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PARTIAL PURIFICATION AND CHARACTERIZATION OF AN ADENOSINE 3′,5′-MONOPHOSPHATE-DEPENDENT PROTEIN KINASE FROM RABBIT GASTRIC MUCOSA

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SUMMARY

An adenosine 3′,5′-monophosphate (cyclic AMP)-dependent protein kinase (ATP:protein phosphotransferase, EC 2.7.1.37) from rabbit gastric mucosa was partially purified and characterized. Purification by $(NH_4)_2SO_4$ precipitation, DEAE-cellulose chromatography, hydroxylapatite chromatography, and gel filtration increased the specific activity of the enzyme 60-fold. This protein kinase could be separated into a cyclic AMP-binding (regulatory) subunit and a catalytic subunit by chromatography on hydroxylapatite in the presence of cyclic AMP. The isolated catalytic subunit did not require cyclic AMP for activity. Recombination of the subunits in the presence of MgATP and bovine serum albumin restored cyclic AMP dependency to the catalytic subunit. Stokes radii of the catalytic subunit, the regulatory subunit, and the holoenzyme were found to be 2.6, 2.7 and 3.9 nm, respectively. The respective values for sedimentation coefficient were found to be 3.7, 3.5 and 5.1 S.

Molecular weights calculated from these data gave values of 39 000 for both the catalytic and regulatory subunits and 82 000 for the holoenzyme.

The enzyme phosphorylated histone, protamine, casein and endogeneous protein of the gastric mucosa. The apparent K_m for ATP was $2 \cdot 10^{-5}$ M in the presence or absence of cyclic AMP. The concentration of cyclic AMP required for half maximal stimulation was 10^{-8} M.

INTRODUCTION

Since the initial discovery of a cyclic AMP-dependent protein kinase (ATP: protein phosphotransferase, EC 2.7.1.37) in rabbit skeletal muscle by Walsh et al. [1], this enzyme has been found in many tissues and evidence has accumulated that it mediates many of the effects of cyclic AMP [2]. By separation of the protein kinase into subunits and recombination of these subunits, it has been demonstrated that cyclic AMP-dependent protein kinases from several tissues consist of two types of subunits [3–11]. These are a catalytic subunit which catalyzes the transfer of phosphate

Abbreviations: cyclic AMP, adenosine 3′,5′-monophosphate; EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*′-tetraacetic acid.

from the terminal phosphate of ATP to protein and a regulatory subunit which binds cyclic AMP and renders the catalytic subunit inactive in the absence of cyclic AMP. In the presence of cyclic AMP the subunits dissociate and the catalytic subunit becomes fully activated as described by Eqn. 1. RC refers to the holoenzyme, R refers to the regulatory subunit and C refers to the catalytic subunit.

$$\underset{\text{(inactive)}}{RC} + \text{cyclic AMP} \rightleftharpoons \underset{\text{(active)}}{C} + \text{R–cyclic AMP} \qquad (1)$$

In 1965, Harris and Alonso [12] reported that cyclic AMP was capable of stimulating gastric secretion by the isolated amphibian gastric mucosa. Subsequently, supportive evidence indicating a role for cyclic AMP in gastric secretion of several species has come from a number of laboratories. The stimulatory effect of cyclic AMP on gastric secretion has been confirmed using cyclic AMP [13–15] and analogues of cyclic AMP [16] but systemic administration of cyclic AMP may cause inhibition of gastric secretion in some instances [17]. Bieck et al. [18] have recently reported that stimulation of gastric secretion in dogs and humans was accompanied by increased concentrations of cyclic AMP in gastric juice. Stimulation of gastric secretion by theophylline [19] or histamine [20] has been associated with increased levels of cyclic AMP in the gastric mucosa. Furthermore, it has been reported that gastric mucosa contains adenylate cyclase activity stimulated by histamine [21, 22] or pentagastrin [21]. The gastric mucosa is also known to contain cyclic nucleotide phosphodiesterase [23–25].

The existence of a cyclic AMP-dependent protein kinase from bovine stomach lining has been demonstrated [26], but it is not clear if this enzyme was prepared from the acid-secreting portion of the stomach. The present study was undertaken to determine if gastric mucosa contains the cyclic AMP-dependent protein kinase and to compare the physical and kinetic properties of this enzyme with those of other cyclic AMP-dependent protein kinases. The partial purification and characterization of this enzyme was described in a preliminary communication [27] and is reported in more detail here.

MATERIALS AND METHODS

Buffers

All phosphate buffers contained equal parts of monobasic and dibasic potassium phosphate unless otherwise indicated. Additions of EDTA were made as a 0.2 M solution of the disodium salt adjusted to pH 7 with NaOH.

[γ-^{32}P]ATP

The method of Glynn and Chappell [28] modified as previously described [29] was used to prepare [γ-^{32}P]ATP.

Protein kinase activity

Protein kinase activity was determined by incorporation of ^{32}P from [γ-^{32}P]-ATP into casein or histone. The pH of the incubation mix was 6.0 for casein and 6.4 for histone phosphorylation. Reactions were initiated by the addition of enzyme and

terminated by pipetting 50 μl aliquots onto 2 cm × 2 cm squares of Whatman 31 ET chromatography paper which were then washed in trichloroacetic acid as described previously [30].

Cyclic AMP binding activity

Binding of cyclic AMP was determined by a modification of the method of Tao et al. [31]. The enzyme fractions were incubated for 1–2 h at 0 °C in a reaction mixture containing 10^{-7} M cyclic [^{3}H]AMP (7–21 cpm/fmole, based on data given by the supplier) and 33 mM phosphate buffer in a total volume of 150 μl. During the course of these experiments, it was discovered that the addition of 60 mM EDTA to the reaction mixtures would sometimes increase the amount of binding activity. EDTA was therefore added in some experiments (Fig. 4, Fig. 6C and Table I). At the end of the incubation period the reaction mix was diluted with 2 or 3 ml of 50 mM phosphate buffer and filtered through a cellulose ester HAWP membrane (Millipore Corporation). The membrane was washed with 8 or 9 ml of phosphate buffer and then either dissolved in 5 ml Bray's solution [32] or dried under a heat lamp and placed in 5 ml of a scintillation solution containing 4 g PPO and 0.45 g *p*-bis-(*o*-methylstyryl)-benzene per l of toluene. The count rates obtained by liquid scintillation counting were the same for both scintillation solutions. Over 90% of the counts remained on the membrane when the toluene based solution was used.

When large amounts (>100 μg) of the 100 000 × *g* supernatant fraction were assayed for binding of cyclic [^{3}H]AMP, the amount of radioactivity bound per mg protein frequently decreased with increasing protein concentration. Furthermore a decrease in bound radioactivity was observed when these samples were incubated overnight. Thin layer chromatography was employed in one experiment to measure the extent of metabolism of cyclic AMP under these conditions. When a 100 000 × *g* supernatant was incubated with cyclic [^{3}H]AMP for 5 h only 13% of the total radioactivity in the reaction mix and 49% of the bound radioactivity co-chromatographed with cyclic AMP. When 60 mM EDTA was added, 42% of the total radioactivity in the reaction mix and 83% of the bound radioactivity co-chromatographed with cyclic AMP. The amount of bound radioactivity was increased 5-fold by the addition of EDTA. In the absence of EDTA, the distribution of radioactive compounds was similar when either [^{3}H]AMP or cyclic [^{3}H]AMP was used, indicating that much of the bound radioactivity from cyclic [^{3}H]AMP was AMP and its metabolites. These metabolites were not identified. EDTA reduced the total amount of bound radio activity when [^{3}H]AMP was used. EDTA therefore increased both the sensitivity and the specificity of the cyclic AMP binding assay. If the 100 000 × *g* supernatant was diluted, the effect of EDTA was markedly reduced and a higher percentage of the bound radioactivity co-chromatographed with cyclic AMP. It is important to minimize degradation of cyclic AMP during the assay of such fractions in recovery studies. Therefore all fractions (Table I) were diluted sufficiently so that the amount of ^{3}H bound was proportional to protein concentration, but the degree of cyclic AMP degradation was not actually measured in these diluted samples.

Purification of catalytic subunit

The catalytic subunit was prepared from rabbit gastric mucosa by a modification of the method of Erlichman et al. [7].

TABLE I

PURIFICATION OF PROTEIN KINASE FROM GASTRIC MUCOSA

Gastric mucosa (67 g) was homogenized and fractionated as described in the text. Fractions were assayed for histone kinase activity and cyclic AMP binding activity as described in the text. 1 unit of activity catalyzes the transfer of 1 pmole of phosphate from ATP to histone per min. The specific activity of the cyclic [^{3}H]AMP was approximately 21 cpm per fmole. The kinase activity ratio is the activity in the absence of cyclic AMP divided by the activity in the presence of cyclic AMP.

Fraction	Protein (total mg)	Specific activity		cpm ^{3}H/unit	Kinase activity ratio
		Kinase (units/μg)	Cyclic [^{3}H]AMP binding (cmp/μg)		
100 000 × *g* supernatant	2440	0.8	67	83	0.30
$(NH_4)_2SO_4$ precipitate	970	1.3	107	84	0.44
DE52 pool	87	6.2	318	51	0.26
Hydroxylapatite pool (concentrated)	6.3	24.9	1887	76	0.21
G-200 pool (concentrated)	0.4	49.3	1111	23	0.33

Kinetic constants

The apparent K_m values for ATP were calculated by the statistical method of Wilkinson [34] on a Hewlett-Packard Model 9810 programmable calculator.

Protein determination

Protein was estimated by absorbance at 280 nm when column fractions were monitored or by a modification [35] of the method of Lowry et al. [36] when specific activities were determined.

Materials

[^{32}P]Orthophosphate was obtained from ICN and cyclic [^{3}H]AMP from Schwarz-Mann. Phosphorylase *b* was generously supplied by Dr D. A. Walsh.

RESULTS AND DISCUSSION

Purification of cyclic AMP-dependent protein kinase

8 white New Zealand rabbits weighing 2 kg each were anesthetized with sodium pentobarbital and exsanguinated. The stomachs were removed, emptied, rinsed in tap water and then rinsed in 0.9% NaCl. The fundic mucosa (about 80 g) was separated from the outer musculature, minced, and homogenized in 2 vol. of 4 mM neutral EDTA containing 1 mM mercaptoethanol. Homogenization was done in a glass homogenizer with a teflon pestle. The homogenization and all subsequent steps were done at 4 °C. The homogenate was centrifuged at 100 000 × *g* for 1 h. Phosphate (1 M) was added to the supernatant to a final phosphate concentration of 0.09 M. The enzyme was precipitated by the addition of an equal volume of saturated $(NH_4)_2SO_4$ (at 4 °C). The precipitate was dialyzed against 5 mM Tris–HCl buffer containing 1 mM

EDTA and 1 mM mercaptoethanol at pH 7.4 for approximately 1 h and then further equilibrated in this buffer by gel filtration on Sephadex G-25. The enzyme was applied to a DE52 column equilibrated in the same buffer and was eluted with a linear NaCl gradient (Fig. 1). The fractions in the major kinase peak were pooled and equilibrated

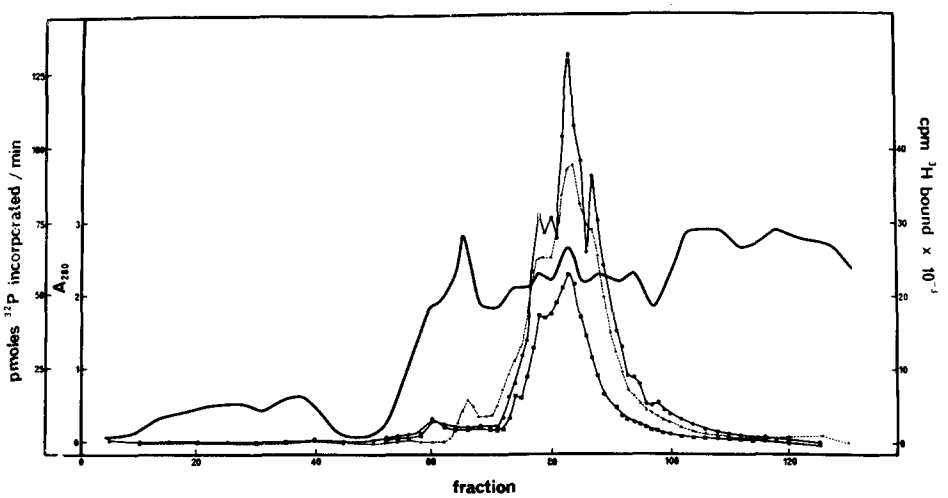

Fig. 1. Chromatography on DEAE-cellulose (DE52). A homogenate of gastric mucosa (85 g) was centrifuged at 100 000 × *g* for 1 h and proteins were precipitated with $(NH_4)_2SO_4$ as described in the text. The precipitate was equilibrated in 5 mM Tris–HCl buffer containing 1 mM EDTA and 1 mM mercaptoethanol at pH 7.4 and applied to a DE52 column (1.5 cm × 30 cm) equilibrated in the same buffer. Proteins were eluted with a linear NaCl gradient to 0.5 M in the same buffer. The gradient began at Fraction 48 and had a total volume of 400 ml. Fractions were analyzed for protein (———), for histone kinase activity in the absence (■—■) or presence (●—●) of cyclic AMP, and for cyclic [^{3}H]AMP binding activity (▲---▲) as described in the text. Fractions 76–90 were pooled for chromatography on hydroxylapatite.

with 5 mM phosphate buffer containing 1 mM mercaptoethanol by gel filtration on Sephadex G-25. The equilibrated sample was applied to a hydroxylapatite column and eluted with a linear phosphate buffer gradient (Fig. 2). Two cyclic AMP-dependent protein kinase peaks and a cyclic AMP-binding peak devoid of kinase activity were resolved. The latter fraction will be referred to as the cyclic AMP-binding protein. The fractions in the major kinase peak were pooled and dialyzed against 10 mM phosphate buffer containing 1 mM EDTA and 1 mM mercaptoethanol. This pool was concentrated to 1–2 ml by placing the dialysis bag before a fan in the cold room for about 40 h. The concentrated enzyme was then further fractionated on a Sephadex G-200 column (Fig. 4A). The fractions containing kinase activity were pooled, dialyzed and concentrated as described above. Usually over half of the enzyme activity was lost during each concentration step. Other methods of concentration, such as $(NH_4)_2SO_4$ precipitation, dialysis against concentrated sucrose, or ultrafiltration resulted in even greater losses of activity.

Table I summarizes the kinase and cyclic AMP-binding specific activities at several stages of the purification scheme. Kinase specific activity was increased

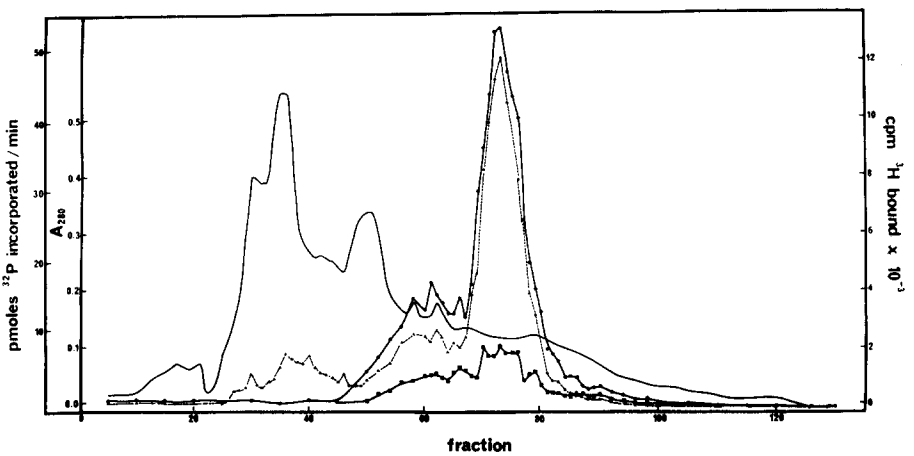

Fig. 2. Chromatography on hydroxylapatite. The kinase fractions from a DE52 column (Fig. 1) were pooled, equilibrated with 5 mM phosphate and 1 mM mercaptoethanol and applied to a hydroxylapatite column (0.9 cm × 15 cm) equilibrated in the same buffer. Proteins were eluted with a linear phosphate gradient to 0.5 M. The gradient began at Fraction 22 and had a total volume of 400 ml. Fractions were analyzed for protein (———), for histone kinase activity in the absence (■—■) or presence (●—●) of cyclic AMP, and for cyclic [^{3}H]AMP binding activity (▲---▲) as described in the text. Fractions 68–80 were pooled for gel filtration. Fractions 33–45 were pooled for regulatory subunit studies.

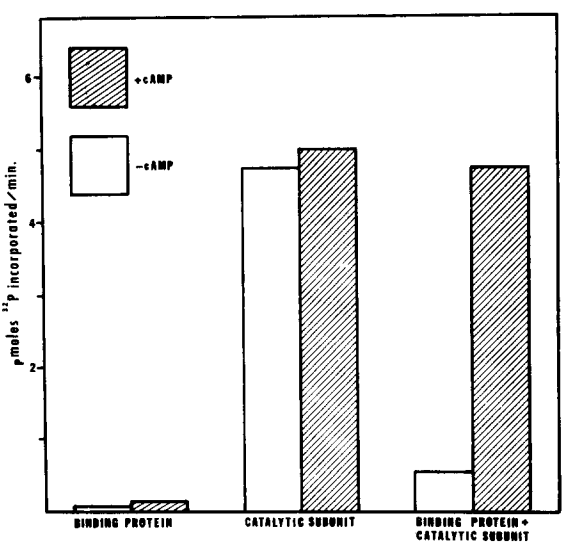

Fig. 3. Restoration of cyclic AMP-dependency of the catalytic subunit by the addition of cyclic AMP-binding protein. The cyclic AMP-binding protein from a hydroxylapatite column (Fig. 2) was preincubated for 20 min at 30 °C with protein kinase catalytic subunit. The preincubation mix was then tested for histone kinase activity in the presence or absence of cyclic AMP in a standard protein kinase assay mixture. From the cyclic AMP-binding activity of the binding protein it was calculated that 50 μl of the kinase reaction mix was capable of binding 11 fmoles of cyclic [^{3}H]AMP when binding protein was added. The control preincubation labeled binding protein was identical to the mixture described except for the omission of catalytic subunit. Likewise, the control preincubation labeled catalytic subunit was identical except for the omission of cyclic AMP-binding protein. cAMP, cyclic AMP.

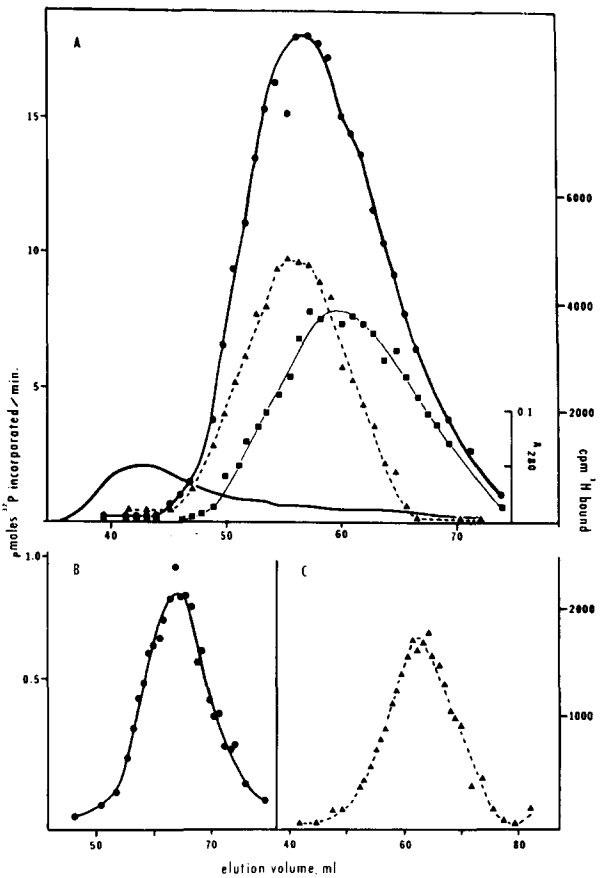

Fig. 4. Gel filtration of holoenzyme, catalytic subunit and regulatory subunit. Gel filtration of the holoenzyme (A) the catalytic subunit (B) and the regulatory subunit (C) was conducted using Sephadex G-200 in a column (2.6 cm × 34 cm) containing glass beads [33]. The column was developed at a flow rate of about 1 ml/min with a buffer containing 10 mM phosphate, 1 mM EDTA and 0.1 M NaCl. The holoenzyme was obtained from a hydroxylapatite column developed without cyclic AMP (Fig. 2). The catalytic and regulatory subunits were obtained from a hydroxylapatite column developed with phosphate buffer containing 1 μM cyclic AMP. Fractions were assayed for protein (———), histone kinase activity in the absence (■—■) or presence (●—●) of cyclic AMP, and for cyclic AMP binding activity (▲---▲).

50–150-fold by this procedure (60-fold in the preparation illustrated in Table I). The amount of cyclic AMP-binding relative to kinase activity consistently decreased 2–4-fold during purification as is indicated in the last column of Table I. It is not clear if this represents a change in subunit structure of the enzyme during purification or whether it is due to difficulties associated with the quantitative assay of cyclic AMP-binding activity. Contamination of the holoenzyme with catalytic subunit would be expected to decrease the amount of cyclic AMP bound per kinase unit and also increase the kinase activity ratio. There was no correlation between the amount of cyclic AMP bound per kinase unit and the kinase activity ratio (Table I). Thus it appears that changes in these two parameters are not due strictly to changes in the relative amount of free catalytic subunit present.

Evidence that the cyclic AMP-binding protein isolated on hydroxylapatite is the regulatory subunit

It was of interest to determine if the cyclic AMP-binding protein isolated on hydroxylapatite (Fig. 2) were the regulatory subunit of the protein kinase. Therefore, the cyclic AMP-binding protein was tested to determine if it could restore cyclic AMP-dependency to the catalytic subunit. Free catalytic subunit and cyclic AMP-binding protein were preincubated for 20 min at 30 °C in a reaction mixture containing 10 mM magnesium acetate, 0.2 mM ATP, 5 mM potassium phosphate buffer, 0.2 mM EDTA, 10 mM NaCl, 0.2 mM mercaptoethanol and 2.0 mg bovine serum albumin per ml. Aliquots of the preincubation mixture were assayed for dependency on cyclic AMP in a standard kinase reaction mixture. The results of one such experiment are shown in Fig. 3. This experiment clearly demonstrates that the cyclic AMP-binding protein has regulatory subunit activity. Bovine serum albumin and MgATP were needed to minimize loss of catalytic subunit activity and to enhance recombination of the subunits. Previous reports have indicated that the regulatory subunit is isolated as a complex with cyclic AMP [5, 37] and that ATP enhances the release of cyclic AMP from this complex [37, 38]. The mechanism by which ATP facilitates recombination of the subunits may therefore involve the release of cyclic AMP from the regulatory subunit.

In another experiment evidence was obtained that the chromatographic behavior of the regulatory subunit and of the cyclic AMP-binding protein are identical. Catalytic and regulatory subunits were separated on a hydroyxlapatite column developed as described above except that 10^{-6} M cyclic AMP was added to the elution buffer (not shown). The major kinase peak obtained from this column was not stimulated by cyclic AMP, even after dialysis, indicating it had been freed of regulatory subunit activity. The peak of cyclic AMP-binding activity eluted at the same phosphate concentration as the cyclic AMP-binding protein observed when cyclic AMP was not present in the developing buffer. This supports the conclusion that the cyclic AMP-binding protein which is isolated in the absence of cyclic AMP is indeed the regulatory subunit. Thus no evidence was found that gastric mucosa contains cyclic AMP-binding proteins other than the regulatory subunit of the cyclic AMP-dependent protein kinase.

Determination of Stokes radii

The Stokes radii of the holoenzyme (RC), the cyclic AMP-binding or regulatory subunit (R) and the catalytic subunit (C) were determined by gel filtration on Sephadex G-200. Fig. 4A illustrates the behavior of the holoenzyme on a G-200 column. This G-200 column was the final step in the enzyme preparation described in Table I. The cyclic AMP-binding activity corresponded well with the net cyclic AMP-dependent kinase activity (not shown) and was used as a measure of the elution volume for the holoenzyme. The cyclic AMP-independent activity eluted at the same position as the shoulder on the elution profile of the activity measured in the presence of cyclic AMP (total activity). Since the Stokes radius of this cyclic AMP-independent activity is apparently lower than that for the holoenzyme, it may represent free catalytic subunit. The catalytic and regulatory subunits obtained from the hydroxylapatite column developed in the presence of cyclic AMP (see above) were used for determination of Stokes radii (Figs 4B and 4C). Stokes radii were calculated by

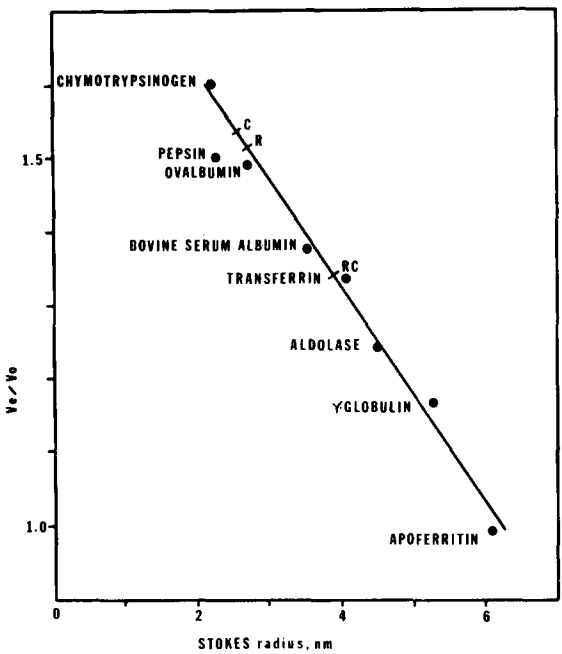

Fig. 5. Determination of Stokes radii of the holoenzyme, catalytic subunit and regulatory subunit. The column described in Fig. 4 was calibrated as described by Andrews [39]. The void volume (V_0), based on blue dextran elution volume, was 41.7 ml. The elution volumes (V_e) for standard proteins of known Stokes radius [39] were determined and V_e/V_0 was plotted against Stokes radius. The values of V_e/V_0 for the holoenzyme (RC), catalytic subunit (C) and regulatory subunit (R) were obtained from the data presented in Fig. 4.

comparing the elution volumes of the catalytic subunit, the regulatory subunit and the holoenzyme to those of standard proteins (Fig. 5) as described by Andrews [39]. By this method the Stokes radii were calculated to be 2.6, 2.7 and 3.9 nm, respectively.

Determination of sedimentation coefficients

The major kinase peak and the cyclic AMP-binding protein obtained from the hydroxylapatite column used in the standard purification scheme (Fig. 2) were used to determine the sedimentation coefficients of the holoenzyme and the regulatory subunit by sucrose density gradient centrifugation (Figs 6A and 6C). The catalytic subunit preparation (Fig. 6B) was the same as that used for the determination of Stokes radius. The sedimentation coefficients of the catalytic subunit, the regulatory subunit and the holoenzyme were found to be 3.7, 3.5, and 5.1 S, respectively.

Calculation of molecular weights from Stokes radii and sedimentation coefficients

The Stokes radii and sedimentation coefficients were used to calculate molecular weights and frictional coefficients using the relationships described by Siegel and Monty [44] and assuming a partial specific volume of 0.725 cm³/g:

$$M_r = 6\pi\eta Nas/(1-\bar{v}\varrho)$$

$$f/f_0 = a/(3\bar{v}Mr/4\pi N)^{1}/_{3}$$

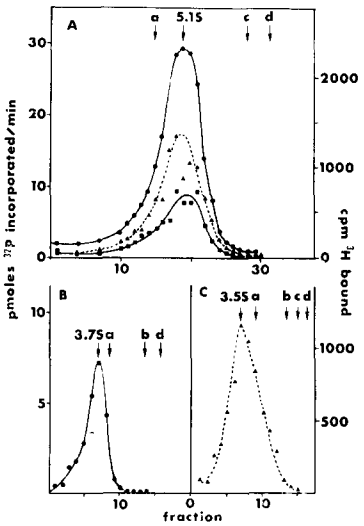

Fig. 6. Density gradient centrifugation of the holoenzyme, catalytic subunit and regulatory subunit. Density gradient centrifugation was performed in 5 to 20% (w/w) sucrose gradients in 50 mM Tris–HCl buffer containing 1 mM EDTA at pH 7.4 using a Beckman SW41 rotor at 40 000 rev./min for 15 h. Holoenzyme (A) and regulatory subunit (C) were obtained from a hydroxylapatite column similar to that illustrated in Fig. 2. Catalytic subunit (B) was the same as for Fig. 4. Fractions were analyzed for histone kinase activity in the absence (■—■) or presence (●—●) of cyclic AMP and for cyclic [^{3}H]AMP binding activity (▲---▲). The arrows indicate the position of the following marker proteins: a, human hemoglobin (4.5 S) [40]; b, human γ-globulin (6.7 S) [41]; c, bovine heart lactate dehydrogenase (7.4 S) [42]; and d, rabbit muscle phosphorylase *b* (8.4 S) [43].

where M_r = molecular weight, η = viscosity of the medium, a = Stokes radius, s = sedimentation coefficient, $\bar{v}$ = partial specific volume, ϱ = density of the medium, f/f_0 = frictional ratio and N = Avogadro's number. The results are summarized in Table II. The molecular weights of the catalytic subunit, the regulatory subunit and the holoenzyme were 39 000, 39 000 and 82 000, respectively. The physical properties of stomach catalytic subunit appear to resemble those of heart catalytic subunit ($s_{20,w}$ 3.6 S; M_r 38 000; f/f_0 1.12) [45]. The properties of the regulatory subunit appear to resemble those of the species designated as R_{IB} from muscle (a = 2.61 nm; $s_{20,w}$ 3.4 S; M_r 37 000) [46]. The calculated molecular weight of the holoenzyme (82 000) agrees well with the sum (78 000) of the calculated molecular weights for the regulatory subunit and

TABLE II

SUMMARY OF PHYSICAL PROPERTIES OF PROTEIN KINASE OF GASTRIC MUCOSA

Subunit	Stokes radius* (nm)	$s_{20,w}$**	Mol. wt***	f/f_0***
Catalytic subunit	2.6	3.7	39 000	1.15
Regulatory subunit	2.7	3.5	39 000	1.22
Holoenzyme	3.9	5.1	82 000	1.37

* Determined from the data in Fig. 5.
** Determined from the data in Fig. 6.
*** Calculated as described by Siegel and Monty [44].

the catalytic subunit indicating that the holoenzyme consists of one regulatory subunit and one catalytic subunit. Preliminary experiments indicate that the molecular weight of the enzyme in crude extracts is 173 000 ($f/f_0 = 1.64$) and that a reduction in molecular weight occurs after application to the hydroxylapatite column. The reduction in amount of cyclic AMP bound per kinase unit (Table I) and the reduction in molecular weight suggests that a change in quaternary structure occurs during the purification of this enzyme. Reduction in molecular weight of the protein kinases from heart [47] and skeletal muscle [46] has also been observed under certain conditions.

Protein substrates

In addition to casein and histone, the enzyme catalyzed the phosphorylation of protamine and endogenous proteins of gastric mucosa. Phosphorylation of all these substrates was stimulated by cyclic AMP. No measurable phosphorylation of phosvitin or ovalbumin could be demonstrated. These protein substrates were tested at concentrations of 6 mg/ml in the standard reaction mixture at pH 6.0. The proteins were precipitated with 25 % trichloroacetic acid. This concentration of trichloroacetic acid was used to insure precipitation of protamine. The highest rate of phosphorylation was observed when histone was the substrate. The dependency on pH and the pH optimum for the phosphorylation of casein (pH 6.0) and histone (pH 6.4) were similar to those reported for the protein kinases of skeletal muscle [30] and adipose tissue [48], respectively. The initial velocity of histone phosphorylation was half maximal at a histone concentration of 0.3 mg/ml in the presence of cyclic AMP. Omission of

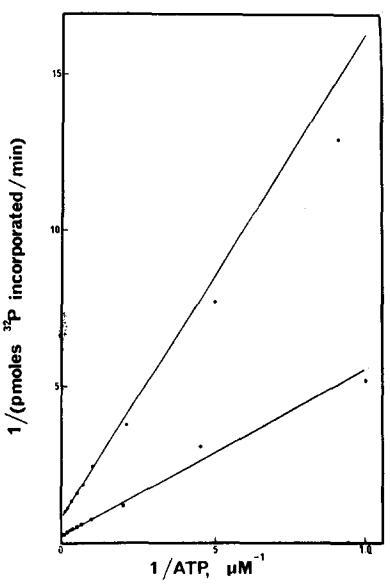

Fig. 7. Determination of apparent K_m values for ATP. Casein phosphorylation was measured in the absence (upper line) or presence (lower line) of 2 μM cyclic AMP at various ATP concentrations. Magnesium acetate was held constant at 10 mM. The method of Wilkinson [34] was used to calculate the apparent K_m values and to determine the slopes and intercepts of the lines shown in these double-reciprocal plots.

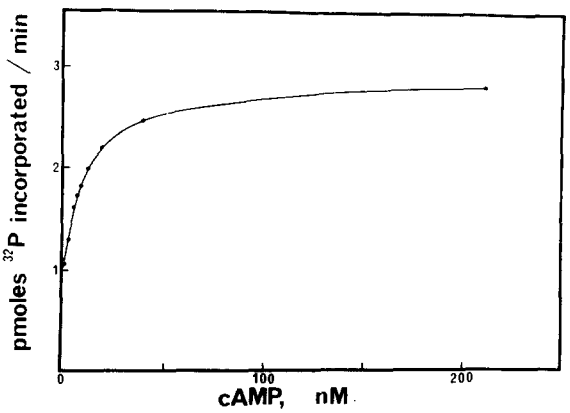

Fig. 8. Effect of the concentration of cyclic AMP on casein phosphorylation. The activity in the absence of cyclic AMP was 0.85 pmoles/min. The data shown were not corrected for this basal activity.

cyclic AMP did not appear to change this value. When histone was the substrate, the rate of protein phosphorylation increased with incubation time if cyclic AMP was absent. This finding is consistent with previous reports that basic proteins can cause dissociation of regulatory and catalytic subunits of cyclic AMP-dependent protein kinases [49, 50] and thereby activate the enzyme in the absence of cyclic AMP.

Determination of apparent K_m values for ATP and cyclic AMP

Apparent K_m values for ATP and cyclic AMP were determined using casein as a substrate. The apparent K_m for ATP was found to be 20 $\pm$ 1 μM in the absence of cyclic AMP and 21 $\pm$ 1 μM in the presence of cyclic AMP (Fig. 7). These values are similar to those reported for the protein kinases of heart [47] and skeletal muscle [30]. Fig. 8 shows the effect of varying cyclic AMP concentration on enzyme activity. Half maximal stimulation was observed when the concentration of cyclic AMP was 10^{-8} M. These results are also similar to those reported for the cyclic AMP-dependent protein kinases from skeletal muscle [30]. In summary, it appears that all the properties of this enzyme which have been carefully studied resemble those of other cyclic AMP-dependent protein kinases.

The presence of the cyclic AMP-dependent protein kinase in the gastric mucosa is consistent with the hypothesis that cyclic AMP plays an important role in the process of gastric secretion. Before it can be concluded that the cyclic AMP-dependent protein kinase is involved in this process it must be demonstrated that this enzyme is present in the chief cells, the parietal cells, or endocrine cells of the gastric mucosa. Furthermore, the elucidation of the exact role of this enzyme in gastric secretion must await the discovery of the physiological substrates of this enzyme. The fact that phosphorylation of endogenous proteins occurs and the demonstration that cyclic AMP increases the activity of carbonic anhydrase [51], an enzyme which has been proposed to play a major role in gastric secretion [52], indicates that such substrates are present in the gastric mucosa.

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