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# INVESTIGATION OF THE ADENOSINE 3',5'-CYCLIC PHOSPHATE BINDING SITE OF PIG BRAIN HISTONE KINASE WITH THE AID OF SOME ANALOGUES OF ADENOSINE 3',5'-CYCLIC PHOSPHATE

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## Summary

2'-*O*-Chloroacetyl cyclic AMP, 2'-*O*-acrylyl cyclic AMP and $N^6$, 2'-*O*-diacrylyl cyclic AMP were synthesized by the reaction of cyclic AMP with chloroacetic and acrylic anhydrides, respectively. Selective O-deacylation of $N^6$, 2'-*O*-diacrylyl cyclic AMP yielded $N^6$-monoacrylyl cyclic AMP. In the reaction of γ-mercaptobutyric acid with 8-bromo cyclic AMP, 8-(γ-carboxypropylthio) cyclic AMP was obtained.

The compounds synthesized and other cyclic AMP analogues (8-bromo cyclic AMP and adenosine 3',5'-cyclic sulphate) were tested for ability to interact with the highly purified pig brain histone kinase.

All compounds under study were found to be activators of the enzyme. The highest activating potency was manifested by 8-bromo cyclic AMP and 8-(γ-carboxypropylthio) cyclic AMP; adenosine 3',5'-cyclic sulphate was the least potent in this respect.

All compounds were shown to inhibit binding of cyclic [$^3$H]AMP to histone kinase. The inhibition was competitive with respect to cyclic AMP in all cases.

All compounds, except for 2'-*O*-chloroacetyl cyclic AMP, were gradually displaced by cyclic [$^3$H]AMP from their complexes with the histone kinase. Tighter binding of 2'-*O*-chloroacetyl cyclic AMP may indicate the formation of a covalent bond between this analogue and the enzyme.

These findings suggest that an active site of the regulatory subunit of the histone kinase contains at least three specific areas responsible for cyclic AMP binding.

## Introduction

In our previous paper we described the isolation procedure and some properties of a histone kinase catalysing the phosphorylation of lysine-rich histones [1]. Like several other protein kinases this enzyme is essentially activated in the presence of adenosine 3′,5′-cyclic phosphate (cyclic AMP). The tentative mechanism of this activation consists of cyclic AMP binding to the regulatory enzyme subunit (R) with a subsequent release of the active catalytic subunit (C).

$$[R \cdot C] + \text{cyclic AMP} \rightleftharpoons [\text{cyclic AMP} \cdot R \cdot C] \rightleftharpoons [R \cdot \text{cyclic AMP}] + C$$

The mechanism of dissociation of the enzyme into subunits under the action of cyclic AMP is still unknown. The role of structural elements of the cyclic AMP molecule in the process of enzyme activation and their importance for binding to the regulatory subunit are not clear as yet.

In recent years the activating capacity of a number of structural analogues of cyclic AMP with respect to protein kinases was studied, and certain conclusions were drawn about the influence of some modification of the cyclic AMP molecule on its activating function [2—7].

Nevertheless, at the present time there is no data available either on the possible cyclic AMP binding areas in the active site of the regulatory subunit or about the nature of functional groups participating in binding and the spatial arrangement of the regulatory site as a whole. This problem might be solved by using cyclic AMP analogues which are able to covalently block certain areas of the enzyme to be activated. We, therefore, undertook the preparation of a number of cyclic AMP derivatives containing reactive groupings in the *exo*-$NH_2$ and 2′-OH groups of the cyclic AMP molecule (see Fig. 1) and to study their interaction with purified histone kinase.

It seemed most promising to use acyl groupings containing alkylating functions, viz. a halogen atom or an activated double bond. Moreover, acylation of the *exo*-$NH_2$ and 2′-OH groups of cyclic AMP can be performed under relatively mild conditions without hydrolysis of the glycosyl bond and without destroying the 3′,5′-cyclic phosphate ring [8—10].

2′-*O*-Chloroacetyl (II), 2′-*O*-acrylyl (III) and $N^6$, 2′-*O*-diacrylyl (IV) deriv-

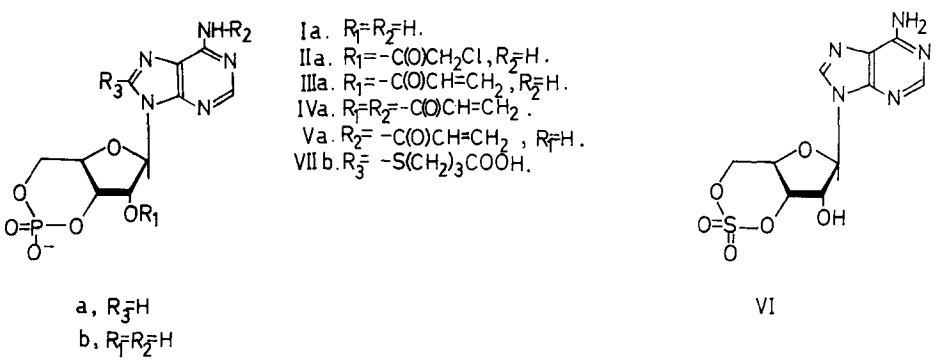


**Fig. 1. Chemical structure of the cyclic AMP analogues under study.**

TABLE I

PHYSICAL PROPERTIES OF SOME DERIVATIVES OF ADENOSINE 3′,5′-CYCLIC PHOSPHATE

| Compound | $R_F$ (A) | $E^*$ (D) cyclic AMP | $\lambda_{max}$ (nm) (pH 6.0) | $\epsilon_{max} \times 10^{-3}$ |
|---|---|---|---|---|
| I. Cyclic AMP | 0.30 | 1.00 | 259 | 14.1 |
| II. 2′-*O*-Chloroacetyl cyclic AMP | 0.53 | 0.98 | 260 | 14.0 |
| III. 2′-*O*-Acrylyl cyclic AMP | 0.46 | 0.98 | 260 | 13.9 |
| IV. $N^6$, 2′-*O*-Diacrylyl cyclic AMP | 0.62 | 0.95 | 270 | 14.2 |
| V. $N^6$-Acrylyl cyclic AMP | 0.48 | 0.98 | 272 | 13.8 |
| VI. Adenosine 3′,5′-Cyclic Sulphate | 0.52 | 0.00 | 259 | 13.6 |
| VII. 8-(γ-Carboxypropylthio) cyclic AMP | 0.50 | 1.98 | 280 | 18.0 |

* $E_{\text{cyclic AMP}}$, electrophoretic mobility relative to cyclic AMP.

atives of cyclic AMP were obtained with 15—30% yields by the method of Michelson [11], modified in this laboratory. The procedure consisted of the incubation of cyclic AMP and anhydrides of chloroacetic or acrylic acid, respectively, in the dioxane/dimethylformamide mixture in the presence of triethylamine.

Hydrolysis of $N^6$, 2′-*O*-diacrylyl cyclic AMP by 0.7 M $NH_4OH$ yielded $N^6$-monoacrylyl cyclic AMP (V).

The degree of activating potency was evaluated not only for the mentioned acyl derivatives of cyclic AMP but also for adenosine 3′,5′-cyclic sulphate (VI) obtained previously in our group (Gulyaev, N.N. and Severin, E.S., unpublished) and for 8-(γ-carboxypropylthio) cyclic AMP (VII) prepared by the reaction of 8-bromo cyclic AMP with γ-mercaptobutyric acid under conditions similar to those described in ref 3.

Cyclic AMP derivatives thus obtained were characterised by elementary analysis, mobility on chromatography and paper electrophoresis, and ultraviolet spectra (Table I).

## Materials and Methods

### *Chemicals*

Cyclic AMP (Sigma), [γ-$^{32}$P] ATP and cyclic [$^3$H] AMP (Amersham) were used.

### *Enzyme preparation and activity assay*

Cyclic AMP-dependent histone kinase was obtained by the procedure described previously [1].

Phosphotransferase (kinase) activity was determined by measuring the amount of $^{32}$P incorporated into histone $F_1$. Incubation mixtures (final volume, 0.2 ml) contained the following components: 50 mM sodium phosphate buffer, pH 6.5; 10 mM $MgCl_2$; 1 mM dithiothreitol; 0.3 mM ethyleneglycolbis-(β-aminoethylene)-*N*,*N*′-tetraacetic acid (EGTA); 2 mM theophylline; 400 μg/ml histone $F_1$; 0.025 mM (0.1 Ci/μmol) [γ-$^{32}$P] ATP. Cyclic AMP or an analogue was added at the concentration indicated under Results. Each assay sample contained 0.5 mg of the purified enzyme preparation.

The activity was measured as described in ref. 1.

Cyclic AMP binding activity was measured in 50 mM citrate buffer, pH 6.2. All operations were performed as described previously [1].

*Determination of inhibition constants*

Inhibition constants ($K_i$) were determined for each analogue in the cyclic [$^3$H]AMP binding test at different concentrations of the analogue. For this purpose we measured the equilibrium values of cyclic [$^3$H]AMP binding to histone kinase without analogues and with different concentrations of the analogues. The ratio of these values was plotted versus the analogue concentration. After determining the tangent of the slope (tg$\alpha$), the inhibition constant was calculated from the following formula:

$$K_i = \frac{K_m}{\text{tg}\,\alpha \cdot (K_m + [S])}$$

where $K_i$ is the inhibition constant for competitive inhibitors; $K_m$ is the cyclic AMP concentration at which the binding of cyclic [$^3$H]AMP to histone kinase reaches half of the maximum value (Michaelis constant); [$S$] is cyclic AMP concentration.

*Synthesis and purification of cyclic AMP analogues*

The following chemicals were used for synthesis: chloroacetic anhydride was obtained from chloroacetyl chloride and anhydrous sodium acetate according to ref. 12; m.p. 46°C.

The anhydride of acrylic acid was prepared by the reaction of acroyl chloride with sodium acrylate according to ref. 13; b.p. 46–47°C (2 mm). $\gamma$-Mercaptobutyric acid was prepared according to Cheney and Piening [14] by the interaction of $\gamma$-bromobutyric acid and thiourea; b.p. 98–99°C (2 mm), $n_D^{20}$ = 1.4910. 8-Bromoadenosine 3′,5′-cyclic phosphate was obtained by direct bromination of cyclic AMP [3].

Analytic chromatography was performed on Whatman No. 1 paper in System A (*n*-butanol/acetic acid/water (5 : 2 : 3, v/v)).

Preparative chromatography was carried out on Whatman 3MM paper (washed with dilute HCl and then with distilled water) in Systems A and B (isopropanol/aqueous ammonia/water (7 : 1 : 2, v/v)).

Preparative thin-layer chromatography was performed on silica gel containing fluorescent indicator in System C (*n*-butanol/acetic acid/water (6 : 1 : 3, v/v)).

Electrophoresis was run on Whatman No. 1 paper at 50 V/cm in System D (citric acid/0.05 M sodium citrate, pH 6.0).

Evaporations were accomplished using a rotating evaporator under reduced pressure with a bath temperature of 35°C.

Analytic samples were dried over $P_2O_5$ at 0.01 mm for 24 h.

Ultraviolet spectra were determined on a recording Specord UV-Vis spectrophotometer.

*Preparation of some derivatives of cyclic AMP. 2'-O-Chloroacetyladenosine 3',5'-cyclic phosphate (II)*

Cyclic AMP (300 mg, 0.9 mmol) was added to a solution of triethylamine (450 mg, 4.5 mmol) in 7 ml of a mixture of abs. dioxane and abs. dimethylformamide (1 : 1, v/v). The resulting mixture was heated at 50° C for 5 min, during which operation the product dissolved almost completely. After cooling, 770 mg (4.5 mmol) of chloroacetic anhydride was added, and the reaction mixture was left at room temperature in the dark with moisture excluded. After 48 h, the solution was evaporated to dryness, and the residue was triturated with ether (5 × 5 ml). To the residual gum 2 ml of a mixture of abs. ethanol and dry ether (1 : 2, v/v) was added. The resulting mixture was shaken for 10 min and filtered. 10 ml of dry ether was added to the filtrate. After 2 h at 4° C, the precipitate formed was collected by centrifugation, washed with dry ether and dried in vacuo over $P_2O_5$. The product was purified once more by a similar procedure; yield about 30%.

Anal. Calcd. for $C_{12}H_{13}N_5O_7PCl \cdot H_2O$ : C, 34.02; H, 3.57; P, 7.31; Cl, 8.37.

Found: C, 33.74; H, 3.37; P, 7.08; Cl, 8.14.

*2'-O-Acrylyladenosine 3',5'-cyclic phosphate (III)*

Cyclic AMP (250 mg, 0.75 mmol) was added to a solution of triethylamine (730 mg, 7.3 mmol) in 13 ml of a 1 : 1 (v/v) mixture of abs. dioxane and abs. dimethylformamide. The mixture was heated at 50° C for 5–10 min. After cooling, 1.1 g (8.5 mmol) of acrylic anhydride was added, and the reaction mixture left at room temperature, protected from light and moisture (if after addition of the anhydride a precipitate was seen, the suspension was mixed to form a clear solution). After 48 h, the solution was evaporated and the residue was triturated with 30 ml of dry ether. The precipitate formed was filtered and washed with dry ether. The resulting powder was partially dissolved in 15 ml of boiling ethanol and filtered. The filtrate was evaporated to half of the initial volume and the product precipitated with excess dry ether (50 ml). After 2 h at 4° C, it was collected by centrifugation, washed with dry ether and dried in vacuo over $P_2O_5$.

Further purification was achieved by paper chromatography in System A. The paper band containing the product was located, cut out and extracted with 50 ml of a 3 : 2 (v/v) mixture of methanol and water. The extract thus obtained was evaporated to dryness and the residue dried over $P_2O_5$ to give III; yield 15–20%.

Anal. Calcd. for $C_{13}H_{14}N_5O_7P \cdot H_2O$: C, 38.91; H, 4.02; P, 7.72; N, 17.46.

Found: C, 38.78; H, 3.89; P, 7.49; N, 17.25.

*$N^6$, 2'-O-Diacrylyladenosine 3',5'-cyclic phosphate (IV)*

Cyclic AMP (250 mg, 0.75 mmol) was added to a solution of triethylamine (730 mg, 7.3 mmol) in 13 ml of a 1 : 1 (v/v) mixture of abs. dioxane and abs. dimethyl formamide. The resulting mixture was heated at 50° C for 5–10 min and, after cooling, 1.1 g (8.5 mmol) acrylic anhydride were added. The reaction mixture was heated at 90° C for 15 min and left at room temperature,

protected from moisture and light. After 72 h, the solution was evaporated to dryness. The residue was triturated with 30 ml dry ether and extracted with 15 ml of boiling abs. ethanol. The product was precipitated as in the procedure for III.

Further purification was achieved by thin-layer chromatography on silica gel in System C.

The silica gel band containing the product was located in ultraviolet light ($R_f$ = 0.35–0.4), separated, dried over NaOH, and extracted with methanol. The extract thus obtained was evaporated to dryness, and the resulting residue crystallized from ethanol/ether (1 : 1, v/v); yield about 15%.

Anal. Calcd. for $C_{16}H_{16}N_5O_8P \cdot H_2O$: C, 42.20; H, 3.99; N, 15.38; P, 6.80.

Found: C, 41.96; H, 3.78; N, 15.14; P, 6.46.

*$N^6$-Acrylyladenosine 3',5'-cyclic phosphate (V)*

70 mg (0.16 mmol) of IV was dissolved in 2 ml of 0.7 M $NH_4OH$. After 5 min at room temperature, 50 ml of ethanol cooled to −40° C was rapidly added, and ammonia removed under reduced pressure. When the pH value was lowered to ≈7, the solution was evaporated to dryness and the residue dried in vacuo over $P_2O_5$. The product was isolated by paper chromatography in System A. Elution was carried out with a 3 : 2 (v/v) mixture of methanol and water. Evaporation of the eluate gave V; yield about 30%.

Anal. Calcd. for $C_{13}H_{14}N_5O_7P \cdot H_2O$: C, 38.91; H, 4.02; P, 7.72.

Found: C, 39.23; H, 4.19; P, 7.94.

*8-(γ-Carboxypropylthio)adenosine 3',5'-cyclic phosphate (VII)*

960 mg (7.5 mmol) of γ-mercaptobutyric acid was added to a solution of methanol (4 ml) containing sodium methoxide (108 mg, 2 mmol) and 8-bromoadenosine 3',5'-cyclic phosphate (200 mg, 0.5 mmol). The reaction mixture was refluxed for 5 h. The solvent was removed by evaporation, and the residue dissolved in 5 ml of water. The solution was treated with an aqueous suspension of Dowex-50 ($H^+$-form) to attain pH ≈ 4. The ion exchange resin was filtered off and washed with water, and the combined filtrates were evaporated to dryness. The residue was triturated with ether (3 × 5 ml) and dried in vacuo.

Further purification was performed by paper chromatography in System B. The paper band containing the product ($R_f$ = 0.3) was cut out and extracted with 50 ml of 0.01 M $NH_4OH$. The extract thus obtained was evaporated to dryness and the evaporation repeated from ethanol. The residue was dried in vacuo over $P_2O_5$ to give VII; yield 30%.

Anal. Calcd. for $C_{14}H_{18}N_5O_8PS \cdot 2H_2O$: C, 34.98; H, 5.06; N, 14.91; P, 6.46; S, 6.67.

Found: C, 35.06; H, 5.11; N, 14.84; P, 6.88; S, 6.90.

## Results

In this work the effect of synthesized cyclic AMP analogues on the cyclic AMP-dependent histone kinase has been studied. Two approaches have been used; (1) We studied the ability of the analogues to activate histone $F_1$ phos-

TABLE II

ACTIVATION OF PIG BRAIN HISTONE KINASE BY 3′,5′-CYCLIC AMP ANALOGUES

| Concn (M) | nmol of $^{32}P$ incorporated into histone $F_1$ in the presence of: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cyclic AMP | 8-Bromo cyclic AMP | 8-(γ-Carboxypropylthio) Cyclic AMP (VII) | 2′-*O*-Chloroacetyl cyclic AMP (II) | $N^6$-Acrylyl cyclic AMP (V) | 2′-*O*-Acrylyl cyclic AMP (III) | $N^6$,2′-*O*-diacrylyl cyclic AMP (IV) | Adenosine-3′,5′-Cyclic Sulphate (VI) |
| $10^{-9}$ | 50 | 64 | 43.5 | 43.5 | 43.5 | 43.5 | 43.5 | 43.5 |
| $10^{-8}$ | 90 | 68 | 43.5 | 69.5 | 43.5 | 43.5 | 43.5 | 43.5 |
| $10^{-7}$ | 188 | 156 | 102 | 77.5 | 81 | 43.5 | 43.5 | 43.5 |
| $10^{-6}$ | 218 | 198 | 187 | 143 | 139 | 43.5 | 43.5 | 43.5 |
| $10^{-5}$ | 182 | 193 | 215 | 177 | 159 | 103.5 | 70 | 57.5 |
| $10^{-4}$ | 123 | 182 | 204 | 185 | 199 | 153.5 | 148.5 | 71.5 |

phorylation catalysed by the histone kinase. (2) We investigated the competitive relationship between the analogues and cyclic AMP with respect to binding to the histone kinase.

*Activation of cyclic AMP-dependent histone kinase by cyclic AMP analogues*

Data concerning the activation of histone kinase by the cyclic AMP analogues as compared to the action of cyclic AMP itself are presented in Table II. All compounds under study appeared to be activators of the enzyme, with the exception of 2′,3′-cyclic GMP and adenosine-5′-chloromethylphosphonate, included into the experiments as negative controls.

One can see from Table II that at most concentrations 8-bromo cyclic AMP and 8-(γ-carboxypropylthio) cyclic AMP (VII) were the most potent whereas adenosine 3′,5′-cyclic sulphate (VI) exhibited the lowest activity.

For a quantitative comparison of the activating potencies of cyclic AMP analogues with respect to the histone kinase, the values of the activation constants ($K'_\alpha$) were estimated. For each analogue, the values of the relative activation constants ($K'_\alpha$ = $K\alpha$(cyclic AMP)/$K\alpha$(analogue) were also estimated (Table III).

8-Bromo cyclic AMP showed the $K\alpha$ value nearest to that of cyclic AMP ($K'_\alpha$ = 0.38) whereas III, IV and VI were about 500—1000 times less active compared to the natural activator. Compounds VII and II showed $K\alpha$ values approx. 10-fold higher than for cyclic AMP ($K'_\alpha$ values were 0.16 and 0.083, respectively).

*Binding of cyclic AMP analogues to histone kinase*

In order to evaluate the affinity of the cyclic AMP analogues under study for histone kinase, cyclic [$^3$H] AMP binding was measured in the presence of these substances. It was found that in the presence of all analogues the equilibrium values of cyclic [$^3$H] AMP binding to histone kinase is decreased, and that the inhibition is competitive with respect to cyclic AMP. Inhibition constants ($K_i$) for cyclic AMP analogues, determined in the cyclic [$^3$H] AMP binding test are listed in Table IV. One can see from this table that the highest affinities for histone kinase are displayed by 8-bromo cyclic AMP and 8-(γ-carboxypropylthio) cyclic AMP ($K_i$ values equal $2.5 \cdot 10^{-8}$ M and $3.4 \cdot 10^{-8}$ M, respectively).

TABLE III

$K'_\alpha$ VALUES FOR 3′,5′-CYCLIC AMP ANALOGUES*

| Compound | $K_\alpha$ | $K'_\alpha = K_\alpha$ (cyclic AMP) / $K_\alpha$ (analogue) |
|---|---|---|
| Cyclic AMP | $2.5 \cdot 10^{-8}$ | 1.0 |
| 8-Bromo cyclic AMP | $6.5 \cdot 10^{-8}$ | 0.38 |
| 8- (γ-Carboxypropylthio) cyclic AMP (VII) | $1.5 \cdot 10^{-7}$ | 0.16 |
| Adenosine 3′,5′-Cyclic Sulphate (VI) | $1.1 \cdot 10^{-5}$ | 0.0023 |
| $N^6$-Acrylyl cyclic AMP (V) | $6.4 \cdot 10^{-7}$ | 0.039 |
| $N^6$,2′-*O*-Diacrylyl cyclic AMP (IV) | $2.1 \cdot 10^{-5}$ | 0.0012 |
| 2′-*O*-Acrylyl cyclic AMP (III) | $1.2 \cdot 10^{-5}$ | 0.0021 |
| 2′-*O*-Chloroacetyl cyclic AMP (II) | $3.0 \cdot 10^{-7}$ | 0.083 |

* $K_\alpha$, concentration of cyclic nucleotide required to give a half-maximal increase in activity.

TABLE IV

$K_i$ VALUES FOR 3′,5′-CYCLIC AMP ANALOGUES AS INHIBITORS OF CYCLIC [$^3$H] AMP BINDING TO HISTONE KINASE*

| Compound | $K_i$ (M) |
|---|---|
| 8-Bromocyclic AMP | $2.5 \cdot 10^{-8}$ |
| 8-(γ-Carboxypropylthio) cyclic AMP (VII) | $3.4 \cdot 10^{-8}$ |
| Adenosine 3′,5′-Cyclic Sulphate (VI) | $1.1 \cdot 10^{-6}$ |
| $N^6$-Acrylyl cyclic AMP (V) | $1.8 \cdot 10^{-7}$ |
| $N^6$,2′-*O*-Diacrylyl cyclic AMP (IV) | $3.2 \cdot 10^{-7}$ |
| 2′-*O*-Acrylyl cyclic AMP (III) | $7.4 \cdot 10^{-7}$ |
| 2′-*O*-Chloroacetyl cyclic AMP (II) | 90% binding degree |

* The $K_i$ values were determined as described in Materials and Methods.

Adenosine 3′,5′-cyclic sulphate showed the lowest affinity for the enzyme ($K_i = 1.1 \cdot 10^{-6}$ M).

To estimate the degree of binding tightness of the analogues to histone kinase we also studied the displacement of those substances from enzyme-inhibitor complexes by cyclic [$^3$H] AMP. It was found that incubation of the cyclic AMP analogues with the enzyme for 5 h, followed by dialysis and cyclic [$^3$H] AMP treatment results in the complete displacement of all analogues, excepting 2′-*O*-chloroacetyl cyclic AMP (II). For the latter, the binding degree was about 90% after a 1-h treatment with cyclic [$^3$H] AMP and did not alter upon further incubation with the natural activator.

## Discussion

From the data presented, some conclusions may be drawn about the importance of distinct regions of the cyclic AMP molecule for the binding to histone kinase and the activation of the enzymatic reaction. Thus, substitution at the 8 position of the purine ring does not substantially affect either the affinity for histone kinase (8-bromo cyclic AMP and VII) or the ability to activate histone phosphorylation; this is in agreement with the data of other authors [3]. On the other hand, introduction of a γ-carboxypropylthiol residue at the same position (compound VII) leads to tighter binding (as compared to that of 8-bromo cyclic AMP) to the enzyme, apparently due to the additional electrostatic interaction of the carboxyl group of VII with a cationic group of the protein. This fact is indicated by a slower displacement of VII (as compared to that of 8-bromocyclic AMP) from its complex with the histone kinase by cyclic [$^3$H] AMP.

The crucial importance of the negatively charged oxygen atom in the 3′,5′-cyclophosphate system of cyclic AMP is proved by the data concerning the interaction of the histone kinase with adenosine 3′,5′-cyclic sulphate (VI) obtained in this work. This compound lacks a negatively charged oxygen atom in the 3′,5′-cyclic sulphate ring (which closely simulates the cyclic phosphate system of cyclic AMP); this is critical for the ability to bind to histone kinase and to activate the histone phosphorylation reaction (Tables III and IV).

The requirement for a negative charge at the cyclic AMP phosphate hy-

droxyl residue suggests that upon the interaction of cyclic AMP with cyclic AMP-dependent enzymes an ionic bond is formed between a cationic locus in the regulatory subunit of histone kinase and the negatively charged oxygen atom of the 6-membered cyclophosphate ring.

The data presented in Tables III and IV show that the introduction of the acrylyl residue into the *exo*-$NH_2$ group of cyclic AMP results in a rather marked decrease of binding and activating potency which is not so critical as in the case of similar substitution at the 2′-OH group. Requirement for the unsubstituted *exo*-$NH_2$ group of cyclic AMP may indicate that this group is involved in the process of binding to the enzyme.

The 2′-OH group should be unblocked in the ribo configuration in order to provide the activating function of cyclic AMP [6]. The data obtained in this work also suggest that the 2′-OH group of cyclic AMP should be free so as to provide the activating function. Thus, introduction of the acrylyl residue in the 2′-OH group of cyclic AMP results in a 500-fold decrease of ability to activate the histone kinase (Table III, compound III). However, substitution of the chloroacetyl moiety which is able to provide covalent binding to the enzyme for the hydrogen atom in the 2′-OH group of cyclic AMP did not result in a substantial decrease in the ability of this compound to activate histone kinase (Table III, compound II). At the same time considerable firmness of 2′-*O*-chloroacetyl cyclic AMP (II) binding to the enzyme may indicate the formation of a covalent bond between II and the protein owing to the alkylation of a nucleophilic group of the histone kinase located in the proximity of the cyclic AMP 2′-OH group.

Thus, one may suggest that an active site of the regulatory subunit of the histone kinase has at least three specific areas of interaction with a cyclic AMP molecule.

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