CHAPTER III

PURIFICATION AND CHARACTERISATION OF A CASEIN KINASE FROM *Candida albicans*

SUMMARY

A cyclic nucleotide independent protein kinase, which is not stimulated by cAMP or cGMP, has been purified and characterized from *Candida albicans* by casein-sepharose affinity chromatography. The purified enzyme exhibits high activity with casein as substrate. Phosvitin can also serve as a substrate, while protamine and histone are comparatively very much less phosphorylated. The purified enzyme appears to be homogenous and migrates as a single band upon neutral polyacrylamide gel electrophoresis. It shows three subunits of 98,000, 61,000 and 55,000 daltons upon SDS-polyacrylamide gel electrophoresis. The K_m for ATP of the purified enzyme is 9.25 μM. The kinase is heat labile and is inactivated by sulphydryl reagents such as p-chloromercuric benzene sulfonic acid.

INTRODUCTION

The abundant distribution of cAMP independent protein kinase has generated growing interest in its function as a controlling factor in cellular mechanisms. A common feature of these enzymes is their preference for acidic proteins like casein and phosvitin as substrates though some of them phosphorylate histone. The casein phosphorylating enzymes, called casein kinases, are present in cytosol and nuclei of many types of cells (210). The specific regulatory role of casein kinases and also, in general, the functional importance of cAMP independent protein kinase need to be studied.

At present the main perspective of studies on these enzymes is concentrated on its regulatory role. The cAMP independent protein kinases have been implicated in the control of intermediary metabolism. Pyruvate dehydrogenase kinase, a cAMP independent protein kinase, is such a regulatory enzyme. This bovine kidney enzyme which catalyzes the phosphorylation of casein (211) also regulates other enzymes by reversible phosphorylation which is a major mechanism of controlling cellular metabolism in response to external stimuli. Another example is the Ca^{++} calmodulin dependent myosin light chain kinase, which has been shown to be essential for the assembly of myosin into filaments and actin activated ATPase activity in smooth muscles and non-muscle tissues (43, 212). A calmodulin dependent protein

kinase independent of cAMP, in rat liver, phosphorylates site 2 of glycogen synthetase without causing activation of the enzyme which normally occurs on phosphorylation, indicating its probable involvement in controlling some other aspect of enzyme activity (213). One important protein kinase controlling cellular action is the epidermal growth factor (EGF) stimulated protein kinase, closely associated with the EGF-receptor complex (74). A similar receptor associated protein kinase is suggested to be active in insulin-receptor complex in insulin action (214). There is a report that protein kinase C, which is activated by Ca^{++} and diacylglycerol, is involved in thrombin induced release of serotonin from platelets. The intercellular target of this enzyme has been implicated to be a 40,000 dalton molecular weight protein (215). An extremely important aspect of cyclic nucleotide independent protein kinase control appears to be in relation to neoplastic transformation. Oncogenic transformation caused by viruses is mediated by a single gene product designated $pp60^{src}$. Protein kinases catalyze the transfer of phosphate groups mostly to serine or phosphoserine or threonine residues in a protein. The $pp60^{src}$ possesses a protein kinase activity specific for tyrosine residue (216, 217). A protein antigenically related to Simian virus (SV40) has been purified from virus infected cells and it has protein kinase activity. No such protein kinase could be detected in uninfected cells (218).

These results are indicative of abnormal protein phosphorylation as being a cause of some viral mediated cancers. Another mechanism, in which the regulatory role of cyclic nucleotide independent protein kinases have been established, is protein synthesis. There are two important events of phosphorylation related to this process. One is the phosphorylation of the initiation factor eIF2 and the other is the phosphorylation of ribosome. The earlier one appears to be of greater importance. In reticulocyte lysates eIF2 phosphorylation by two protein kinases causes inhibition of protein synthesis. One, which is present in the heme deficient lysate, is called the heme controlled repressor and the other is dsRNA activated protein kinase (219). This later enzyme is also present in interferon treated cells and hence it represents a facet of the antiviral action of interferon. The main target of ribosome phosphorylation is the protein S6 (220). The phosphorylation of S6 can be altered, *in vivo*, by hormones, growth factors,mitogens and protein synthesis controlling agents. However, no clear correlation could be demonstrated between protein synthesis and ribosome phosphorylation. Recent studies have indicated that the amino acyl tRNA synthetase complex might be the target for controlling protein synthesis. The inactivation of this complex by glucagon and isobutyl methyl xanthine in isolated hepatocytes, indicated the involvement of cAMP. However, the inhibition of cAMP dependent protein kinase

did not in any way affect this inactivation. Yet, the tRNA synthetase complex was shown to be totally inhibited by phosphorylation and reactivated by dephosphorylation, *in vitro*. Hence, it has been proposed that a cAMP independent protein kinase mediates this inactivation and this may prove to be an important site of metabolic control (221). An inhibitor of protein synthesis in wheat germ which phosphorylated casein and crude wheat germ ribosomes, was shown to be a cAMP independent protein kinase (222). This inhibitor was shown to affect the translation of Bromo Mosaic Virus (BMV) RNA1 and RNA2, without affecting the translation of RNA4. It was found that the formation of 80S initiation complex with RNA1 and RNA2was inhibited by the inhibitor,and 32,000 dalton and 76,000 dalton ribosome associated polypeptides were phosphorylated. Dephosphorylation reversed the inhibition (223). This is, however, in contrast to the report in retic system where the 40S initiation complex formation is inhibited by phosphorylation of eIF2 (178).

Tables IV and V show the casein kinases which have been isolated from different organisms and their properties. It is amply explicit from these studiesthat though casein kinases have been clearly shown to occur in a large number of tissues and systems and important endogenous substrates have been identified, no concrete conclusion has been arrived at with regards to its exact role in cellular control.

TABLE IV

PHYSIOCHEMICAL PROPERTIES OF CASEIN KINASE, TYPE I

Source	Name	Quaternary structure*	Subunit MW	K_m ATP (uM)	Modified amino acid
Reticulocyte, rabbit	Casein kinase I	M	37,000	13	Ser
Erythrocyte, human	None	ND	30,000-32,000	20	ND
Erythrocyte membrane, rabbit	Membrane kinase II	ND	30,000	65	ND
Erythrocyte membrane, human	None	ND	32,000-34,000	11	ND
Thymus, calf	Casein kinase I	M	37,000	22	ND
Skeletal muscle,rabbit	Glycogen synthase kinase I	ND	34,000	15	ND
Novikoff ascites tumor	Kinase I	M	37,000	20	ND
Liver, rat	C_2	ND	ND	10	Ser
Liver nuclei, rat	Nuclear kinase, A_2, KlVb	Dimer, ND	28,000 ND	6-50	Ser,ND
Adrenal cortex,bovine	Casein kinase A	ND	ND	15	ND
Oviduct nuclei,chick	None	ND	ND	13	ND
Yeast	None	M	42,000-50,000	33-200	Ser
Cauliflower nuclei	None	M	39,000	20	ND
Soybean nuclei	None	M	55,000	8	ND

* ND, not determined; M, monomer.

After Hathaway and Traugh (210).

TABLE V

PHYSIOCOCHEMICAL PROPERTIES OF CASEIN KINASE, TYPE II

Source	Name	Quaternary structure*	Subunit M W	Nucleotide Donor	Nucleotide K_m (uM)	Modified amino acid
Reticulocyte, rabbit	Casein kinase II		43,000 24,000	ATP GTP	10 40	Thr
Erythrocyte, human	None	ND	35,000 25,000	ATP GTP	15 26	ND
Erythrocyte membrane, rabbit	Membrane kinase I	ND	ND	ATP GTP	62 48	ND
Liver, rat	C_1	ND	ND	ATP	6	Thr
Liver, nuclei, rat	Nuclear kinase, A_1	,ND	42,000 39,000 26,000	ATP GTP	6-22 30	Thr, ND
Novikoff ascites tumor	Kinase II		44,000 40,000 26,000	ATP GTP	4 7	ND
Thymus, calf	Casein kinase II		44,000 40,000 26,000	ATP GTP	14 30	ND
Brain, calf	None	ND	41,000 26,000	ND	ND	ND
Adrenal cortex, bovine	Casein kinase G	ND	ND	ATP GTP	8 15	ND
Liver rooster	Phosvitin kinase	ND	ND	ATP GTP	8 ND	ND
Oviduct nuclei, chick	None	ND	ND	ATP	9	ND

* ND, not determined.

After Hathaway and Traugh (210)

Recently a casein kinase has been purified from barley embryo which was shown to be a potent inhibitor of protein synthesis in a reticulocyte lysate *in vitro* translation system,at low concentrations. Such an effect has not been demonstrated earlier (224). The existence of cAMP independent protein kinase in general and casein kinases in particular, in chromatin, ribosome, nuclei - i.e. important cellular organelles and their ability to phosphorylate important cellular proteins distinctly points towards an involvement in the control of important cellular reaction. This generated our interest in this class of enzymes and we have purified and characterized a casein kinase from the pathogenic yeast *Candida albicans*, as the initial step towards a better understanding of these enzymes in various control systems.

Casein kinases have been studied in *Saccharomyces cerevisiae* but not in *Candida albicans*. The earliest description of such an enzyme was from Baker's yeast (225). Since then, casein kinases have been purified from the mitochondria and cytosol of *Saccharomyces cerevisiae*, as shown in Table VI.

TABLE VI

CASEIN KINASES FROM *Saccharomyces cerevisiae*

Source (Ref)	Number and Type	Substrate Phosphorylated	Nucleotide used	No.of Sub-unit	Molecular weight and other properties
Cytosol (91)	One	Casein and Phosvitin	-	One	42,000 ± 1,500
Post ribosomal (226) Supernatant	One (PKI)	Acidic proteins L2, L3 and ribosomal wash containing initiation factors	ATP	One	50,000
Mitochondria (227) (inner membrane)	One Casein kinase type I	Serine residue of casein	ATP	One	42,000
Cytosol (228)	Two a.Casein kinase type I	Casein	ATP	ND	30,000 not inhibited by heparin.
	b.Casein kinase type II		ATP and GTP	Two	130,000 strongly inhibited by heparin. Subunits of 37,000 25,000

ND - not determined

RESULTS

Purification of Casein Kinase from *Candida albicans* :

A protein kinase,from the cytosol of *Candida albicans*, was purified by affinity chromatography on casein sepharose. The crude cell extract was loaded directly on the column, previously equilibrated with 100 ml of 20 mM Tris-HCl buffer at pH 7.5. After washing the column with 50 ml buffer, the bound proteins were eluted with 0.5 M KCl. A single peak of protein with casein kinase activity was eluted (Fig. 18). This enzyme was then characterized for its various properties.

Polyacrylamide Gel Electrophoresis of the Enzyme :

The casein kinase migrates as a single band in a non-denaturing gel, which indicates its homogenity (Fig. 19A). On SDS-polyacrylamide gel, it showed three protein bands (Fig. 19B). Using marker proteins as standard, the molecular weight of the subunits was determined to be 98,000, 61,000 and 55,000 daltons (Fig. 20).

Substrate Specificity of the Enzyme :

Table VII shows that both casein and phosvitin were phosphorylated by the enzyme while histone and protamine were poor substrates. In the absence of added substrate, there was no phosphorylation and, this suggests that the enzyme did not undergo autophosphorylation. On increasing concentrations of casein a steady increase in enzyme

FIGURE 18 : PURIFICATION OF CASEIN KINASE FROM [illegible] BY AFFINITY CHROMATOGRAPHY.

Cells from 3-4 litres of exponential culture were harvested and extracted in 20 mM Tris-HCl buffer (pH 7.5) containing 1 mM 2-mercaptoethanol. The crude extract was applied to a casein sepharose column (1x8.5 cm) previously equlibrated with the same buffer and washed till all the unbound proteins were removed. Then the bound proteins were eluted with 0.5 M KCl. Protein concentrations and casein kinase activity was determined as described in 'Materials and Methods'.

o———o protein concentration

enzyme activity

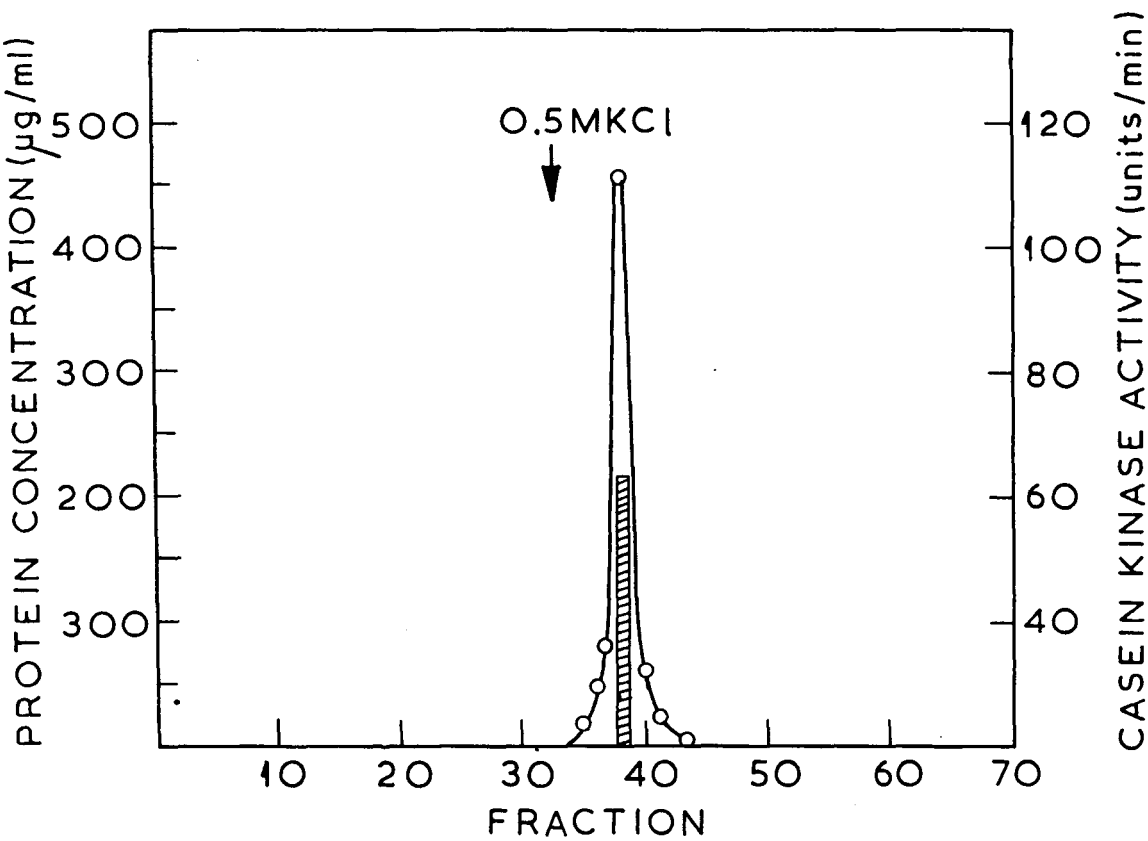

PROTEIN CONCENTRATION (µg/ml)
500
400
300
200
300
0.5MKCl
120
100
80
60
40
CASEIN KINASE ACTIVITY (units/min)
10
20
30
40
50
60
70
FRACTION

FIGURE 19 : POLYACRYLAMIDE GEL ELECTROPHORESIS OF CASEIN KINASE.

Electrophoresis was carried out in the absence (A) and presence (B) of SDS, on 7.5% and 10% polyacrylamide gels respectively, according to the procedures described in Materials and Methods. In (B) lane 1 shows marker proteins and lane 2 shows casein kinase. Markers used were a) Phosphorylase b 94,000 b) Bovine Serum albumin 67,000; c) Ovalbumin 43,000; d) Carbonic anhydrase 30,000; e) Soybean Trypsin Inhibitor 20,000; f) α -Lactalbumin 14,000.

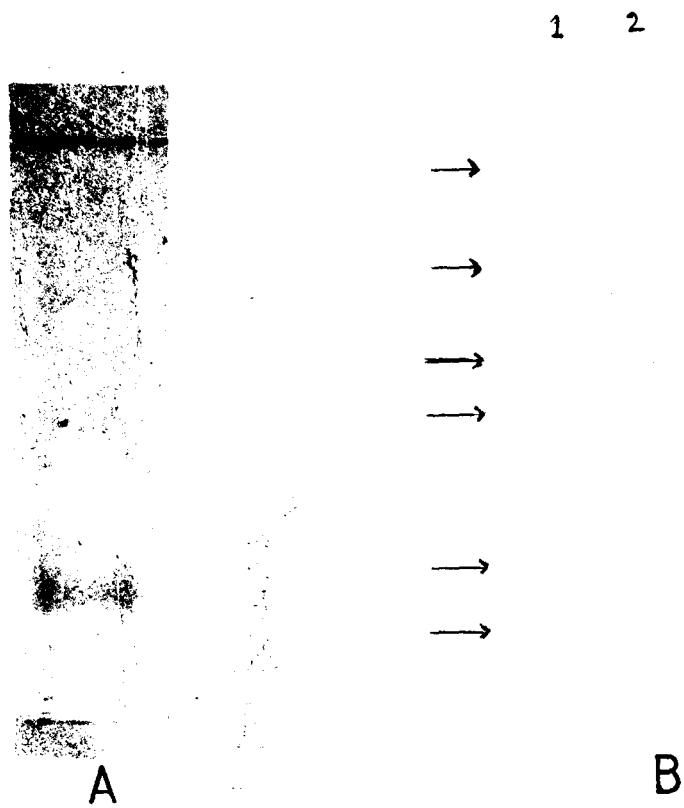

1
2
A
B

FIGURE 20 : DETERMINATION OF MOLECULAR WEIGHT OF THE SUBUNITS OF CASEIN KINASE.

Molecular weight markers were run along with the casein kinase on the SDS-PAGE and the molecular weight of the subunits was determined, from their relative mobility as compared to the marker proteins. Arrows show the distance of migration of the subunit proteins.

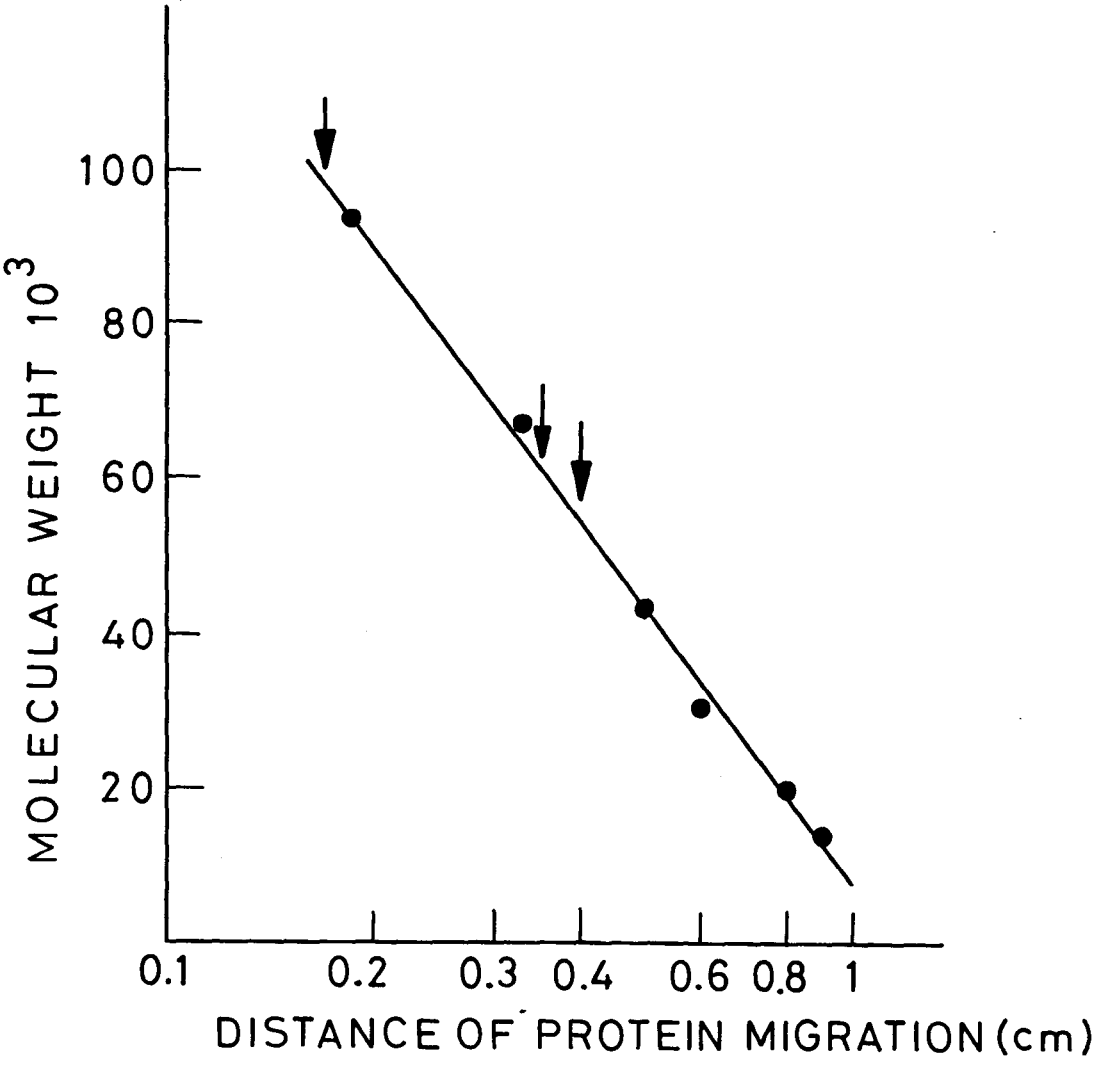

MOLECULAR WEIGHT 10^3
100
80
60
40
20
0.1
0.2
0.3
0.4
0.6
0.8
1
DISTANCE OF PROTEIN MIGRATION (cm)

FIGURE 21 : EFFECT OF SUBSTRATE CONCENTRATION ON CASEIN KINASE ACTIVITY.

Activity of casein kinase was assayed using various concentrations of casein (10-50 µg) in the assay mixture.

Units = number of p moles of ^{32}P transferred to casein.

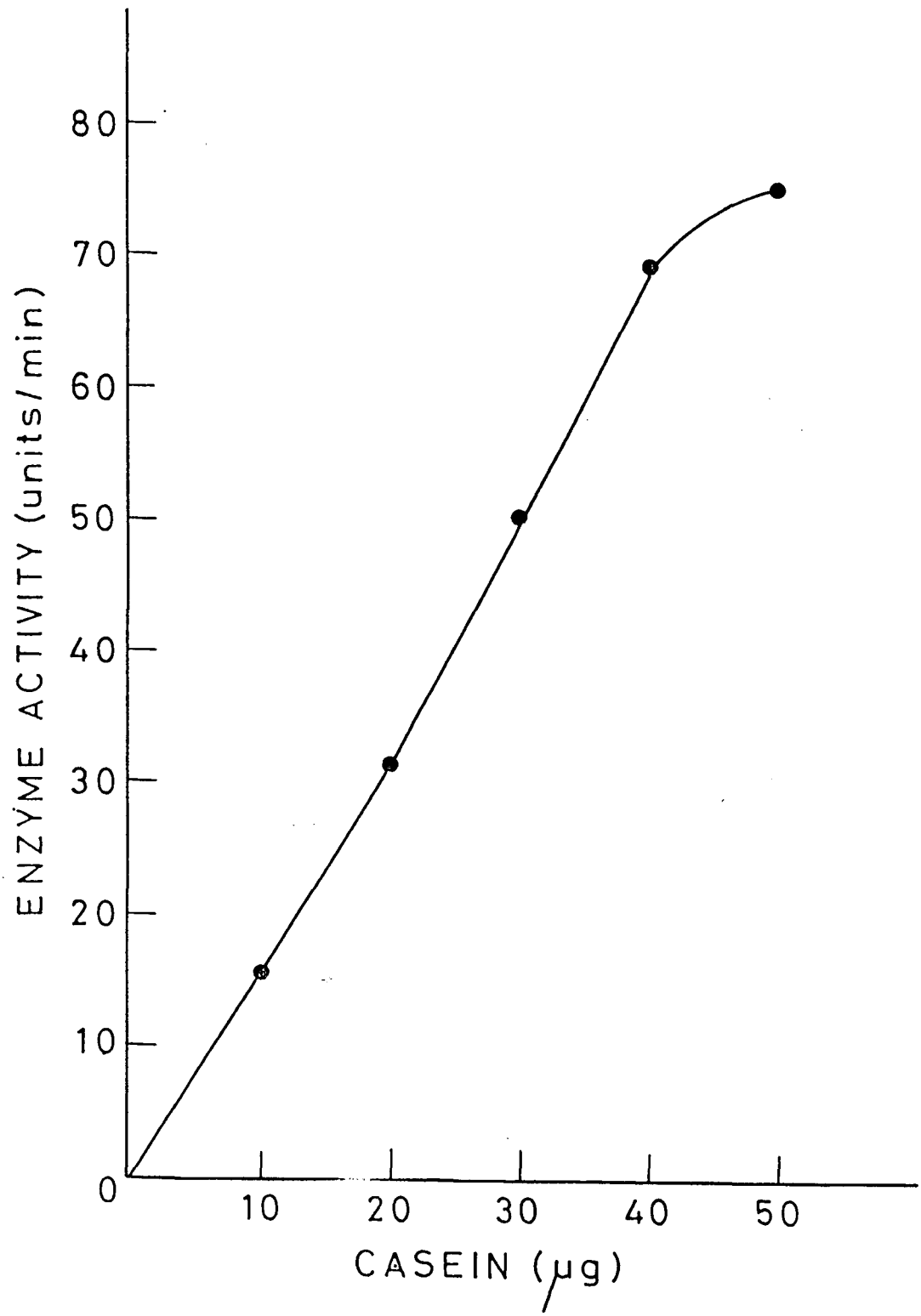

ENZYME ACTIVITY (units/min)
0
10
20
30
40
50
60
70
80
10
20
30
40
50
CASEIN (µg)

TABLE VII : Activity of casein kinase was assayed using different substrates, in the assay mixture. Enzyme activity was also assayed in the absence of added substrate.
Units = pmoles of ^{32}P transferred to casein.

TABLE VII

PHOSPHORYLATION OF DIFFERENT SUBSTRATES BY CASEIN KINASE

	Enzyme Activity (Units/min.)
Without substrate	0.81
With substrate :	
a) Casein	70.41
b) Phosvitin	33.14
c) Histone	1.25
d) Protamine	0.67

activity was observed (Fig. 21).

Ion Dependence :

The requirement for divalent cation for the enzyme activity was determined. In the absence of ion a very low level of activity was observed. Table VIII shows that magnesium is essential for the reaction. Manganese could replace magnesium partly. However, both calcium and zinc were comparatively ineffective. Fig. 22 shows the activity of casein kinase with increasing concentration of magnesium. Data suggests that higher concentrations of magnesium (5 mM) inhibit the enzyme activity. A rat liver nuclei casein kinase was also inhibited at high concentration of magnesium, but above 20 mM (229).

Time Course of Reaction :

In Fig. 23 the time course of reaction of the casein kinase is shown. The enzyme activity increases and is linear upto 15 min of incubation at 30°C. Therefore, all assays were done upto 10 min.

Effect of Cyclic Nucleotides of Enzyme Activity :

Casein kinases are reported to be cyclic nucleotide independent protein kinases. The effect of cAMP and cGMP on the casein kinase from [illegible] is shown in Table IX. The result clearly demonstrates that neither cAMP

nor cGMP affect enzyme activity at concentrations which are capable of stimulating cAMP dependent protein kinase activity. Hence, it is a cyclic nucleotide independent protein kinase.

Effect of ATP on Enzyme Activity :

The enzyme activity was assayed using varying concentrations of ATP (Fig. 24). By a double reciprocal plot, the K_m for ATP was determined to be 9.25 μM (Fig. 24 inset).

Effect of Sulphydryl Reagents and Stability :

Both the sulphydryl reagents, NEM and PCMBS inhibited enzyme activity. At a concentration of 2.0 mM about 70% inhibition was seen with both PCMBS and NEM. The enzyme was heat labile, since heat treatment at 80°C for 10 minutes completely destroyed the enzyme activity as shown in Table X. Freezing and thawing also reduced the enzyme activity. It could also cause complete loss of activity. Activity could be best maintained by storing between 0-4°C in an unfrozen state. However, the activity goes down to zero within two weeks.

Effect of Polyamines on Enzyme Activity :

Polyamines have been shown to stimulate the activity of one class of casein kinase (type II) in presence of Mg^{++} (230). As shown in Fig. 25 both spermine and spermidine strongly inhibit the enzyme activity. However, the inhibitory

TABLE VIII : Casein kinase activity was assayed using different ions in the assay mixture, and also in the absence of any added ion.
Units = pmoles of ^{32}P transferred to casein.

TABLE VIII

EFFECT OF DIFFERENT IONS ON THE ACTIVITY OF CASEIN KINASE

ion	Enzyme Activity (Units/min)
Mg^{++}	59.13
Mn^{++}	22.5
Ca^{++}	13.59
Zn^{++}	13.6
None	14.3

FIGURE 22 : EFFECT OF Mg^{++} CONCENTRATION ON CASEIN KINASE ACTIVITY.

Activity of Casein kinase was determined using various concentrations of Mg^{-+} (1-5 mM) in the assay mixture.

Units = number of p moles of ^{32}P transferred to casein.

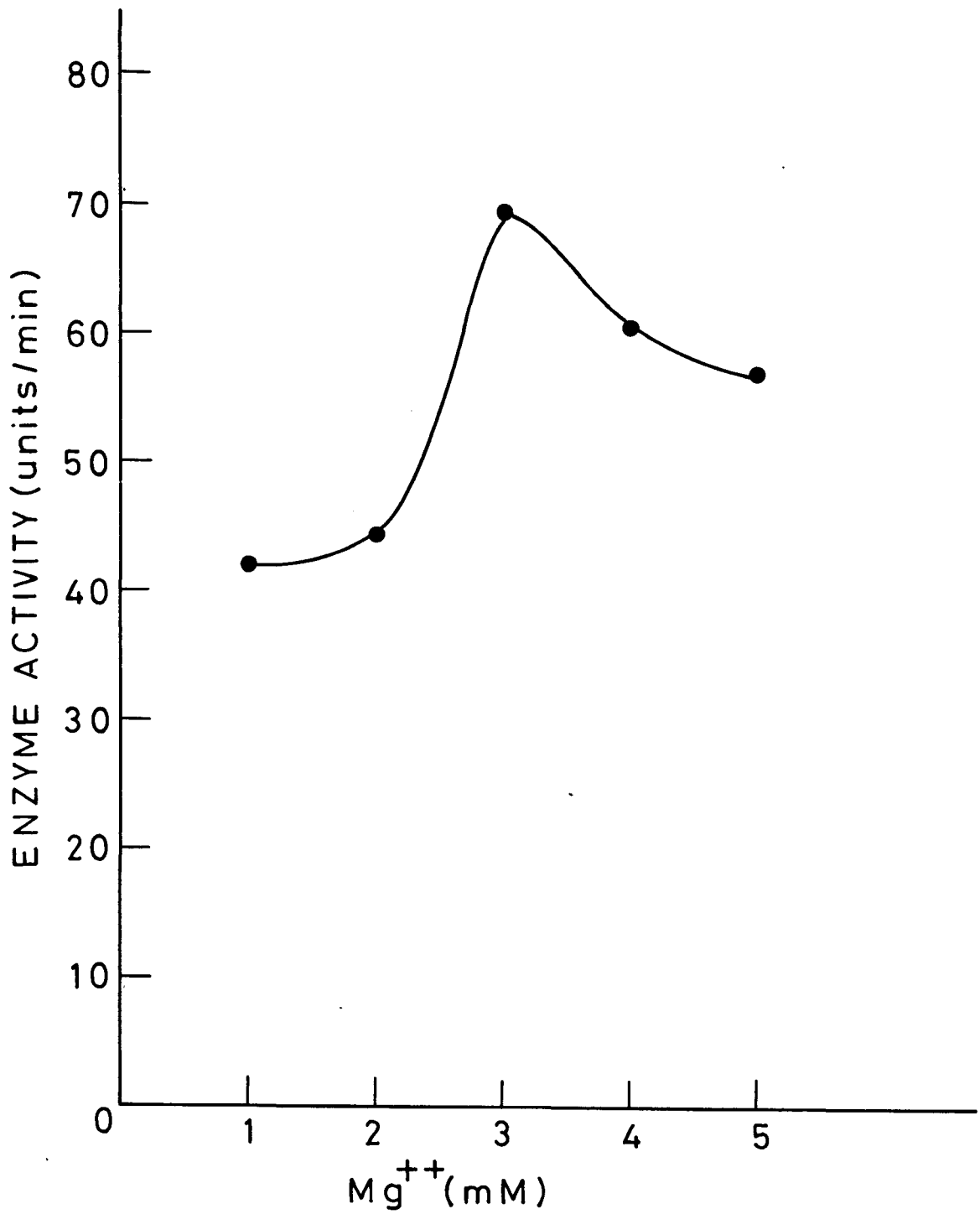

80
70
60
50
40
30
20
10
0
ENZYME ACTIVITY (units/min)
1
2
3
4
5
Mg++(mM)

FIGURE 23 : EFFECT OF THE TIME OF INCUBATION ON CASEIN KINASE ACTIVITY.

Casein kinase activity was assayed upto different times of incubation (0-20 min). Units = number of p moles of ^{32}P transferred to casein.

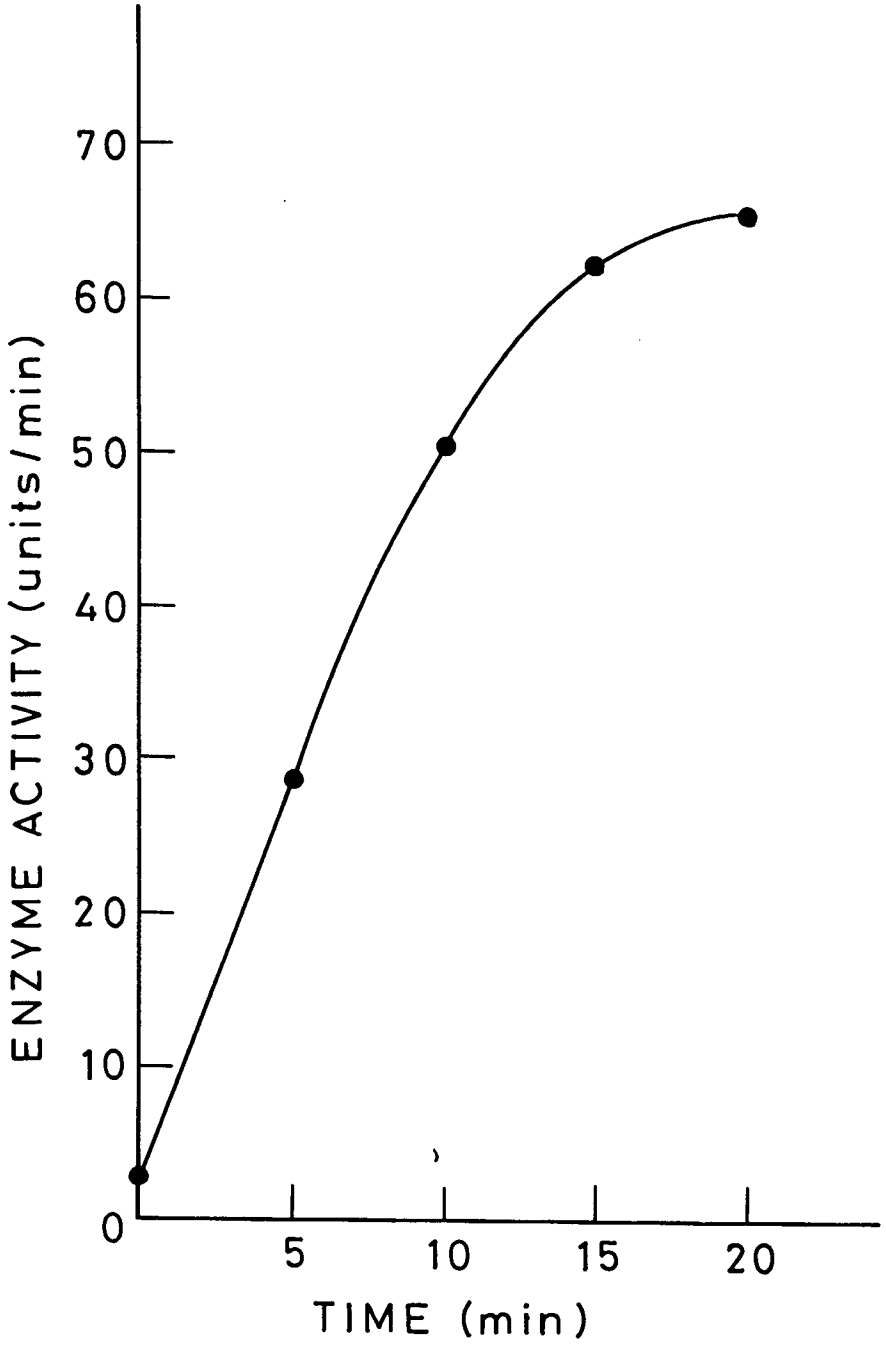

70
60
50
40
30
20
10
0
ENZYME ACTIVITY (units/min)
5
10
15
20
TIME (min)

FIGURE 24 : EFFECT OF ATP CONCENTRATION ON CASEIN KINASE ACTIVITY.

Activity of Casein kinase was assayed using various concentrations of (^{32}P) ATP (0-24.36 μM) in the assay mixture. Inset shows a Lineweaver Burk plot. Units = number of p moles of ^{32}p transferred to casein.

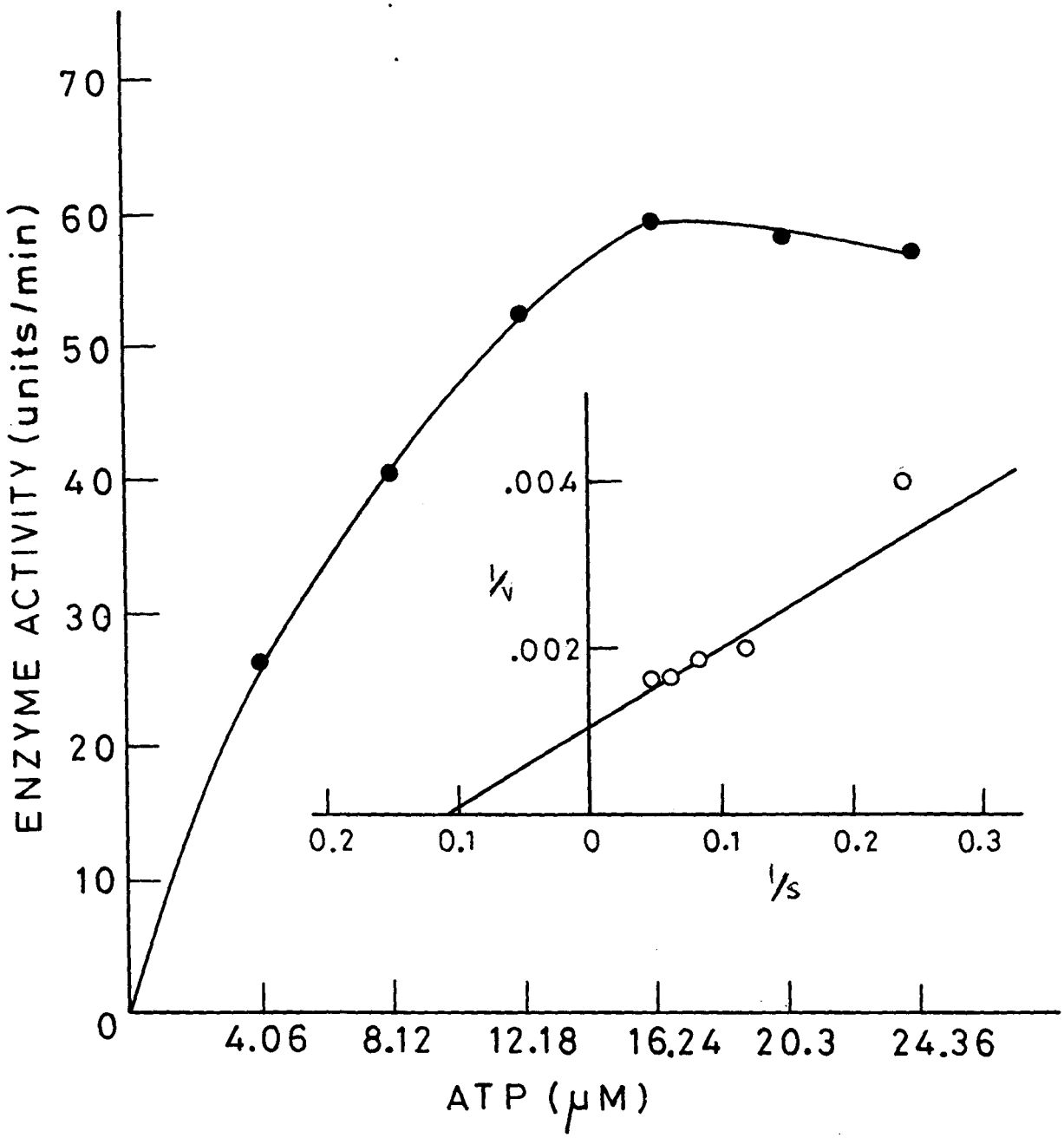

ENZYME ACTIVITY (units/min)
70
60
50
40
30
20
10
0
4.06
8.12
12.18
16.24
20.3
24.36
ATP (μM)
.004
.002
1/v
0.2
0.1
0
0.1
0.2
0.3
1/s

TABLE IX : Casein kinase activity was assayed in the presence and absence of cyclic nucleotides.
Units = pmoles of ^{32}P transferred to casein.

TABLE X : Casein kinase activity was determined in the presence of PCMBS and NEM in the concentrations given. One sample was heated at 30°C for 10min and then used to assay its activity.
Units = pmoles of ^{32}P transferred to casein.

TABLE IX

EFFECT OF CYCLIC NUCLEOTIDES ON THE ACTIVITY OF CASEIN KINASE

Cyclic nucleotide	Enzyme Activity (Units/min)
cAMP (10 μM)	69.44
cGMP (10 μM)	76.37
None	75.86

TABLE X

EFFECT OF SULPHYDRYL REAGENTS AND HEAT TREATMENT ON THE ACTIVITY OF CASEIN KINASE

	Enzyme Activity (Units/min.)	% inhibition
Without Inhibitor	89.75	100
With Inhibitor :		
(1) PCMBS (0.5 mM)	45.59	49.2
(2) PCMBS (1.0 mM)	34.65	61.4
(3) PCMBS (2.0 mM)	26.24	70.8
(4) NEM (1.0 mM)	43.68	51.3
(5) NEM (2.0 mM)	24.16	73.1
Heat treatment at 80°C for 10 min	0.063	99.3

FIGURE 25 : EFFECT OF POLYAMINES AND HEPARIN ON CASEIN KINASE ACTIVITY.

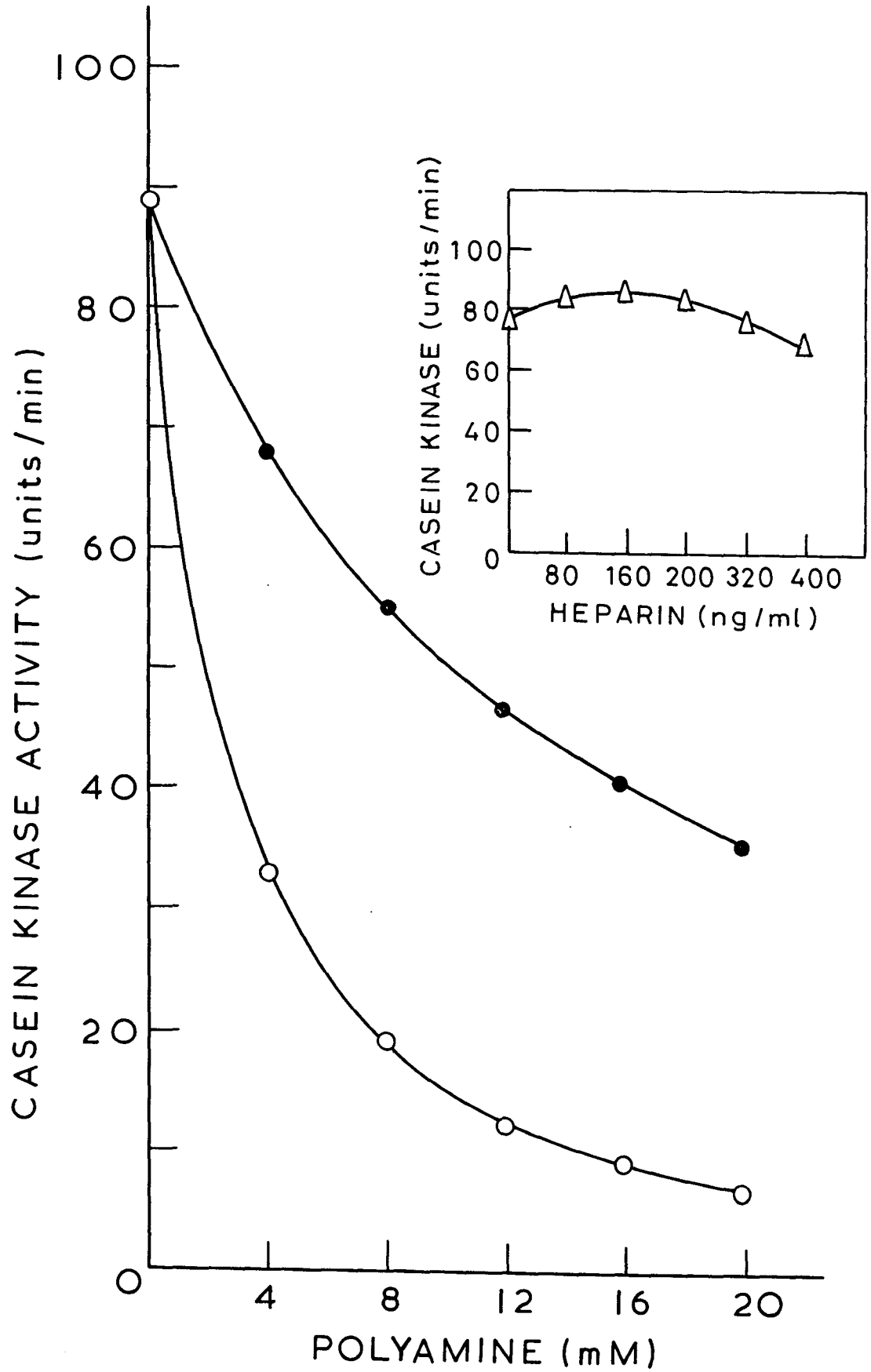
Casein kinase activity was assayed in the presence of varying concentration of polyamines (0-20 mM).

o———o with Spermine

●———● with Spermidine

Inset show the effect of different concentrations of heparin (80-400 ng/ml) on casein kinase activity.

Units = p moles of ^{32}P transferred to casein.


CASEIN KINASE ACTIVITY (units/min)
100
80
60
40
20
0
4
8
12
16
20
POLYAMINE (mM)
CASEIN KINASE (units/min)
100
80
60
40
20
0
80
160
200
320
400
HEPARIN (ng/ml)

effect of spermine appeared to be more than that of spermidine.

Effect of Heparin on Enzyme Activity :

Heparin, a glucosaminoglycan, inhibits the activity of type II casein kinase drastically (230). Casein kinases are classified on the basis of differential response to heparin. Increasing concentration of heparin was ineffective in bringing about any alteration in the activity of the *Candida* casein kinase (Fig. 25 inset). Even a high concentration of heparin (400 mM) was ineffective. This is in contrast to the inhibitory effect reported on some casein kinases.

DISCUSSION

Cyclic AMP independent protein kinases are certainly an important group of regulatory enzymes. Casein kinases are of interest in this group due to their wide distribution. The casein kinases isolated from tissues other than mammary glands are called non-physiological casein kinases. Two such protein kinases exist, which are designated casein kinase I and II.

CK-S (or CKI) and CK-TS(or CKII) have been demonstrated in rat liver (90). In yeast also, there appears to be casein kinases similar to these mammalian casein kinases.

In *Saccharomyces cerevisiae*, a casein kinase similar to CK-S of rat liver was reported earlier (227) in mitochondria. More recently, a casein kinase of the CK-TS type has been reported in cytosol (228) . The purification procedures used in these cases involve various steps including salting out techniques and column chromatography. We have, here, for the first time, purified a casein kinase from *Candida albicans*. Our method of purification is very rapid and consists of a single step affinity chromatography. After the elimination of particulate fraction, the soluble fraction was used to isolate the casein kinase. A similar procedure has been used earlier for the purification of a protein kinase from rat liver nuclei (231) . A direct fractionation of the crude nuclear extract on an affinity column (casein sepharose) showed a single peak of bound protein, which was identified to be a casein kinase and it was eluted with 0.6 M NaCl. Our casein kinase also eluted in a single peak (Fig. 18) and migrated as a single protein band on a neutral polyacrylamide gel (Fig.19A). In SDS-PAGE i.e. under denaturing conditions, it showed three protein bands (Fig.19B)of 98,000, 61,000 and 55,000 dalton molecular weights (Fig.20). However, our casein kinase sometimes migrated as two bands in tube gels, but in slab gels we observed three bands.

Substrate specificity of the casein kinase, shown in Table VII shows a similarity with many other casein kinases. Casein and phosvitin are usually good substrates for casein

kinase. In general, acid proteins or non-histone proteins have been shown to be better substrates for casein kinases. Some casein kinases have been shown to undergo autophosphorylation (92, 93). But our casein kinase showed no phosphorylating activity in the absence of casein, indicating that there is no autophosphorylation. However, the implication and functions of autophosphorylation or absence of it is not known.

Cyclic AMP independent protein kinases in some system are sensitive to sulphydryl reagents (232), whereas, in others it is insensitive (233). Our casein kinase could be inhibited by the reagents such as PCMBS and NEM. The enzyme is also heat labile and heating at 80°C for 10 minutes completely inactivates it.

One property of casein kinase that is being studied is the modulation of its activity by polyamines and heparin. Both polyamines and heparin have been implicated in protein synthesis. While polyamines have been established to stimulate protein synthesis in a manner yet unclear heparin is distinctly inhibitory in action. Casein kinase activity appears to be greatly susceptible to alteration by polyamines and heparin. However, whether this reflects on its role in translation control or not cannot yet be predicted. Recently evidence has been obtained regarding a direct inhibitory effect of a barley embryo casein kinase on translation in retic lysate (224). Casein kinases can be classified

according to the effect of heparin on them. The type I casein kinases are rather insensitive to heparin, whereas the type II enzymes are drastically inhibited by heparin. On the basis of this distinction our casein kinase appears to be of the type I class, since, as shown in Fig. 25 inset, heparin did not effect the enzyme activity upto a concentration which is inhibitory for both rat liver and yeast casein kinase II. The type I casein kinases have been abundantly reported in every class of eukaryotic organism, whereas type II casein kinases have been shown mostly in mammalian and avian tissues (210). A type II casein kinase has been isolated from the cytosol of *Saccharomyces cerevisiae*. In this, two casein kinases were shown in the cytosol one of which was insensitive to heparin (the first peak on phosphocellulose column), while the other was markedly inhibited. The heparin insensitive fraction in the cytosol has been predicted to be identical to the mitochondrial casein kinase of yeast reported earlier.

The effect of polyamines on our casein kinases is unusual. Though polyamines have been reported to stimulate the activity of type II casein kinases in the presence of Mg^{++} and low concentration of substrate (230) their effect on type I casein kinase is not clear. However, a cAMP independent protein kinase phosphorylating casein and phosvitin was found in wheat germ which was strongly inhibited by spermine but slightly stimulated by low concentrations

of spermidine. This enzyme was also inhibited by heparin at 100 µg/ml concentration [234]. The implication of the inhibition of this [illegible] casein kinase I by spermine and spermidine is not clear. Considering the stimulatory action of polyamines on protein synthesis mentioned earlier and the reported inhibition of protein synthesis by casein kinase in barley embryo, it is tempting to speculate that this property may reflect a role of the casein kinase in protein synthesis. The exact nature of this remains to be elucidated using this enzyme in an [illegible] translation system.