

**YEAST Cu,Zn-SUPEROXIDE DISMUTASE (SOD)
AS AN INHIBITORY SUBUNIT OF PROTEIN KINASE CK2 α '**

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Protein kinase CK2 (previously known as casein kinase type 2 because of its ability to phosphorylate the artificial substrate casein *in vitro*), participates in and regulates a broad spectrum of cellular targets [1]. Many of these proteins are implicated in a wide variety of cellular functions, such as signal transduction, gene expression, and protein synthesis.

The CK2 holoenzyme has a tetrameric structure and is composed of two catalytic (α and α') and two regulatory β -subunits. In *Saccharomyces cerevisiae* this enzyme differs from kinases isolated from animal sources. The sequences of the yeast catalytic α and α' subunits (44 and 39 kDa respectively) and regulatory β and β' subunits (41 and 32 kDa) have been determined [2]. The amino acid sequences of the yeast $\alpha\alpha'\beta\beta'$ holoenzyme are homologous to CK2 from other organisms. The β subunit stabilizes the α subunit against thermal inactivation and stimulates kinase activity against most of protein substrates. The regulatory subunit contains an N-terminal autophosphorylation site, an internal acidic region, and a C-terminal Zn²⁺-binding motif engaged in dimerization of CK2 β [1].

Together with four other second messenger-independent protein kinases, namely PK60S and RAP kinases I-III, CK2 has been shown to regulate activity of the ribosome by phosphorylation of acidic ribosomal P-proteins of the 60S subunit [3]. Phosphorylation of P-proteins and their exchange between ribosome and cytoplasm takes place. Changes in ribosomal stalk conformation and composition can affect expression of specific proteins by affecting the initiation and elongation factors. It has been shown that, in yeast, phosphorylation of P-proteins is correlated with the state of growth of cells and activity of SOD1 [4].

Using a peptide derived from the N-terminal end of SOD as an affinity ligand, the protein kinase known as PK60S was purified to homogeneity and characterized. The purified enzyme has properties similar to those reported for protein kinase type 2. Peptide mass fingerprinting (PMF) identified PK60S as the catalytic α' subunit of CK2 (CK2 α'). SOD1 inhibits CK2 α' activity and inhibition is competitive with respect to the protein substrates – P-proteins and 80S ribosome – with K_i values of 3.5 μ M for recombinant acidic ribosomal protein P2B and 0.6 μ M for 80S ribosomes. As previously shown, differences in activity of yeast CK2 α' , depending on the growth phase of the cells, were

observed. This observation is in close correlation between the state of phosphorylation of ribosomal P proteins isolated from logarithmic and diauxic shift phase cells and presence of the SOD1 protein.

Based on these observations, we presume that SOD1 protein may form some kind of regulatory complex with CK2 α' . To confirm this presumption, pure combined α' subunit of CK2 and SOD were centrifuged in a 10 – 40% glycerol gradient. This showed that CK2 α' interacts with SOD1 to form a complex of molecular weight of ~73 kDa which suggests its structure as CK2 α' (SOD1)₂. In parallel control gradients, α' subunit of CK2 migrates as a single protein of molecular weight of ~39 kDa, and SOD1 as a ~35 kDa dimer.

Attention was then directed to the effect of the SOD on activity of both forms of yeast CK2 phosphorylating acidic protein substrates (casein, 80S ribosome and ribosomal P proteins). We examined whether yeast dismutase inhibits phosphorylation of the five P1/P2 polypeptides and whether SOD inhibits both enzymes. Results obtained showed that inhibition of phosphorylation by SOD applies only to the free catalytic α' subunit and is similar for all P1/P2 ribosomal proteins.

Probably there are some proteins other than SOD present in different cell compartments regulating free CK2 α or/and α' catalytic subunits in a manner similar to the regulatory B/B'/B'' subunits of protein phosphatase type 2A.

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