Differential Labeling and Identification of the Cysteine-containing Tryptic Peptides of Catalytic Subunit from Porcine Heart cAMP-dependent Protein Kinase*

(Received for publication, November 14, 1980, and in revised form, December 15, 1980)

Norman C. Nelson and Susan S. Taylor

From the Department of Chemistry, University of California, San Diego, La Jolla, California 92039

The cysteine-containing tryptic peptides from the catalytic subunit of cAMP-dependent protein kinase were isolated and characterized with the aid of the sulphydryl specific reagent iodoacetic acid. When the catalytic subunit was reacted with $[^3]Cl$iodoacetic acid, cleaved with trypsin, and chromatographed on Sephadex G-50, three distinct components of radioactivity (C-1, C-2, and C-3) were identified. These components were further purified using a combination of high performance liquid chromatography and paper chromatography. Subsequent analysis revealed that only two of these components (C-2 and C-3) contained carboxymethylcysteine. The sequences of these two peptides are as follows: (C-2) -Cys-Gly-Lys-Glu-Ph-Ser-Glu-Phe; (C-3) Thr-Trp-Thr-Leu-Cys-Gly-Asn-Pro-Gln-Tyr-X-Ala-Pro-Glu-Ile-Ile-Leu-X-Lys. The third component (C-1) was shown definitely not to contain carboxymethylcysteine.

The alkylation of the catalytic subunit with $[^3]Cl$iodoacetic acid was also studied with respect to inhibition of the enzymatic activity. Incubation of the enzyme with a 1500-fold molar excess of $[^3]Cl$iodoacetic acid at 37°C resulted in essentially complete loss of phosphotransferase activity within 2 min. These conditions resulted in the complete alkylation of C-1, C-2, and C-3. Incubation of the enzyme with a 500-fold molar excess of $[^3]Cl$iodoacetic acid at 37°C resulted in essentially no loss of phosphotransferase activity, even after 90 min of incubation. Under these conditions, only C-1 was alkylated, with no significant alkylation of C-2 or C-3 occurring. This eliminates the possibility of C-1 being involved in the phosphotransferase activity of the enzyme. The substrate MgATP protected the enzyme against loss of activity due to modification with iodoacetic acid and blocked the alkylation of not only C-2 and C-3, but also C-1. These data suggest that both cysteine residues contained in the catalytic subunit are located in or near the active site of the molecule.

The dissociated catalytic subunit of cAMP-dependent protein kinase (EC 2.7.1.37) is a monomeric protein having a molecular weight of approximately 40,000 (1–4). It is this dissociated form of the catalytic subunit that is fully active as an ATP-protein phosphotransferase. The holoenzyme, on the other hand, is an inactive tetramer containing two regulatory and two catalytic subunits. Although at least two major and distinct forms of the holoenzyme exist, the dissociated catalytic subunits from both have been shown to be highly homologous in marked contrast to the regulatory subunits (5).

Characterization of the phosphorylation sites on native protein substrates as well as kinetic studies with synthetic peptides (6–9) have demonstrated certain features that are common to recognition sites for this enzyme. In particular, one and frequently two basic amino acid residues followed by a hydrophobic residue usually precede the site of phosphorylation, which can be either a serine or a threonine hydroxyl group. In native protein substrates, no more than two intervening sequences have been found between the basic residues and the phosphorylated residue.

Various affinity-labeling studies have identified potential residues that may play a role in the catalytic functioning of this enzyme. p-Fluorosulfonylbenzoyl C'-adenosine irreversibly inactivated the catalytic subunit, and a single modified lysine residue was identified (10). Treatment of the catalytic subunit with N*-tosyl-L-lysine chloromethyl ketone was also shown to specifically and irreversibly inactivate the catalytic subunit although no specifically modified amino acid residue was identified (11). Kochetkov et al., using a brain histone kinase, identified a lysine residue that was modified with the dihaloethane analog of ATP (12). Witt and Roskoski utilized acetic anhydride to inhibit the catalytic subunit and suggested the involvement of a tyrosine residue (13).

In addition to these affinity-labeling studies, alkylation with a variety of reagents has indicated that at least one, and possibly two, cysteine residues are situated near the active site of the enzyme (1–3, 11, 14, 15). The present study was designed to identify the cysteine-containing tryptic peptides from porcine heart catalytic subunit, to distinguish the cysteine residues on the basis of reactivity, and to determine which of these cysteine residues was in close proximity to the ATP-binding site on the catalytic subunit.

**Experimental Procedures**

**Assays.—** Protein kinase was assayed as described previously (4) according to a modification of the procedure of Was tallia et al. (16), using histone type II (Sigma) as substrate. One unit of activity represents 1 nmol of $^32$P incorporated into protein per min.

**Purification of the Catalytic Subunit of cAMP-dependent Protein Kinase.—** The catalytic subunit used for these studies was prepared from fresh porcine heart tissue (predominantly type II protein kinase) as described previously (5), with minor modifications. The Whatman DE11 column was eluted with a 15-liter gradient from 40 mm phosphate buffer (pH 6.5), to 40 mm phosphate buffer (pH 6.5) + 350 mm NaCl. The pooled kinase peak was immediately diluted to 1 nmoi of $^32$P incorporated into protein per min.

**Procedure.** The supernatant was poured off and the resin was poured into a column and washed extensively with 40 mm phosphate buffer (pH 6.5) followed by extensive washing with 15 mm phosphate buffer (pH 6.1). The catalytic subunit was then eluted with 3 liters of $2.2 \times 10^{-4}$
Cysteine Peptides of cAMP-dependent Protein Kinase Catalytic Subunit

Cyanogen Bromide Cleavage—Reduced and carboxymethylated protein was dialyzed against water and lyophilized to dryness. The residue was dissolved in 70% formic acid and incubated with a 50-fold molar excess of CNBr (MCB) for 24 h according to Steers et al. (28). The CNBr mixture was diluted 2-fold with water before application to the Sephadex-G20 column.

Radioactivity—Radioactive peaks for the various peptide maps were determined by either counting direct aliquots (up to 0.4 ml) in 5 ml of Bray's solution (180 g of naphthalene, 12 g of PPO, 0.5 g of POPP, 300 ml of absolute methanol, brought to 3 liters with dioxane) or by drying down aliquots (up to 1.6 ml), redissolving, and counting these in 5 ml of a solution of 15 g of PPO and 0.9 g of POPP in 3 liters of toluene. All samples were counted in a Beckman LS-223 liquid scintillation system.

Carboxypeptidase Treatment—Treatment with carboxypeptidase was carried out according to Ambler (29). Incubations were carried out at 37° C for 1 h unless otherwise stated.

RESULTS

Amino Acid Composition—The amino acid composition of protein kinase II catalytic subunit isolated from porcine heart was determined on the basis of hydrolyses carried out for 24, 48, and 72 h. In order to quantitate the cysteine content, the protein was first oxidized with performic acid, thereby converting the cysteines to cysteic acid. The results shown in Table I indicated that each monomeric catalytic subunit contained two to three cysteine residues.

Peptide Mapping—In order to specifically identify the cysteine-containing tryptic peptides, the catalytic subunit was reduced, alkylated with [14C]iodoacetic acid, and digested with trypsin. The resulting mixture of tryptic peptides was resolved using several different methods. Initial mapping of the tryptic peptides using two-dimensional paper chromatography gave the peptide map indicated in Fig. 1. Autoradiography of this map showed three well resolved radioactive spots designated as C-1, C-2, and C-3. Differential staining for histidine and arginine indicated that the radioactive spots did not contain either of these two residues. Peptide C-3 was distinguished by its fluorescent and positive Ehrlich stain indicating the presence of tryptophan.

When this mixture of tryptic peptides was subjected to high performance liquid chromatography on a Waters C-18-Bondapak reverse phase column and eluted as described under "Experimental Procedures," the profile shown in Fig. 2 was

| Amino acid | Mole amino acid/mole catalytic subunit | 24 h | 48 h | 72 h | Best estimate |
|------------|--------------------------------------|------|------|------|---------------|
| Cysteic acid | 3.39 | 2.40 | 2.5 | 2-3 |
| Aspartate | 37.08 | 34.45 | 35.18 | 15 |
| Threonine | 13.52 | 11.76 | 8.08 | 14 |
| Serine | 15.53 | 12.10 | 11.56 | 16 |
| Glutamate | 41.89 | 39.40 | 40.53 | 41 |
| Proline | 17.77 | 22.00 | 18.75 | 19 |
| Glycine | 23.83 | 25.26 | 24.41 | 24 |
| Alanine | 23.08 | 22.87 | 22.39 | 23 |
| Valine | 19.45 | 17.74 | 18.80 | 19 |
| Methionine | 5.80 | 5.02 | 6 |
| Leucine | 14.98 | 18.99 | 18.09 | 18 |
| Phenylalanine | 31.10 | 31.96 | 31.47 | 32 |
| Tyrosine | 12% | 12% | 12% | 12% |
| Histidin | 9.19 | 10.36 | 9.86 | 10 |
| Arginine | 3.48 | 36.89 | 36.33 | 36 |
| Tryptophan | 3% | 3% | 3% | 3% |

* Based on 40,000 g/mol of molecular weight for catalytic subunit.

* Determined from other analyses.
peptides C-2 and C-3 are indicated in Table II. The sequences of these peptides are indicated in Table III and were determined by a combination of manual and solid phase sequencing procedures.

NH₂-terminal dansylation, amino acid composition, and paper electrophoresis showed that C-1 could not be purified using the HPLC method described above. A variety of other methods were therefore used in order to further characterize C-1. Although acid hydrolysis demonstrated that the radioactive pool from HPLC was not homogeneous, glycine was identified as the major component. Paper electrophoresis at pH 1.9 with subsequent radioactivity scanning revealed a

obtained. When the fractions were measured for radioactivity, three peaks were identified. Electrophoresis of an aliquot of each of these three peaks at pH 6.5 under conditions identical with those used for peptide mapping above identified the three radioactive peaks as C-1, C-2, and C-3 corresponding to the peptide map in Fig. 1.

When the complete mixture of tryptic peptides was chromatographed on Sephadex G-50, the profile shown in Fig. 3 was observed. Again, three well resolved peaks of radioactivity were identified. Electrophoresis of these radioactive peaks at pH 6.5 identified the first peak, corresponding to the largest peptide fraction, as C-3, the middle peak as C-2, and the third peak, corresponding to the smallest Sephadex fraction, as C-1. The radioactive peaks were also subjected to HPLC as shown in Fig. 4. The first and second peaks run coincidentally with the peaks previously identified with HPLC (Fig. 2) as C-3 and C-2, respectively. The third peak of radioactivity from the Sephadex column eluted from HPLC at the same position C-1 had been eluted previously, and is not shown in Fig. 4. These data confirm the identification of the radioactive peaks as C-3, C-2, and C-1, respectively, in order of their elution from Sephadex G-50.

Purification and Characterization of the [¹⁴C]Iodoacetic Acid-labeled Peptides—The three peaks of radioactivity from the Sephadex G-50 column were pooled, lyophilized, and applied to the C₄ BONDAPAK column. In each case, the radioactive peak was pooled, desalted on Sephadex G-10, and further characterized. The amino acid compositions of the peptides C-2 and C-3 are indicated in Table II. The sequences of these peptides are indicated in Table III and were determined by a combination of manual and solid phase sequencing procedures.
radioactive peak that migrated slightly faster than glycine ($R_F$ glycine = 0.55, $R_F$ radioactive peak = 0.62). When the sample was acid-hydrolyzed and then electrophoresed under the same conditions, the radioactive peak was coincident with glycine. Chromatography of the sample without hydrolysis revealed a radioactive peak with $R_F = 0.16$ compared to glycine with an $R_F = 0.17$. As stated earlier, the sample ran as a neutral spot on paper electrophoresis at pH 6.5, both with and without hydrolysis. In all cases, there was no radioactivity detected at the carboxymethylcysteine position. As a control, aliquots of C-2 and C-3 were acid-hydrolyzed and electrophoresed, in which case radioactivity ran coincident with carboxymethylcysteine.

**Effect of Alkylation on Catalytic Activity**—Iodoacetic acid has been shown to be an effective irreversible inhibitor of the catalytic subunit (3). As seen in Fig. 5, incubation of the catalytic subunit with a 500-fold molar excess of iodoacetic acid had no effect on enzymatic activity whereas incubation with 1500-fold excess led to almost immediate inactivation. At intermediate concentrations (1250-fold), a more gradual inhibition was observed, which was essentially complete after 30 min. In the presence of 0.1 mM MgATP, the catalytic subunit was completely protected from irreversible inactivation (Fig. 6). Peters et al., using beef heart catalytic subunit, identified one of these three sulfhydryl groups in that enzyme as very reactive and could be titrated with DTNB with no concomitant loss of activity (2). A similar observation was made by Shaltiel (11). In this study we established that incubation of the porcine catalytic subunit with a 500-fold excess of iodoacetic acid for 30 min resulted in no loss of activity (Fig. 5). In order to establish whether the porcine catalytic subunit also had a reactive sulfhydryl group that could be alkylated with no concomitant loss of activity, the effect of a 500-fold excess of iodoacetic acid on the alkylation of C-1, C-2, and C-3 was determined. As indicated in Fig. 7, if the native catalytic subunit was incubated with a 1500-fold excess of iodoacetic acid, conditions which rapidly inactivate the enzyme, all three radioactive peaks were observed. On the other hand, incubation of the catalytic subunit with a 500-fold excess of iodoacetic acid c-2

**TABLE II**

| Amino acid | C-2 | C-3 |
|------------|-----|-----|
| Carboxymethylcysteine | 1 | 1 |
| Aspartate | 1 | 1 |
| Threonine | 2 | 2 |
| Serine | 1 | 1 |
| Glutamate | 2 | 2 |
| Proline | 2 | 2 |
| Glycine | 1 | 1 |
| Alanine | 1 | 1 |
| Isoleucine | 2 | 2 |
| Leucine | 3 | 3 |
| Tyrosine | 1 | 1 |
| Phenylalanine | 2 | 2 |
| Lysine | 1 | 1 |
| Tryptophan | 1 | 1 |

**TABLE III**

| Amino acid sequence of the two cysteine-containing tryptic peptides from the catalytic subunit |
|---------------------------------|
| C-2: Cys-Gly-Lys-Glu-Phe-Ser-Glu-Phe |
| Dansyl-Edman: | | |
| $^1$C radioactivity (cpm): 2625 525 0 0 |

| | C-3: Thr-Trp-Thr-Leu-Cys-Gly-Asp-Pro-Gln-Tyr-X-Ala-Pro-Glu-Ile-Ile-Leu-X-Lys |
| Dansyl-Edman: | | |
| $^1$C radioactivity (cpm): 0 0 0 0 650 145 60 0 |

$^a$ The NH$_2$-terminal residue as well as internal lysine residues remain coupled to glass.
peptides of catalytic subunit with a 1500-fold molar excess of iodoacetic acid in the native enzyme. Catalytic enzyme in 50 mM Tris, 10% glycerol, pH 7.8, was incubated with a 1500-fold molar excess of \([^{14}C]\)iodoacetic acid for 40 min at 37 °C. The reaction was stopped by the addition of a 10-fold molar excess of β-mercaptoethanol over iodoacetic acid and the mixture was dialyzed against 100 mM Tris, 0.5 mM EDTA, pH 8.5. Any unreacted sulfhydryl groups were then reacted with nonradioactive iodoacetic acid as described under “Experimental Procedures.” The mixture was dialyzed exhaustively and digested with TPCK-trypsin for 4 h at 37 °C (see “Experimental Procedures”). An aliquot was eluted using HPLC and 200 μl of the indicated tubes were measured for radioactivity as in Fig. 2.

Fig. 7. Alkylation pattern of the cysteine-containing tryptic peptides of catalytic subunit with a 1500-fold molar excess of iodoacetic acid in the native enzyme. Catalytic enzyme in 50 mM Tris, 10% glycerol, pH 7.8, was incubated with a 1500-fold molar excess of \([^{14}C]\)iodoacetic acid for 40 min at 37 °C. The reaction was stopped by the addition of a 10-fold molar excess of β-mercaptoethanol over iodoacetic acid and the mixture was dialyzed against 100 mM Tris, 0.5 mM EDTA, pH 8.5. Any unreacted sulfhydryl groups were then reacted with nonradioactive iodoacetic acid as described under “Experimental Procedures.” The mixture was dialyzed exhaustively and digested with TPCK-trypsin for 4 h at 37 °C (see “Experimental Procedures”). An aliquot was eluted using HPLC and 200 μl of the indicated tubes were measured for radioactivity as in Fig. 2.

Fig. 8. Alkylation pattern of the cysteine-containing tryptic peptides of catalytic subunit with a 10-fold molar excess of iodoacetic acid. Catalytic enzyme in 50 mM Tris, 10% glycerol, pH 7.8, was incubated with a 10-fold molar excess of \([^{14}C]\)iodoacetic acid for 15 min at 37 °C followed by reaction of the remaining free sulfhydryl groups with a 2000-fold molar excess of nonradioactive iodoacetic acid for 7 min at 37 °C. The molecule was unfolded with guanidine HCl, dialyzed exhaustively, and digested with TPCK-trypsin for 4 h at 37 °C (see “Experimental Procedures”). An aliquot was eluted using HPLC and 200 μl of the indicated tubes were measured for radioactivity as in Fig. 2.

acid, an amount insufficient to inactivate the enzyme, resulted in incorporation of radioactivity into only C-1 with no significant alkylation of C-2 and C-3 (Fig. 8). These results identified C-1 as being the most reactive group on porcine catalytic subunit towards iodoacetic acid alkylation and also eliminated C-1 as being directly or peripherally involved in the catalytic activity of the enzyme.

To determine the location of the cysteine residues contained in C-2 and C-3 with respect to the ATP-binding site in catalytic subunit, \([^{14}C]\)iodoacetic acid alkylation was carried out in the presence of MgATP. As indicated in Fig. 6, MgATP was shown to be effective in protecting the catalytic subunit from inhibition with iodoacetic acid. Therefore, a parallel experiment was run under conditions (cited in Fig. 5) where C-1, C-2, and C-3 were readily alkylated in the native enzyme, this time in the presence of MgATP. Again, no loss of activity was observed as compared to the control without iodoacetic acid. Furthermore, whereas, in the absence of ATP, all activity was lost and three peaks of radioactivity were observed, in the presence of ATP, no incorporation of radioactivity was observed (Fig. 9).

The reactivities of the cysteine residues contained in C-2 and C-3 towards alkylation with iodoacetic acid were also compared. The differences in reactivity were measured by comparing the per cent alkylation of the two cysteines as a function of time. Alkylation at pH 8.5 yielded essentially no difference in reactivity between C-2 and C-3 (data not shown). At pH 7.5, however, a distinct difference in the reactivities of the two sulfhydryl groups towards iodoacetic acid was observed (Fig. 10). Under the particular conditions of the reaction, the sulfhydryl group contained in C-3 reacted with iodoacetic acid at a higher rate than the sulfhydryl contained in C-2. Differences in per cent alkylation between C-2 and C-3 were observed over the entire time course with the largest difference—68% labeling of C-3 compared to 25% labeling of C-2—occurring at 120 min.

Cyanogen Bromide Cleavage of the Catalytic Subunit—
The catalytic subunit of prucine cAMP-dependent protein kinase contains 6 methionine residues. When the protein was reduced, carboxymethylated, cleaved with cyanogen bromide, and subjected to gel filtration, the results seen in Fig. 11 were obtained. Seven cyanogen bromide peptides were obtained: I, II, IV, III, A and B, and V, A and B. Fractions III and V from the Sephadex G-50 column were each resolved into two components, A and B (Fig. 12), following HPLC carried out according to Fullmer and Wasserman (17). When the protein was alkylated with \([^{14}C]\)iodoacetic acid prior to cyanogen bromide cleavage, the radioactivity was associated either with peaks I and II or eluted at the included volume of the column. CNBr I was a large fragment (>100 residues) having an NH₂-terminal phenylalanine. Digestion of CNBr I with trypsin followed by HPLC revealed that C-3 was located in this...
fragment. CNBr II was identified as the COOH-terminal cyanogen bromide fragment, by the absence of homoserine in the amino acid composition. In addition, treatment with carboxypeptidases A and B released phenylalanine. On sodium dodecyl sulfate-urea gels run according to the method of Swank et al. (30), this CNBr fragment had a molecular weight of 12,000 to 13,000. Tryptic digestion coupled with HPLC revealed that this fragment contained C-2, which was also identified as the COOH-terminal tryptic peptide by the release of phenylalanine with carboxypeptidase A and B treatment. When an aliquot of the third radioactive peak was run on HPLC, all the radioactivity eluted at the position C-1 had previously been shown to elute. When an aliquot was electrophoresed at pH 6.5, the radioactivity ran with a fluorescamine-positive, neutral spot. This spot was eluted and then electrophoresed at pH 1.9, either with or without prior hydrolysis. In both cases, the radioactivity ran coincident with glycine. An aliquot of this eluted sample was also dansylated (see “Experimental Procedures”) and then chromatographed, again either with or without prior hydrolysis. In both cases, glycine was the major dansyl spot and autoradiography identified this glycine spot as the only radioactive component on the plate. This third peak of radioactivity was further characterized by running a hydrolyzed aliquot on the amino acid analyzer, collecting the eluent in fractions, and counting these fractions for radioactivity (the eluent was not allowed to react with ninhydrin as the ninhydrin solution quenches radioactivity; known retention times were used to identify any peaks). The only radioactive peak eluted at the glycine position. In a different CNBr digest, when an aliquot of the third radioactive peak (corresponding to C-1) was electrophoresed at pH 1.9, radioactivity was detected only slightly off the origin (RF = 0.045) with none being detected at the glycine position. The difference between digests was that in this case the enzyme was in 70% formic acid for 48 h, as compared to the usual 24 h.

**DISCUSSION**

The number as well as the role of the cysteine residues in the catalytic subunit of cAMP-dependent protein kinase has been somewhat unclear. On the basis of amino acid composition alone, a value of 2 to 3 cysteines per catalytic subunit has been reported in most cases (1-3, 11, 14). An exception is the bovine liver enzyme where Sugden et al. (3) report only 1; however, this value may be low in that bovine heart enzyme contains 2 cysteines (31). In the case of the catalytic subunit from porcine heart type II cAMP-dependent protein kinase, amino acid analysis of the performic acid-oxidized protein also indicated a value of 2 to 3 cysteine residues/subunit. Further evidence indicating that there were 3 cysteines/subunit was derived from mapping of the tryptic peptides of [14C]iodoacetic acid-reacted protein, where three radioactive components were observed. These three components were clearly

---

**Fig. 10.** Alkylation of C-2 and C-3 with iodoacetic acid in the native enzyme. Time points represent per cent alkylation of the cysteines contained in C-2 and C-3 with [14C]iodoacetic acid in the native enzyme at pH 7.5 and 1 min (Tris buffer). Each time point aliquot was digested with TPCK-trypsin followed by separation of C-2 and C-3 using HPLC. Amount of protein present was determined by amino acid analysis.

**Fig. 11.** Separation of the CNBr peptides from reduced and [14C]-carboxymethylated catalytic subunit by Sephadex G-50 chromatography. Radioactivity was determined by counting 100-μl aliquots of the indicated tubes (3.0 ml total volume) as described under “Experimental Procedures.”

**Fig. 12.** Further separation of CNBr peaks III and V from Sephadex G-50 by HPLC. Aliquots from CNBr peaks III and V from the Sephadex G-50 elution shown in Fig. 11 were further separated by HPLC as described under “Experimental Procedures.” The doublets seen in the fraction 5 separation correspond to the homoserine and the homoserine lactone form of the peptide as described by Kerlavage et al. (35).
separated from one another using several different chromato-
graphic techniques and, in addition, were shown to be distinct
from carboxymethylcysteine-containing peptides, thus indi-
cating a value of two cysteine residues/catalytic subunit. This
isolated and characterized, only two, C-2 and C-3, were found
Titani
To differentiate between C-2 and C-3 with respect to inacti-
ation and characterization of tryptic peptides from [I4C]iodoa-
cetic acid reacted protein. It was shown that C-1 could be
react  faster  with iodoacetic  acid than  the  sulfhydryl  contained
reactivity in the native enzyme towards alkylation with io-
desoxycholate. One or more of these residues.
C-2 and C-3 were further differentiated on the basis of reactivity in the native enzyme towards alkylation with io-
doactic acid. The sulfhydryl in C-2 was shown to react faster with iodoacetic acid than the sulfhydryl contained in C-2 at pH 7.5 and a conductivity around 1 mho. Essential-
ly no difference in reactivities was observed at pH 8.5. This difference in reactivities between the two sulfhydryl groups suggests the possibility that one sulfhydryl group could be completely alkylated without significant alkylation of the other. Such an alkylation pattern could provide a method for determining the role of each sulfhydryl group in the inacti-
vation of the catalytic subunit upon alkylation with iodoacetic
acid, i.e. if only one sulfhydryl group is responsible for the
inactivation, or if, indeed, both sulfhydryl groups have to be
alkylated to achieve complete inactivation of the enzyme.
Experiments of such a nature are currently in progress.

Acknowledgment—We wish to acknowledge the technical assistance of B. Resseto.

REFERENCES
1. Bechtel, P. J., Beavo, J. A., and Krebs, E. G. (1977) J. Biol. Chem. 252, 2681-2687
2. Peters, K. A., Demaille, J. G., and Fischer, E. H. (1977) Biochemistry 16, 5691-5697
3. Sugden, P. H., Holladay, L. A., Reimann, E. M., and Corbin, J. D. (1976) Biochem. J. 159, 409-422
4. Taylor, S. S., and Stafford, P. H. (1978) J. Biol. Chem. 253, 2284-2297
5. Zoller, M. J., Kerlavage, A. R., and Taylor, S. S. (1979) J. Biol. Chem. 254, 2408-2412
6. Kemp, B. E., Bylund, D. B., Huang, T. S., and Krebs, E. G. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 3448-3452
7. Yeaman, S. J., Cohen, M., Watson, D. C., and Dixon, G. H. (1977) Biochem. J. 162, 411-421
8. Kemp, B. E., Graves, D. J., Benjamini, E., and Krebs, E. G. (1977) J. Biol. Chem. 252, 4888-4894
9. Zetterquist, O., Ragnarsson, U., Humbel, B., Berglund, L., and Engstrom, L. (1976) Biochem. Biophys. Res. Commun. 70, 696-698
10. Zoller, M. J., and Taylor, S. S. (1979) J. Biol. Chem. 254, 8363-8368
11. Kupfer, A., Gani, V., Jimenez, J. S., and Shaltiel, S. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3073-3077
12. Kochekov, S. N., Bulgargina, T. V., Sashchenko, L. P., and Severin, E. S. (1977) Eur. J. Biochem. 81, 111-118
Cysteine Peptides of cAMP-dependent Protein Kinase Catalytic Subunit

13. Witt, J. J., and Roskoski, R., Jr. (1975) Biochemistry 14, 4503-4507
14. Armstrong, R. N., and Kaiser, E. T. (1978) Biochemistry 17, 2840-2843
15. Hartl, G. Thomas (1980) Fed. Proc. 39, 2094
16. Wastila, W. B., Stull, J. T., Mayer, S. E., and Walsh, D. A. (1971) J. Biol. Chem. 246, 1996-2003
17. Fullmer, C. S., and Wasserman, R. H. (1979) J. Biol. Chem. 254, 7208-7212
18. Heilmann, J., Barollier, J., and Watze, E. (1957) Z. Physiol. Chem. 309, 219-223
19. Easly, C. W., Zegers, B. J., and Vijlder, M. (1975) Biochim. Biophys. Acta 175, 211-213
20. Crestfield, A. M., Moore, S., and Stein, W. H. (1963) J. Biol. Chem. 238, 622-627
21. Hirs, C. H. W. (1956) J. Biol. Chem. 219, 611-621
22. Gray, W. R., and Hartley, B. S. (1963) Biochem. J. 89, 379
23. Hartley, B. S. (1971) Biochem. J. 119, 805-822
24. Laursen, R. A., Bonner, A. G., and Horn, M. J. (1975) in Instrumentation in Amino Acid Sequence Analysis (Perham, R. N., ed) pp. 73-110, Academic Press, London
25. Potter, R. L., and Taylor, S. S. (1979) J. Biol. Chem. 254, 2413-2418
26. Machleidt, W., Wachter, E., Scheulen, M., and Otto, J. (1973) FEBS Lett. 37, 217-220
27. Bhown, A. S., Mole, J. E., Weissinger, A., and Bennett, J. C. (1978) J. Chromatog. 148, 532-535
28. Steers, E., Craven, G. R., Anfinsen, C. B., and Bethune, J. L. (1965) J. Biol. Chem. 240, 2478-2484
29. Ambler, R. P. (1967) Methods Enzymol. 11, 155-166
30. Swank, R. T., and Munkres, K. D. (1971) Anal. Biochem. 39, 462-477
31. Shoji, S., Ericsson, L. H., Demaille, J. D., Walsh, K. A., Neurath, H., Fischer, E. H., and Titan, K. (1980) Fed. Proc. 39, 2006
32. Shoji, S., Titan, K., Demaille, J. G., and Fischer, E. H. (1979) J. Biol. Chem. 254, 6211-6214
33. Maggio, E. T., Kenyon, G. L., Markham, G. D., and Reed, G. H. (1977) J. Biol. Chem. 252, 1202-1207
34. Schwartz, K. J., Nakagawa, Y., and Kaiser, E. T. (1976) J. Am. Chem. Soc. 98, 6369
35. Kerlavage, A. R., and Taylor, S. S. (1980) J. Biol. Chem. 255, 8483-8488