Biochem. J. (1976) **157**, 117–126
Printed in Great Britain

Adenosine 3′:5′-Cyclic Monophosphate-Dependence of Protein Kinase Isoenzymes from Mouse Liver

By PER MAGNE UELAND and STEIN OVE DØSKELAND
Cell Biology Research Group, Preclinical Institutes, University of Bergen, Bergen, Norway

(*Received* 9 *January* 1976)

Conditions influencing the cyclic AMP-dependence of protein kinase (ATP–protein phosphotransferase, EC 2.7.1.37) during the phosphorylation of histone were studied. Protein kinase from mouse liver cytosol and the two isoenzymes [PK (protein kinase) I and PK II] isolated from the cytosol by DEAE-cellulose chromatography were tested. A relation between concentration of enzyme and cyclic AMP-dependence was observed for both isoenzymes. Moderate dilution of isoenzyme PK II decreased the stimulation of the enzyme by cyclic AMP. Isoenzyme PK I could be diluted 200 times more than isoenzyme PK II before the same decrease in cyclic AMP-dependence appeared. Long-term incubation with high concentrations of histone increased the activity in the absence of cyclic AMP relative to the activity in the presence of the nucleotide. This was more pronounced for isoenzyme PK II than for isoenzyme PK I. The cyclic AMP concentration needed to give half-maximal binding of the nucleotide was the same as the cyclic AMP concentration (K_a) at which the protein kinase had 50% of its maximal activity. The close correlation between binding and activation is also found in the presence of KCl, which increased the apparent activation constant (K_a) for cyclic AMP. With increasing [KCl], a progressively higher proportion of the histone phosphorylation observed in cytosol was due to cyclic AMP-independent (casein) kinases, leading to an overestimation of the degree of activation of the cyclic AMP-dependent protein kinases present. The relative contributions of cyclic AMP-dependent and -independent kinases to histone phosphorylation at different ionic strengths was determined by use of heat-stable inhibitor and phosphocellulose chromatography.

We previously described a differential effect of $(NH_4)_2SO_4$ and acid precipitation on the two main cyclic AMP-dependent protein kinase isoenzymes (PK I and PK II) resolved from mouse liver cytosol by DEAE-cellulose chromatography (Døskeland & Ueland, 1975*a*). Under the conditions then used (absence of Mg-ATP), one of the isoenzymes (PK I) was preferentially dissociated by $(NH_4)_2SO_4$. A preferential dissociation of PK I by salt has been found for this isoenzyme from several other sources (Corbin *et al.*, 1975). This effect of salt is, however, not seen in the presence of Mg-ATP (Corbin *et al.*, 1975).

The aim of the present work was to compare the two isoenzymes in the presence of Mg-ATP. Enzyme concentration, histone concentration, incubation time and ionic strength of the incubation mixture were varied. We especially wanted to see if there were differences in the cyclic AMP-dependence of the two isoenzymes when the concentration of enzyme and of other components in the incubation medium approached those believed to exist in the cell.

The relation between cyclic AMP activation of, and cyclic AMP binding to, protein kinase was studied for both isoenzymes under varying conditions.

The cyclic AMP-independent protein kinases of mouse liver cytosol were also studied, as their presence influenced the determination of the basal activity of cyclic AMP-dependent protein kinase at high ionic strength.

Materials and Methods

Chemicals

[γ-^{32}P]ATP (approx. 18 Ci/mmol) and cyclic [^{3}H]-AMP (27 Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Type II histone, cyclic AMP, ATP and EGTA [ethanedioxybis(ethylamine)tetra-acetic acid] were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. DEAE-cellulose (DE-52) and phosphocellulose (P-II) were obtained from Whatman Biochemicals Ltd., Maidstone, Kent, U.K. Casein (Hammerstein) and other chemicals, which were of analytical grade, were from Merck, Darmstadt, Germany.

Heat-stable inhibitor protein

This, prepared as described (Døskeland & Ueland, 1975*a*), was further purified by fractionation with 20–40%-satd. $(NH_4)_2SO_4$ and desalted on a Sephadex G-25 column (0.9cm×15cm) equilibrated with 15mM-Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid] buffer adjusted to pH7.0 by adding 2M-NaOH. A portion (4μg) of this inhibitor preparation in 150μl of incubation mixture gave maximal inhibition of 150 units (1 unit = 1pmol of phosphate incorporated/min) of cyclic AMP-dependent protein kinase.

Protein kinase assay

The incubation mixture contained 15mM-Hepes, pH7.0, 10mM-magnesium acetate, 0.3mM-EGTA, 30μM-ATP, γ-[^{32}P]ATP (1μCi/ml), histone (type II; Sigma Chemical Co.), at the concentrations indicated in Figure legends, or casein (6mg/ml). Inhibitor, KCl or cyclic AMP was added when indicated. The reaction was started by addition of enzyme. A unit of enzyme activity is the amount of enzyme incorporating 1pmol of phosphate into histone (0.6mg/ml) per min at 30°C. Activity ratio is defined as the ratio of protein kinase activity in the absence of cyclic AMP to that in the presence of the nucleotide (2μM). Throughout this work, activation of protein kinase means increase in the activity ratio.

The protein kinase activity of all the preparations used was linear with respect to time and enzyme concentration (in the presence of cyclic AMP).

Binding of cyclic [^{3}H]AMP

This was assayed as described in the legend to Fig. 7. The incubations were terminated by pipetting a 200μl portion of the reaction mixture into 2ml of ice-cold 80%-satd. $(NH_4)_2SO_4$ (Døskeland & Ueland, 1975*b*). The precipitates (containing the bound radioisotope) were collected by suction through Millipore filters (HAWP; 0.45μm), washed with 3×3ml of 60%-satd. $(NH_4)_2SO_4$, and put into scintillation vials containing 1ml of 1% sodium dodecyl sulphate. The contents were well mixed and 7ml of scintillation fluid (Unisolve; Koch–Light, Colnbrook, Bucks., U.K.) was added. Radioactivity was measured in a Nuclear–Chicago Isocap 300 liquid-scintillation counter.

Protein

This was measured by the method of Klungsøyr (1969) by using bovine serum albumin as a standard.

Preparation of cytosol

Adult male NMRI mice were killed by cervical dislocation, the livers rapidly removed and put into ice-cold 15mM-Tris/HCl buffer (pH7.6)/6mM-EDTA/5mM-2-mercaptoethanol/0.25M-sucrose (buffer A). The livers were minced with scissors and then homogenized in buffer A (1:7, w/v) with two strokes at 465rev./min in a Teflon/glass homogenizer. The 20000$g_{av.}$ supernatant of the homogenate was spun at 100000$g_{av.}$ for 1h. The cytosol so obtained was applied to a column (2.6cm×40cm) of Sephadex G-25 equilibrated with 10mM-Tris/HCl buffer, pH7.5, containing 4mM-EDTA and 5mM-mercaptoethanol (buffer B) and eluted in the same buffer.

Preparation of isoenzymes by DEAE-cellulose chromatography

Cytosol from 45g of liver was applied to a column (2.4cm×31cm) of DEAE-cellulose (DE-52) equilibrated with buffer B, and eluted with a 900ml linear gradient of 0–300mM-KCl. The isoenzymes (Døskeland & Ueland, 1975*a*) are referred to as 'PK I' and 'PK II' in the order of elution from the column.

Separation of casein kinase from cyclic AMP-dependent protein kinase by phosphocellulose (P-11) chromatography

Fractions 28–32 from the DEAE-cellulose column (Fig. 1) were pooled and made 250mM in KCl. Then 50ml of this preparation was applied to a column (0.9cm×4cm) of phosphocellulose (P-11) previously equilibrated with buffer B containing 250mM-KCl. The column was then washed with 20ml of the same buffer; 95% of the histone kinase activity was recovered in the first 50ml of effluent, which contained no detectable casein kinase activity. This preparation is referred to as isoenzyme PK II unless otherwise indicated. Some 60% of the casein kinase applied was eluted with 30ml of buffer B containing 900mM-KCl.

All preparations of enzyme were passed through a column (0.9cm×6cm) of Sephadex G-25 equilibrated with 15mM-Hepes buffer adjusted to pH7.0 by adding 2M-NaOH (buffer C) before being assayed.

Results

Study on the interference by cyclic AMP-independent (casein) kinase

During preliminary experiments on the effect of salt on the activity ratio of cyclic AMP-dependent protein kinase, we observed a progressive apparent activation, between 0 and 300mM-KCl, of protein kinase in cytosol and crude preparations of isoenzyme PK II (Fig. 2).

A kinase that preferentially phosphorylated casein partially co-chromatographed with isoenzyme PK II on DEAE-cellulose (Fig. 1*b*). This enzyme, presumably so-called 'casein kinase' (Takeda & Nishizuka, 1974) was independent of cyclic AMP and stimulated by KCl, whereas the cyclic AMP-dependent protein

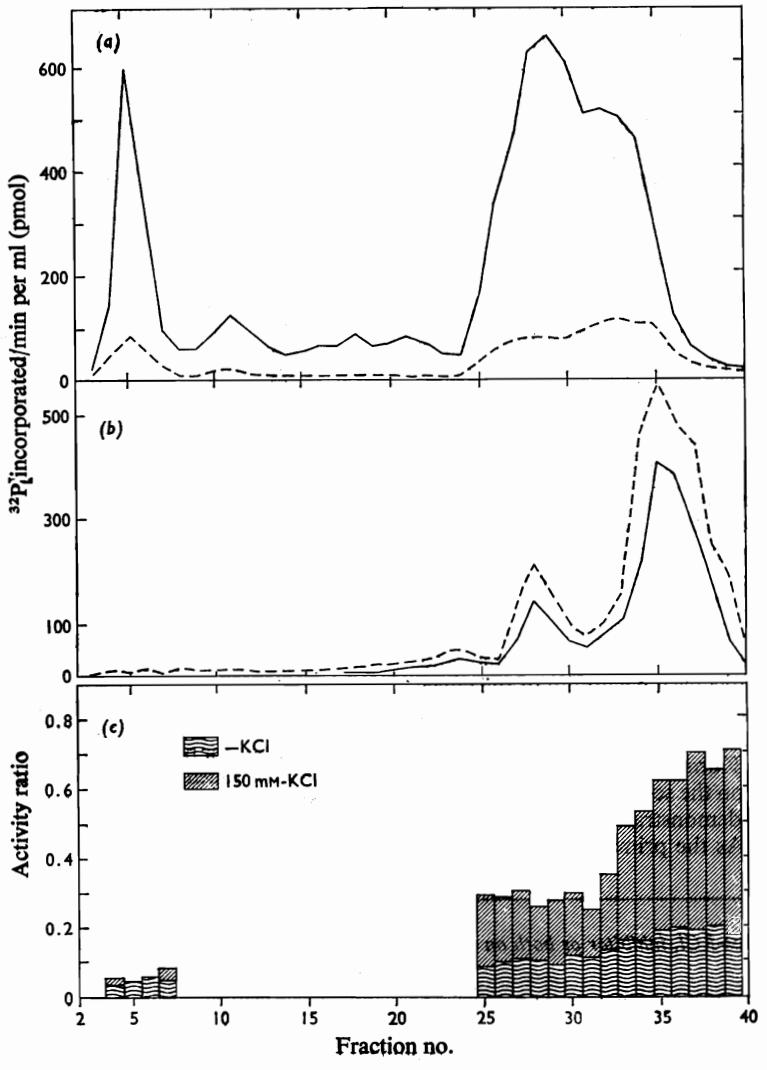

Fig. 1. *Chromatography of liver cytosol on DEAE-cellulose and measurement of activity ratio of protein kinase in separate fractions in the absence and presence of* 150 mM-*KCl*

Portions of the fractions (3ml) obtained by DEAE-cellulose chromatography of cytosol were passed through columns (0.4cm×3.2cm) of Sephadex G-25 equilibrated with buffer C. The protein was collected quantitatively in 500μl of effluent, and assayed for protein kinase activity. (*a*) —— indicates phosphorylation of histone (0.6mg/ml) in the presence of cyclic AMP (2μM). ---- shows the same as above but 150mM-KCl was added to the incubation mixture. Activity in the absence of cyclic AMP is not indicated. (*b*) ----, Phosphorylation of casein (6mg/ml) in the presence of 150mM-KCl. ——, Activity in the absence of KCl. (*c*) The activity ratio of protein kinase of the DEAE-cellulose fractions with histone as substrate in the absence and presence of 150mM-KCl.

kinase was strongly inhibited by KCl (Fig. 1*a*). An opposite effect of KCl on these two types of protein kinase has also been noted for the enzymes from human lymphocytes (Murray *et al.*, 1972; Kemp *et al.*, 1974). Low cyclic AMP-dependence of histone phosphorylation in the presence of KCl was observed in the fractions containing casein kinase (Figs. 1*a*, 1*b* and 1*c*). This suggested that casein kinase might be

responsible for the apparent activation of cyclic AMP-dependent protein kinase by salt (Fig. 2).

The activity of casein kinase with histone as substrate, after the cyclic AMP-dependent protein kinase had been removed by phosphocellulose chromatography, was about 4% of the activity found when casein was the phosphate acceptor. Phosphorylation of histone by casein kinase was stimulated (about 30%) by 150mM-KCl (results not shown). Cyclic AMP-dependent protein kinase containing no casein kinase was not activated by salt.

The specific inhibitor of cyclic AMP-dependent protein kinase (Ashby & Walsh, 1972) only partly suppressed the histone phosphorylation by a preparation of isoenzyme PK-II containing casein kinase in the presence of 150mM-KCl (Table 1). This is further evidence that casein kinase may account for a considerable part of the cyclic AMP-independent phosphorylation of histone at high ionic strength. The experiments reported below were therefore conducted on preparations of isoenzyme PK II from which casein kinase had been separated by phosphocellulose chromatography.

The finding that KCl stimulated cyclic AMP-independent casein kinase with both histone and casein as substrate indicates a generality of the effect of KCl on casein kinase. One study on casein kinases showed that the effect of KCl was to prevent substrate inhibition by casein (Kumar & Tao, 1975), whereas a direct effect of KCl on the sedimentation behaviour of casein kinase was demonstrated in another study (Kemp *et al.*, 1974). As the primary concern of this investigation was cyclic AMP-dependent phosphorylation, the casein kinases were not further studied.

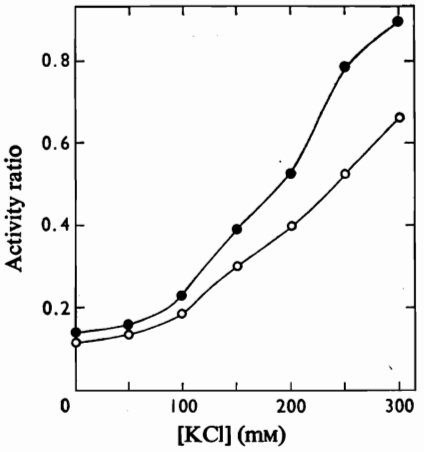

Fig. 2. *Activity ratio of protein kinase as a function of the concentration of KCl*

Mouse liver cytosol (○) and fraction 33 [isoenzyme PK II (●)] from the DEAE-cellulose column (Fig. 1*a*) were assayed for protein kinase activity in the absence and presence of added cyclic AMP (2μM). The concentrations of histone and protein kinase were 0.6mg/ml and 400 units/ml respectively. The reaction was allowed to proceed for 10min.

Table 1. *Effect of 150mM-KCl, inhibitor or both on the activity ratio of isoenzyme PK II before and after phosphocellulose chromatography*

Fractions 33–35 from the DEAE-cellulose column (Fig. 1) were pooled and divided into two parts. One portion was chromatographed on a phosphocellulose column as described in the Materials and Methods section. Both portions were then desalted by passage through a column of Sephadex G-25 equilibrated with buffer C. Phosphate incorporated into histone (0.6mg/ml) was determined in the absence and presence of 2μM-cyclic AMP. The reaction was run for 10min. Inhibitor protein and KCl were added to the incubation mixture as indicated in the Table.

Preparation of enzyme	Addition to assay buffer	Phosphate incorporated (pmol/min per ml) −Cyclic AMP	Phosphate incorporated (pmol/min per ml) +Cyclic AMP	Ratio (−/+)
Fractions 33–35 from the DEAE-cellulose column (Fig. 1*a*)				
A	None	48	372	0.13
B	150mM-KCl	37	76	0.48
C	Inhibitor (50μg/ml)	16	27	0.60
D	Inhibitor (50μg/ml)+150mM-KCl	31	30	1
B–D		6	46	0.13
Same preparation as above, after removal of casein kinase by phosphocellulose chromatography				
E	None	34	305	0.11
F	150mM-KCl	3	30	0.10
G	Inhibitor (50μg/ml)	0.3	1	0.30

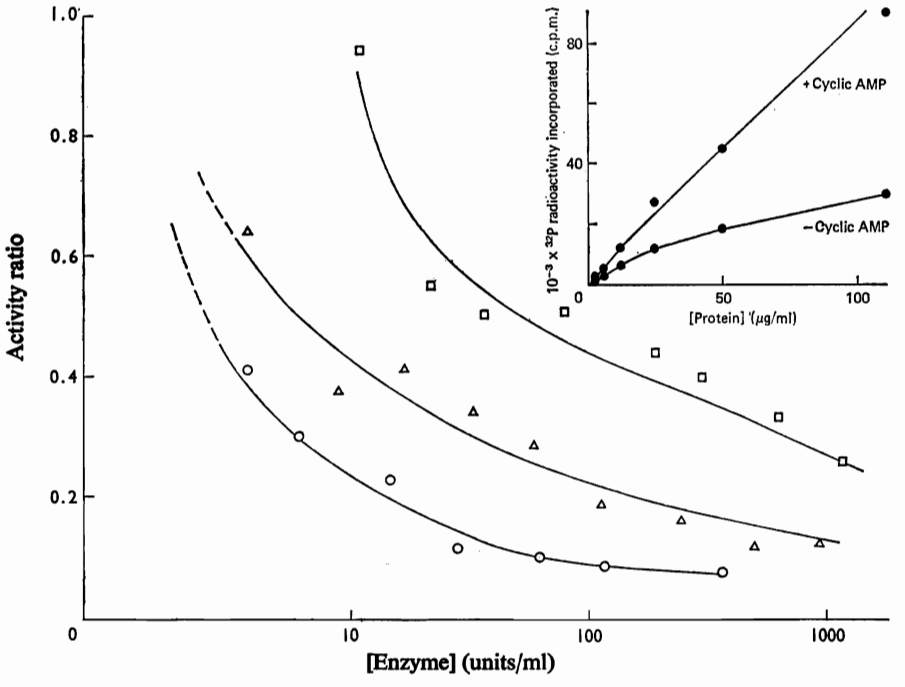

Fig. 3. *Cyclic AMP-dependence of isoenzymes PK I* (○) *and PK II* (□) *and protein kinase of cytosol* (△) *as a function of enzyme concentration*

Incubations were performed for 10min in the presence of 3mg of histone/ml and the concentrations of enzyme indicated on the abscissa. The results are plotted as activity ratio versus concentration of enzyme. Inset: the results obtained for the preparation of isoenzyme PK II plotted as concentration (μg of protein/ml) versus activity in the absence and presence of 2μM-cyclic AMP.

Cyclic AMP-dependence of protein kinase isoenzymes under various conditions

The cyclic AMP-dependence of protein kinase in cytosol and in the preparations of isoenzyme PK I and especially isoenzyme PK II decreased when measured at progressively lower concentration of enzyme (Fig. 3). The protein kinase of cytosol and isoenzyme PK I had to be diluted 10 and 200 times more respectively than isoenzyme PK II to show the same degree of activation. The same results were obtained for isoenzymes PK I and PK II before and after phosphocellulose chromatography.

The loss of cyclic AMP-dependence by dilution was intermediary for protein kinase in liver cytosol compared with isoenzymes PK I and PK II. This might be expected, since cytosol contained both isoenzymes.

Increasing concentrations of histone induced an activation of both isoenzymes PK I and PK II. Isoenzyme PK II was activated to a much greater extent than isoenzyme PK I (Fig. 4). This effect of histone declined when the concentration of enzyme was increased, but was present over a 400-fold range of enzyme concentration (Fig. 5). The activation of isoenzyme PK II by high concentration of histone was partly prevented by including 150mM-KCl in the assay mixture (Fig. 6).

The activity ratio increased progressively during the incubation of both isoenzyme PK I and PK II. This loss of cyclic AMP-dependence with time was found at all concentrations of histone tested, but was more pronounced at high concentrations (Fig. 4, inset). When the incubation was performed for only 2min in the presence of 0.17mg of histone/ml and at a high enzyme concentration (600 units/ml), an extremely low activity ratio (less than 0.02) was observed for both isoenzymes (results not shown).

Isoenzyme PK II was eluted as a broad peak compared with isoenzyme PK I and the casein kinase (Figs. 1*a* and 1*b*). This might indicate enzyme heterogeneity. Isoenzyme PK II from rabbit erythrocytes has been partially separated into two peaks by chromatography on QAE [diethyl-(2-hydroxypropyl)aminoethyl]-Sephadex (Tao & Hackett, 1973).

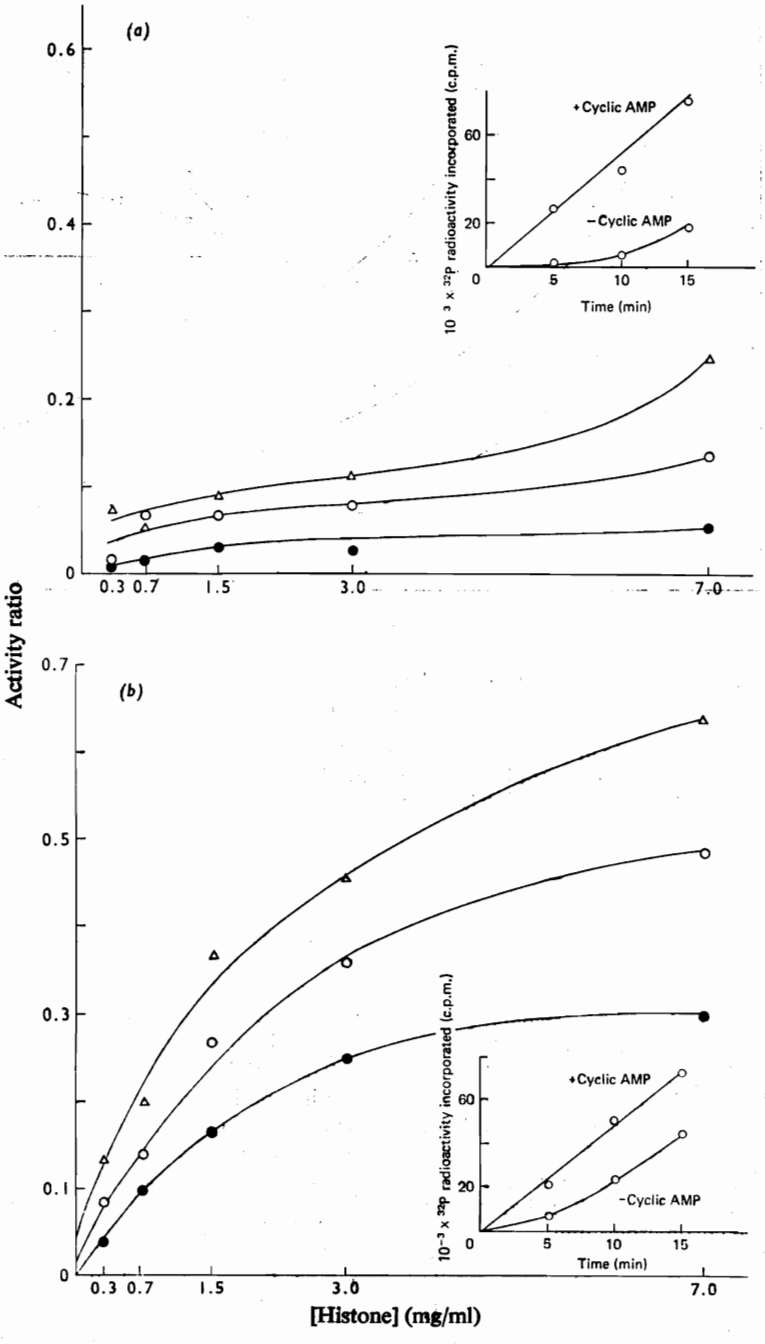

Fig. 4. *Effect of histone on isoenzymes PK I (a) and PK II (b) during the assay*

Preparations of isoenzymes PK I (350 units/ml) and PK II (370 units/ml) were incubated for 5 min (●), 10 min (○) and 15 min (△) in the presence of various concentrations of histone. Inset: plot of enzyme activity with and without added cyclic AMP (2 μM) in the presence of 7 mg of histone/ml, versus time of incubation.

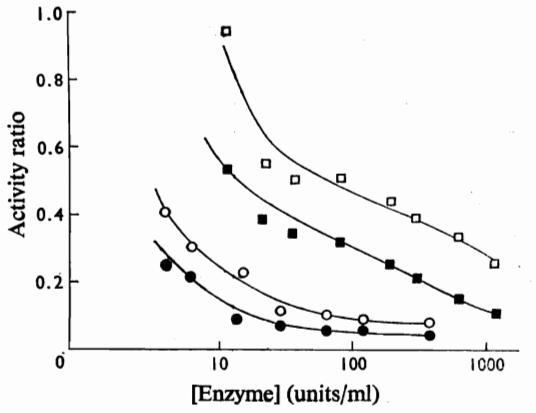

Fig. 5. *Effect of histone on isoenzymes PK I and PK II at various concentrations of enzyme*

Preparations of isoenzymes PK I (○, ●) and PK II (□, ■) were assayed in the presence of 0.5 mg of histone/ml (●, ■) and 3 mg of histone/ml (○, □) with and without 2 μM-cyclic AMP. The reaction proceeded for 10 min in the presence of the concentrations of enzymes indicated on the abscissa. The results are plotted as activity ratio versus units of protein kinase/ml of assay mixture.

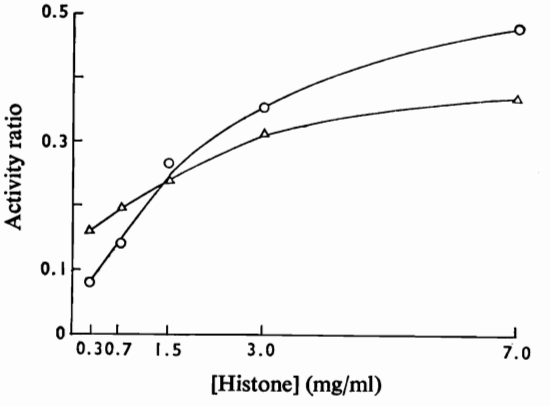

Fig. 6. *Effect of* 150 mM-*KCl on the histone activation of isoenzyme PK II*

The preparation of isoenzyme PK II (370 units/ml) was assayed for 10 min with and without 2 μM-cyclic AMP as described in the legend to Fig. 4. The reaction was run in the absence (○) and presence (△) of 150 mM-KCl. The results are plotted as activity ratio versus concentration of histone.

The effect of dilution of enzyme, concentration of histone and incubation time on the cyclic AMP-dependence was the same for different fractions of isoenzyme PK II from the DEAE-cellulose column (fractions 26, 30 and 36; Fig. 1*a*). Thus in these respects no functional heterogeneity of isoenzyme PK II could be detected.

Correlation between cyclic AMP activation of protein kinase and binding of cyclic AMP

Under the conditions of the experiment depicted in Fig. 7, the apparent activation constant (K_a) for cyclic AMP was 15 nM in the absence of added KCl. In the presence of 150 mM-KCl, it was 45 nM. The value of K_a was intermediate at 50 mM-KCl. The cyclic AMP concentration needed to give half-maximal binding showed a parallel shift from 15 nM to 45 nM. These values were obtained from four separate experiments and varied in the range 12–20 nM in the absence of added salt, and 40–50 nM in the presence of 150 mM-KCl. A typical experiment is shown in Fig. 7. Under all conditions tested, the binding of cyclic [^{3}H]AMP and the activation of protein kinase followed each other closely. There were no significant differences between isoenzymes PK I and PK II (Fig. 7) and cytosol in the pattern of activation and binding, in either the absence or the presence of added KCl. The decreased responsiveness of protein kinase to cyclic AMP observed in the presence of KCl is presumably due to decreased interaction between histone and the protein kinase, as KCl had no such effect on the enzyme during preincubation in the assay medium without histone. The same results were obtained when NaCl was used instead of KCl. These experiments were performed at sufficiently low concentration of enzyme to obtain an apparent activation constant (K_a) not affected to any significant extent by the enzyme concentration (only a small fraction of the total cyclic AMP was bound).

Discussion

Two main forms of cyclic AMP-dependent protein kinase seem to be widely distributed in mammalian tissue (Corbin *et al.*, 1975; Døskeland *et al.*, 1975); their properties have been compared (Corbin *et al.*, 1975; Hofmann *et al.*, 1975). Evidence has been presented that the isoenzymes differ in their regulatory subunit, whereas the catalytic subunit seems to be the same (Bechtel *et al.*, 1975; Fakunding & Means, 1975; Kumon *et al.*, 1972).

Several workers (Corbin *et al.*, 1975; Tao & Hackett, 1973; Kumon *et al.*, 1972), including ourselves (Døskeland *et al.*, 1975; Døskeland & Ueland, 1975*a*), have found that isoenzyme PK II is less dependent than isoenzyme PK I on cyclic AMP for activity under standard assay conditions. On this basis one might speculate whether isoenzyme PK II accounts for a continuous cyclic AMP-independent phosphorylation in the cell (Døskeland *et al.*, 1975).

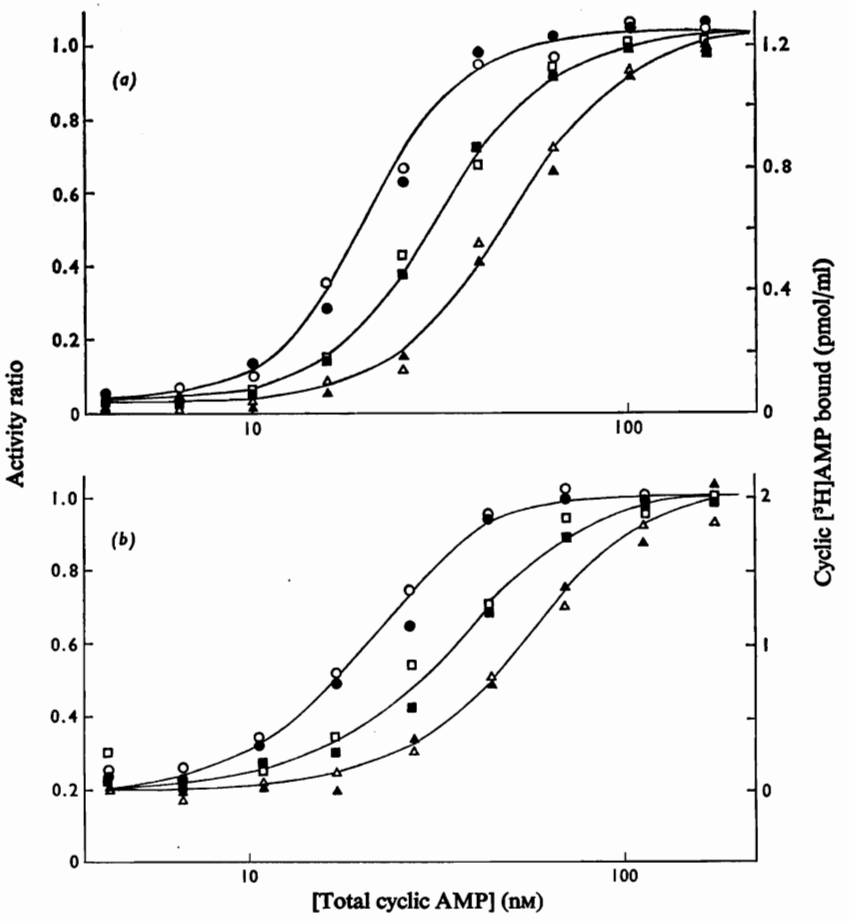

Fig. 7. *Relation between protein kinase activity ratio and cyclic [³H]AMP binding*

The protein kinase was incubated in 15 mM-Hepes buffer, pH 7.0, either without added KCl (○, ●) or in the presence of 50 mM-KCl (□, ■) or 150 mM-KCl (△, ▲). Cyclic [³H]AMP was present in the concentrations indicated on the abscissa. After 10 min of incubation, a 200 µl sample was taken for measurement of cyclic [³H]AMP bound (closed symbols), and 100 µl for determination of the degree of activation of the kinase (open symbols). The concentration of isoenzyme PK I (*a*) was 180 units/ml; that of isoenzyme PK II (*b*) was 240 units/ml.

In the present work a very low enzymic activity was observed for either isoenzyme in the absence of cyclic AMP, provided that the concentration of enzyme was kept sufficiently high. This, taken together with the fact that the concentration of protein kinase in the cell is high (Beavo *et al.*, 1974), argues against the possibility that isoenzyme PK II in the absence of cyclic AMP stimulation possesses any significant activity *in vivo*. Likewise, the low degree of activation of both isoenzymes by histone at high enzyme concentrations (Fig. 5) does not support the suggestion (Miyamoto *et al.*, 1971) that substrate dissociation of protein kinase may be a physiological regulatory device. In addition, a near-physiological concentration of KCl (150 mM) counteracts the activation of protein kinase by histone (Fig. 6).

Whether or not undissociated holoenzyme, especially isoenzyme PK II (Corbin *et al.*, 1975), as assayed in the absence of cyclic AMP, possesses any catalytic activity, has been discussed (Traugh *et al.*, 1974). The present experiments argue against the possibility that the undissociated PK II holoenzyme catalyses the phosphorylation of histone to any significant extent. The activation of isoenzyme PK II on dilution (Fig. 3) suggests that this activation is due to dissociation according to mass-action. Further, most of the acti-

vity of isoenzyme PK II in the absence of cyclic AMP could be inhibited by the heat-stable inhibitor protein (Table 1, G). As the inhibitor is considered specific for the free catalytic subunit (Ashby & Walsh, 1972; Walsh & Ashby, 1973), this result suggests that the major part of even the cyclic AMP-independent activity is catalysed by the free catalytic subunit. Finally, one would expect the phosphorylation reaction catalysed by an enzymically active holoenzyme to be linear with time; the basal activity was found to deviate from linearity with respect to time, especially at high concentration of histone (Fig. 4, inset).

At each concentration of enzyme tested, further activation was induced by increasing the concentration of histone (Figs. 4 and 5). This finding indicates that dilution itself does not fully activate (dissociate) the holoenzyme, but makes it more susceptible to a time-dependent histone–enzyme interaction. Complex-formation between the acidic regulatory subunit (Corbin *et al.*, 1974) and histone, which carries a net positive charge at neutral pH (Hnilica, 1972), may decrease the concentration of free regulatory subunit (R) and thereby promote the continuous dissociation of the holoenzyme (RC) by changing the position of the equilibrium ($RC = R+C$) in favour of the free regulatory (R) and catalytic (C) subunits. At low concentrations of enzyme spontaneous dissociation will be facilitated. According to this hypothetical scheme, the preferential activation of isoenzyme PK II on dilution may be explained either by less tight binding of R and C in the PK II holoenzyme, or by higher affinity of the regulatory subunit of isoenzyme PK II for histone.

It is noteworthy that whereas isoenzyme PK I is more easily dissociated than isoenzyme PK II by histone in the absence of Mg-ATP (Corbin *et al.*, 1975; S. O. Døskeland, P. M. Ueland & H. J. Haga, unpublished work), the reverse is true under the conditions of the present study (Fig. 4). No activation of either isoenzyme by salt (Table 1, Figs. 6 and 7) was found.

To our knowledge, the binding of cyclic [^{3}H]AMP to protein kinase has not been measured under the conditions for assay of protein kinase activity. We found it of special interest to compare the binding of the nucleotide with its ability to activate the kinase, as preliminary experiments had shown that the amount of cyclic AMP required to activate the kinase varied with the ionic strength of the assay medium. The results (Fig. 7) showed a close correlation between the binding of cyclic [^{3}H]AMP and activation, which was similar for both isoenzymes. This is unlikely to be a coincidence, as the concentration of cyclic [^{3}H]AMP required to give half-maximal binding and half-maximal activation increased in parallel as a result of the inclusion of 150mM-KCl in the medium.

We believe that the crucial importance of enzyme concentration, especially that of isoenzyme PK II (Fig. 3), for the degree of cyclic AMP-dependence measured, may have methodological implications when the cyclic AMP sensitivity of kinase in different cell extracts is compared, e.g. transformed as against parent-cell lines (Simantov & Sachs, 1975; Troy *et al.*, 1973), hepatoma as against normal liver (Criss & Morris, 1973), or different phases of ontogenesis (Lee & Jungmann, 1975). A decrease in cyclic AMP-sensitivity of total kinase may be due to a relative increase in the amount of isoenzyme PK II present, which has in fact been described to occur on malignant transformation of hepatocytes (Mackenzie & Stellwagen, 1974).

In the present study we have used histone as the substrate for protein kinase. Histone is the most commonly used substrate for this enzyme (Corbin & Reimann, 1974), and has been shown to be subject to cyclic AMP-dependent phosphorylation *in vivo* (Langan, 1973). Factors determining the cyclic AMP-dependence of protein kinase during the phosphorylation of other substrate proteins remain to be investigated.

We are grateful to Dr. Ian F. Pryme for correcting the English content of the manuscript. This work was supported by grants from the Norwegian Cancer Society (Landsforeningen mot Kreft) and the Norwegian Research Council for Sciences and the Humanities.

References

Ashby, C. D. & Walsh, D. A. (1972) *J. Biol. Chem.* **247**, 6637–6642

Beavo, J. A., Bechtel, P. J. & Krebs, E. G. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3580–3583

Bechtel, P. J., Beavo, J. A., Hofmann, F., Dills, W. L. & Krebs, E. G. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 543

Corbin, J. D. & Reimann, E. M. (1974) *Methods Enzymol.* **38G**, 287–290

Corbin, J. D., Soderling, T. R., Sugden, P. H., Keely, S. L. & Park, C. R. (1974) in *Regulation of Function and Growth of Eukaryotic Cells by Intracellular Cyclic Nucleotides (Proc. NATO Meet. Cyclic Nucleotides, Wepion, Belgium, Sept. 1974)* in the press

Corbin, J. D., Keely, S. L. & Park, C. R. (1975) *J. Biol. Chem.* **250**, 218–225

Criss, W. E. & Morris, H. P. (1973) *Biochem. Biophys. Res. Commun.* **54**, 380–386

Døskeland, S. O. & Ueland, P. M. (1975*a*) *Int. J. Biochem.* **6**, 181–190

Døskeland, S. O. & Ueland, P. M. (1975*b*) *Biochem. Biophys. Res. Commun.* **66**, 606–613

Døskeland, S. O., Kvinnsland, S. & Ueland, P. M. (1975) *J. Reprod. Fert.* **44**, 207–216

Fakunding, J. L. & Means, A. R. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 543

Hnilica, L. S. (1972) in *The Structure and Biological Function of Histone* (Hnilica, L. S., ed.), p. 48, C.R.C. Press, Cleveland

Hofmann, F., Beavo, J. A., Bechtel, P. J. & Krebs, E. G. (1975) *J. Biol. Chem.* **250**, 7795–7801

Kemp, B. E., Froscio, M., Rogers, A. & Murray, A. W. (1974) *Biochem. J.* **145**, 241–249

Klungsøyr, L. (1969) *Anal. Biochem.* **27**, 91–98

Kumar, R. & Tao, M. (1975) *Biochim. Biophys. Acta* **410**, 87–98

Kumon, A., Nishiyama, K., Yamamura, H. & Nishizuka, Y. (1972) *J. Biol. Chem.* **247**, 3726–3735

Langan, T. A. (1973) *Adv. Cyclic Nucleotide Res.* **3**, 99–153

Lee, P. C. & Jungmann, R. A. (1975) *Biochim. Biophys. Acta* **399**, 265–276

Mackenzie, C. W. & Stellwagen, R. H. (1974) *J. Biol. Chem.* **249**, 5755–5762

Miyamoto, E., Petzold, G. L., Harris, J. S. & Greengard, P. (1971) *Biochem. Biophys. Res. Commun.* **44**, 305–312

Murray, A. W., Froscio, M. & Kemp, B. E. (1972) *Biochem. J.* **129**, 995–1002

Simantov, R. & Sachs, L. (1975) *J. Biol. Chem.* **250**, 3236–3242

Takeda, M. & Nishizuka, Y. (1974) in *Lipmann Symposium (Energy, Regulation and Biosynthesis in Molecular Biology)* (Richter, D., ed.), pp. 623–635, Walter de Gruyter, Berlin and New York

Tao, M. & Hackett, P. (1973) *J. Biol. Chem.* **248**, 5324–5332

Traugh, J. A., Ashby, C. D. & Walsh, D. A. (1974) *Methods Enzymol.* **38G**, 290–299

Troy, F. A., Vijay, I. K. & Kawakami, T. G. (1973) *Biochem. Biophys. Res. Commun.* **52**, 150–158

Walsh, D. A. & Ashby, C. D. (1973) *Recent Prog. Horm. Res.* **29**, 329–359