PHOSPHOLIPID-SENSITIVE Ca^{2+}-DEPENDENT PROTEIN KINASE IN TESTIS: ITS LOCALIZATION, PURIFICATION AND ENDOGENOUS SUBSTRATES, AND INHIBITION BY GOSSYPOL

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In order to study the physiological roles of phospholipid-sensitive Ca^{2+} -dependent protein kinase in testis, its localization, its substrates and their indentification and its inhibition by gossypol were examined.

The enzyme activity was found in pig and rat testis total particulate fractions and pig epididymal fluid, but little in the pig and rat testis cytosolic fractions and mature spermatozoa obtained from pig epididymis. Similarly, its at least three endogenous substrates (83K, 33K and 26K) and five substrates (>100K, 83K, 60K, 43K and 19K) were detected in the testis total particulate fractions and the epididymis, respectively, but little or no substrates in the testis cytosolic fraction and mature spermatozoa.

The enzyme was purified from the total particulate fraction of pig testis by the steps of ; DEAE-cellulose, Sephacryl S-200, CM-cellulose chromatographies and phosphatidylserine-Affigel 10 affinity chromatography. The enzyme was about 80–90% homogenous and its molecular weight was estimated to be 56,000 by SDS-polyacrylamide gel electrophoresis.

The three substrates detected in the total particulate fraction were also observed in nuclear fraction of pig testis, and of the substrates, a 26K protein was identified as high mobility group (HMG) 1 protein (one of the major chromatin-associated non-histone proteins). Exhaustive phosphorylation of HMG 1 by the enzyme revealed that 1 mol of phosphate was incorporated/mol HMG 1. Apparent Km value for HMG 1 was determined to be 3.36 μM.

Gossypol, a potent inhibitor of spermatogenesis, inhibited dose-dependently Ca^{2+} -dependent activity of the enzyme, without affecting the basal activity. IC_{50} value (concentration causing 50% inhibition) was estimated to be 88 μM, when HMG 1 was used as the substrate. Inhibition by gossypol was competitive with respect to phosphatidylserine.

These results suggested that phospholipid-sensitive Ca^{2+}-dependent protein phosphorylation system in testis is involved in the regulation of spermatogenesis.