE-52 and Phosphocellulose P-11 from Whatman; CNBr-activated sepharose and protein A-sepharose from Pharmacia; heparin-agarose from Sigma; polylysine-sepharose was prepared by coupling 20 mg of polylysine to 1 g of CNBr-activated sepharose-4B, following the procedure described by the manufacturer. [$\gamma$-32P]ATP and [$\gamma$32P]GTP from Amersham International; R3E3TE3 from Peninsula Laboratories and goat-anti-rabbit IgG from Nordic Immunology. Polyclonal antibody against bovine thymus casein kinase was a generous gift of Dr. Dahmus. All other reagents were of the highest grade commercially available.

**Buffers**

Lysis buffer (STEM): 6% sucrose, 50 mM Tris.Cl, pH 7.5, 5 mM EDTA, 5 mM 2-mercaptoethanol. Chromatography buffer (GTEM): 20% glycerol, 50 mM Tris.Cl, pH 7.5, 5 mM EDTA and 5 mM 2-mercaptoethanol. Immunoblot buffer (NET): 140 mM NaCl, 5 mM EDTA, 10 mM Tris.Cl, pH 7.5, 0.2% gelatin and 0.5% Triton X-100.

**Strain**

The biological material used in this work is the Dicytostelium discoideum axenic strain AX-2 grown in HL-5 medium [32].

**Quiescence/proliferation transition**

Cells were grown exponentially in HL-5 medium up to cell densities of $8 \times 10^6$ cell/ml. At cell densities above $1 \times 10^7$ cells/ml, growth is arrested (stationary phase). Reinitiation of growth in a synchronous fashion was