Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 821–825, January 1990
Biochemistry

Stimulation of casein kinase II by epidermal growth factor: Relationship between the physiological activity of the kinase and the phosphorylation state of its β subunit

(hormonal regulation/phosphoserine/phosphothreonine/human A-431 carcinoma cells)

PAT ACKERMAN*, CLAIBORNE V. C. GLOVER†, AND NEIL OSHEROFF*‡

*Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232-0146; and †Department of Biochemistry, University of Georgia, Athens, GA 30602

Communicated by Edwin G. Krebs, November 7, 1989 (received for review June 5, 1989)

ABSTRACT **To determine relationships between the hormonal activation of casein kinase II and its phosphorylation state, epidermal growth factor (EGF)-treated and EGF-naive human A-431 carcinoma cells were cultured in the presence of [^{32}P]orthophosphate. Immunoprecipitation experiments indicated that casein kinase II in the cytosol of EGF-treated cells contained approximately 3-fold more incorporated [^{32}P]phosphate than did its counterpart in untreated cells. Levels of kinase phosphorylation paralleled levels of kinase activity over a wide range of EGF concentrations as well as over a time course of hormone action. Approximately 97% of the incorporated [^{32}P]phosphate was found in the β subunit of casein kinase II. Both activated and hormone-naive kinase contained radioactive phosphoserine and phosphothreonine but no phosphotyrosine. On the basis of proteolytic mapping experiments, EGF treatment of A-431 cells led to an increase in the average [^{32}P]phosphate content (i.e., hyperphosphorylation) of casein kinase II β subunit peptides which were modified prior to hormone treatment. Finally, the effect of alkaline phosphatase on the reaction kinetics of activated casein kinase II indicated that hormonal stimulation of the kinase resulted from the increase in its phosphorylation state.**

Casein kinase II is a ubiquitous serine/threonine kinase which is located in the cytosol as well as the nucleus of eukaryotic cells (reviewed in refs. 1–3). Although the detailed physiological function of the kinase has yet to be delineated, both *in vivo* and *in vitro*, it is capable of phosphorylating a number of proteins which play critical roles in regulating cellular growth and metabolism (1–3).

Casein kinase II from most sources exists as a heterotetramer with an $\alpha_2\beta_2$ structure (1, 4, 5). The α subunit ranges in molecular mass from 35 to 44 kDa (1–3). On the basis of activity and modification studies, it has been identified as the enzyme's catalytic subunit (6–8). In addition, α is homologous with the catalytic domains of several other serine/threonine kinases (9), especially with the catalytic domain of the yeast cell division cycle protein kinase CDC28 (10). The β subunit ranges in molecular mass from 24 to 29 kDa (1–3) and is the acceptor site for an intramolecular autophosphorylation reaction (4, 5, 8, 11). Neither the function of this subunit nor the purpose of its autophosphorylation is known. However, the β subunit appears to be required for optimal kinase activity (7).

Casein kinase II is cyclic nucleotide independent and calcium/calmodulin insensitive (1–3). Thus, for many years the enzyme was believed to be unregulated in the cell. Recent evidence indicates that this is not the case. The physiological activity of casein kinase II is transiently stimulated by a number of polypeptide hormones, including insulin (12, 13), insulin-like growth factor I (13), and epidermal growth factor (EGF) (12, 14). A similar activation is observed upon serum stimulation of deprived cells (15).

Following a 50-min treatment of human A-431 carcinoma cells with EGF, cytosolic casein kinase II activity rises approximately 4-fold (14). Kinase activity returns to baseline (i.e., the level found in cytosol of hormone-naive cells) when cytosol from EGF-treated cells is incubated with alkaline phosphatase prior to assay (14). This suggests that the hormonal regulation of casein kinase II depends on a terminal phosphorylation event: modification of either the kinase itself or a stimulatory factor.

To determine whether EGF stimulation of casein kinase II results from a change in the phosphorylation state of the kinase, A-431 cells were cultured in the presence of [^{32}P]orthophosphate. EGF treatment of cells increased the radioactive phosphoserine/phosphothreonine content of the kinase's β subunit ≈3-fold. Moreover, removal of these phosphate groups by alkaline phosphatase converted the reaction kinetics of EGF-stimulated casein kinase II to those of the hormone-naive enzyme. Thus, hormonal activation of casein kinase II appears to result from a hyperphosphorylation of the kinase.

EXPERIMENTAL PROCEDURES

Materials. EGF was isolated from mouse submaxillary glands by the procedure of Savage and Cohen (16). Rabbit antiserum directed against *Drosophila* casein kinase II was prepared as previously described (17). Tris, insolubilized (agarose-linked) calf alkaline phosphatase, protein and phosphorylated amino acid standards, *Staphylococcus aureus* V8 protease, and *Streptomyces griseus* protease were from Sigma; Formalin-fixed *Staph. aureus* cells (Pansorbin) were from Calbiochem; α-chymotrypsin and trypsin (TPCK-treated) were from Worthington; ATP was from Pharmacia LKB; the peptide phosphate acceptor Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu was from Peninsula Laboratories; and [γ-^{32}P]ATP (3000 Ci/mmol; 1 Ci = 37 GBq) and [^{32}P]orthophosphate (carrier-free in aqueous solution) were from Amersham.

Cell Culture. Human A-431 carcinoma cells were cultured as previously described (14). Unless stated otherwise, EGF-treated cells were incubated with the hormone (200 ng/ml final concentration) for 50 min prior to harvest. A 50-min point was employed as it represents the time after hormone treatment at which casein kinase II activity is maximal (14). When appropriate, cells were metabolically labeled by adding

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: EGF, epidermal growth factor.

‡To whom reprint requests should be addressed.

[^{32}P]orthophosphate (10 μCi/ml final concentration) to the growth medium 12 hr prior to harvest.

Preparation of Cytosolic Extracts. Cells were harvested and cytosolic extracts were prepared as described by Ackerman and Osheroff (14).

Casein Kinase II Activity Assay. The casein kinase II activity in cytosolic extracts was determined by the ability to phosphorylate the peptide Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu. This peptide is a specific phosphate acceptor for casein kinase II in a variety of systems (12, 18–20), including human A-431 cell cytosol (14). Unless stated otherwise, assays were carried out in 30 μl of 50 mM Tris·HCl, pH 8.2/50 mM NaCl/25 mM KCl/10 mM $MgCl_2$/10 μM sodium orthovanadate containing 15 μM [γ-^{32}P]ATP (2 Ci/mmol) and 0.15 mM peptide substrate. Reactions were initiated by the addition of 1 μl ($\approx$9 μg total protein) of A-431 cytosolic extract (obtained from cells harvested at 3.5×10^7 cells per plate) and were allowed to proceed for 1 min at 23°C. When the kinetics of casein kinase II activity were examined, reaction mixtures contained either 15 μM [γ-^{32}P]ATP and peptide substrate in the concentration range 0.1–5 mM or 0.15 mM peptide and [γ-^{32}P]ATP in the concentration range 3–60 μM. In all cases, phosphorylation was stopped by spotting reaction mixtures onto Whatman P81 ion-exchange paper. Papers were washed five times with 0.5% phosphoric acid and twice with 95% ethanol and dried. Phosphorylation was quantitated by using a Beckman LS 5000 TD liquid scintillation counter and Ecolume liquid scintillant (ICN). Assays always were carried out in duplicate and radioactivities were measured twice and averaged. When appropriate, cytosol (24 μl) was treated with alkaline phosphatase (0.5 unit, 4 μl of packed beads) prior to assay (14).

Immunoprecipitation of Casein Kinase II from Cytosolic Extracts. Human casein kinase II was isolated from A-431 cytosolic extracts by a modification (21) of the procedure of Soderquist and Carpenter (22). Briefly, cytosolic extracts of EGF-treated or naive cells were incubated with 1/10th vol of rabbit antiserum to *Drosophila* casein kinase II (17). This antiserum displays specificity for the mammalian kinase (17), even in crude extracts of human cells (14). Casein kinase II was precipitated by the addition of Formalin-fixed *Staph. aureus* cells (10% final concentration). Immunoprecipitates were washed four times with 10 mM Tris·HCl, pH 8.5/1.0% Triton X-100/1.0% sodium deoxycholate/0.1% SDS/1 mM EDTA/0.02% sodium azide, resuspended in 100 μl of 250 mM Tris·HCl, pH 6.8/8% SDS/20% (vol/vol) 2-mercaptoethanol/40% (vol/vol) glycerol/0.004% bromphenol blue, and heated to 90°C for 30 min. *Staph. aureus* cells were removed by centrifugation. Radioactivity was determined by Cherenkov counting using a Beckman LS 5000 TD liquid scintillation counter. Alternatively, samples were subjected to electrophoresis on a denaturing 10% polyacrylamide gel (23) followed by autoradiography using Kodak XAR film and a DuPont Lightning Plus intensifying screen. The radioactive label in the enzyme was quantitated with a Biomed Instruments (Fullerton, CA) model SL-504-XL scanning densitometer.

Identification of Phosphorylated Amino Acid Residues. Immunoprecipitated [^{32}P]phosphate-labeled casein kinase II was purified by electrophoresis on a denaturing 10% polyacrylamide gel (23). The band which corresponded to the β subunit of the kinase was localized by autoradiography, excised, and subjected to acid hydrolysis as previously described (21). The hydrolysate was analyzed by two-dimensional thin-layer electrophoresis as described by Hunter and Sefton (24). Phosphorylated amino acid standards were localized by ninhydrin staining and [^{32}P]phosphate was localized by autoradiography.

Partial Proteolytic Mapping of Phosphorylated Casein Kinase II β Subunit. Metabolically [^{32}P]phosphate-labeled β subunit of casein kinase II was isolated as described above. The subunit was digested by the procedure of Cleveland *et al.* (25), using (final concentrations) α-chymotrypsin at 120 μg/ml, trypsin at 40 μg/ml, *Staph. aureus* V8 protease at 10 μg/ml, or *S. griseus* protease at 20 μg/ml. Samples were subjected to electrophoresis on a denaturing 12.5% polyacrylamide gel (23) and radioactive bands were visualized by autoradiography.

RESULTS

Effect of EGF on the *in Vivo* Phosphorylation State of Casein Kinase II in A-431 Cells. Incubation of human A-431 carcinoma cells with EGF at 200 ng/ml transiently stimulates cytosolic casein kinase II activity $\approx$4-fold (14). To determine whether the phosphorylation state of casein kinase II correlates with its physiological activity, A-431 cells were cultured in the presence of [^{32}P]orthophosphate. The A-431 cell line was employed for this study because of its abundance of EGF receptors (26).

Fig. 1 shows the effect of EGF concentration on the activity and [^{32}P]phosphate content of casein kinase II in A-431 cytosol at 50 min post hormone treatment. A 50-min time point was employed for this study because it represents the time after hormone treatment at which kinase activity is maximal (14). Kinase activity increased maximally 4-fold and plateaued at EGF concentrations between 140 and 200 ng/ml. Parallel results were observed with the enzyme's [^{32}P]phosphate content, which plateaued at a 3-fold increase. At all levels of hormone examined, a strong correlation between the activity and [^{32}P]phosphate content of casein kinase II was observed (Fig. 1 *Inset*). Kinase activity and phosphorylation were also followed over a time course of EGF (200 ng/ml) action (Fig. 2). As found previously (14), the activity of casein kinase II rose 4-fold, peaked at 50 min post hormone treatment, and returned to baseline by $\approx$120 min. Over the same time course, the [^{32}P]phosphate content of the enzyme rose 3-fold, peaked at 50 min, and also returned to baseline by $\approx$120 min. As above, a strong correlation was found between kinase activity and [^{32}P]phosphate content at all times examined (Fig. 2 *Inset*). The finding that casein kinase II [^{32}P]phosphate levels not only rose with kinase activation but fell with kinase deactivation as well provides strong evidence

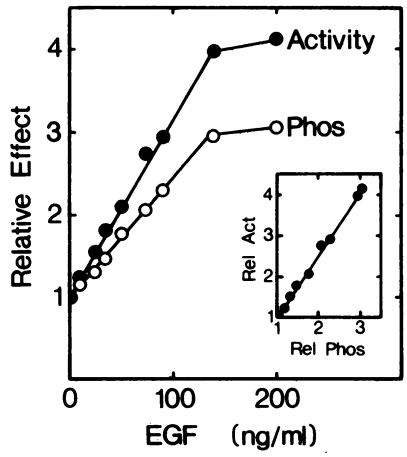

FIG. 1. Effect of EGF concentration on the activity and [^{32}P]phosphate content of casein kinase II in metabolically labeled human A-431 carcinoma cells. Cells were incubated with EGF for 50 min. ●, Relative kinase activity; ○, relative [^{32}P]phosphate content of casein kinase II immunoprecipitates (as determined by Cherenkov counting). (*Inset*) Correlation between relative kinase activity (Rel Act) and relative [^{32}P]phosphate content (Rel Phos).

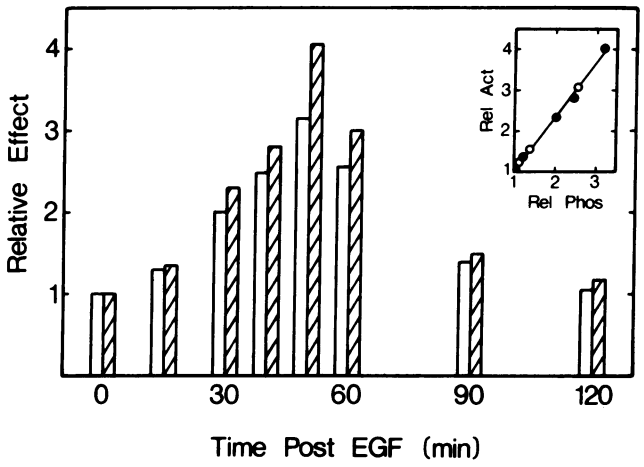

FIG. 2. Variations in casein kinase II activity and [^{32}P]phosphate content over a time course of EGF (200 ng/ml) action in metabolically labeled A-431 cells. Hatched bars represent relative kinase activity and open bars represent relative [^{32}P]phosphate content in casein kinase II immunoprecipitates. (*Inset*) Correlation between relative kinase activity (Rel Act) and relative [^{32}P]phosphate content (Rel Phos). ●, Data points from 0 to 50 min after EGF; ○, data points from 60 to 120 min after EGF.

that the enzyme's activity is linked to its phosphorylation state.

To further analyze the above results, radioactive casein kinase II immunoprecipitates from naive and EGF-treated (200 ng/ml for 50 min) cells were subjected to electrophoresis on a denaturing polyacrylamide gel. As seen in an autoradiogram of the gel (Fig. 3), the only radioactive bands which were observed corresponded to the α and β subunits of casein kinase II. Immunoblot analysis of parallel gels confirmed the identification of the kinase subunits (not shown). Moreover, densitometric scanning of blots indicated that comparable amounts of casein kinase II polypeptides had been immunoprecipitated from EGF-treated and naive cells.

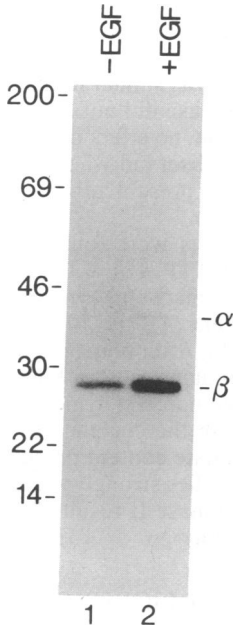

FIG. 3. Immunoprecipitation of ^{32}P-phosphorylated casein kinase II from metabolically labeled A-431 cells. Immunoprecipitates were subjected to electrophoresis on a denaturing polyacrylamide gel. An autoradiogram is shown. Cells were grown in the absence (lane 1) or presence (lane 2) of EGF at 200 ng/ml for 50 min. The molecular masses of marker proteins (in kDa) are shown at left and the positions of the α and β subunits of human casein kinase II are shown at right.

As determined by densitometric scanning of the autoradiogram shown in Fig. 3, ≥97% of the observed radioactivity was incorporated into the β subunit of the kinase. Furthermore, the [^{32}P]phosphate content of the casein kinase II β subunit from hormone-treated cell cytosol was ≈3-fold higher than that of the β subunit from EGF-naive cells. Thus, the *in vivo* stimulation of casein kinase II by EGF is accompanied by an increased ^{32}P-phosphorylation (i.e., hyperphosphorylation) of the kinase's β subunit.

Sites of Casein Kinase II Phosphorylation. Two experiments were carried out as a first step toward characterizing the site(s) of phosphorylation on the β subunit of casein kinase II that correlates with the enzyme's hormonal regulation. In both cases, the casein kinase II β subunit from [^{32}P]phosphate-labeled EGF-treated or naive cells was isolated by immunoprecipitation and gel electrophoresis prior to analysis. (In these experiments, radioactivities in the β subunits from +EGF and −EGF cytosols were normalized prior to digestion.)

First, labeled β subunits were subjected to acid hydrolysis and two-dimensional thin-layer electrophoresis (24) to determine the type of amino acid(s) which was phosphorylated *in vivo*. Results are shown in Fig. 4. In both the hormone-treated and naive kinases, radioactive phosphoserine and phosphothreonine, but no phosphotyrosine, were observed. This is consistent with the previous finding that activated casein kinase II does not absorb to anti-phosphotyrosine antibodies (14). As determined by scintillation counting of amino acids excised from thin-layer plates, the phosphothreonine to phosphoserine ratio remained constant at ≈0.2:1 in both samples, despite the overall increase in the [^{32}P]phosphate content of the hormone-stimulated kinase. This ratio is similar to that reported for the autophosphorylation of bovine casein kinase II (phosphothreonine-to-phosphoserine ratio, 0.25:1) (5).

Second, to determine the effect of EGF on the *in vivo* sites of casein kinase II phosphorylation, labeled β subunits isolated from hormone-treated and naive A-431 cells were subjected to partial proteolytic digestion (Fig. 5). While such a procedure does not allow the sites of modification to be identified at the amino acid level, it readily allows a comparison between the peptides phosphorylated in the presence and absence of hormone.

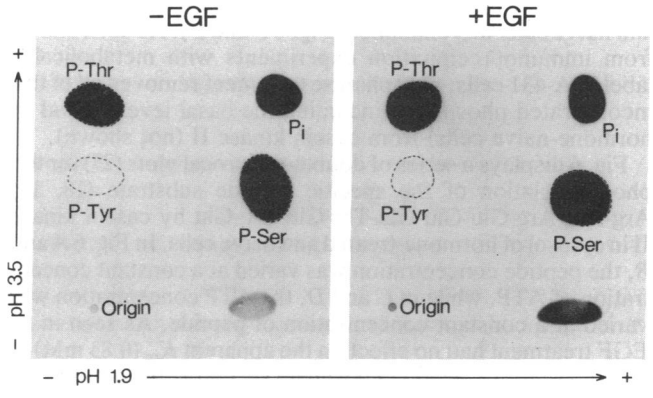

FIG. 4. Identification of the amino acid residues phosphorylated in metabolically labeled hormone-naive (*Left*) and EGF-treated (*Right*) A-431 cells. The β subunit of casein kinase II was subjected to partial acid hydrolysis, mixed with phosphorylated amino acid standards (P-Ser, P-Thr, and P-Tyr), and analyzed by two-dimensional thin-layer electrophoresis at pH 1.9 and 3.5. Autoradiograms of the thin-layer plates are shown. Broken circles indicate the positions of amino acid standards. P_i designates the location of inorganic phosphate.

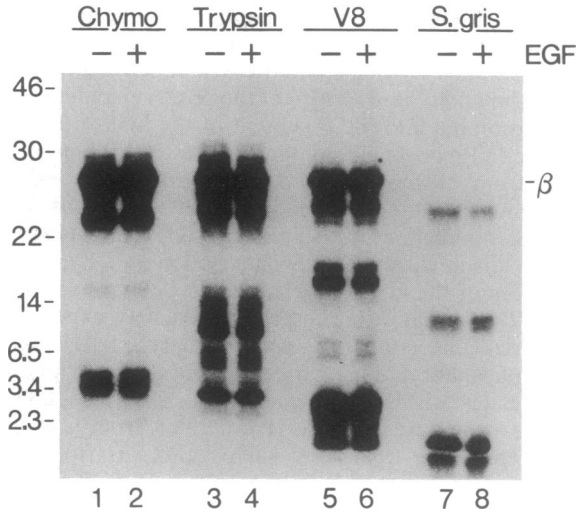

FIG. 5. Partial proteolytic maps of ^{32}P-phosphorylated casein kinase II. The enzyme's β subunit was isolated from metabolically labeled hormone-naive (odd-numbered lanes) or EGF-treated (even-numbered lanes) A-431 cells. Maps were prepared by digestion with α-chymotrypsin (lanes 1 and 2), trypsin (lanes 3 and 4), *Staph. aureus* V8 protease (lanes 5 and 6), or *S. griseus* protease.(lanes 7 and 8). The molecular masses of marker proteins (in kDa) are shown at left, and the position of the β subunit of human casein kinase II is shown at right.

Four proteases with different cleavage specificities were employed for this experiment; α-chymotrypsin, trypsin, *Staph. aureus* V8 protease, and *S. griseus* protease. For each protease, a unique banding pattern of phosphorylated peptides was generated after electrophoresis on a denaturing polyacrylamide gel and autoradiography. For all of the proteases employed, maps of EGF-stimulated and naive casein kinase II β subunit were identical (Fig. 5). Thus, EGF treatment of A-431 cells does not result in the modification of new peptides on casein kinase II. Instead it appears that EGF causes an increase in the average [^{32}P]phosphate content of peptides which were modified prior to the exposure of cells to the hormone.

Effect of Alkaline Phosphatase on the Kinetics of EGF-Stimulated Casein Kinase II. To more fully characterize the relationship between phosphorylation and the hormonal stimulation of casein kinase II, the effect of alkaline phosphatase on the reaction kinetics of the kinase in EGF-treated and naive cells was examined (Fig. 6 *B* and *D*). As determined from immunoprecipitation experiments with metabolically labeled A-431 cells, phosphatase treatment removed all of the incorporated phosphate (including the basal levels found in hormone-naive cells) from casein kinase II (not shown).

Fig. 6 displays a series of double-reciprocal plots (27) for the phosphorylation of the specific peptide substrate (18, 19) Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu by casein kinase II in cytosol of hormone-treated and naive cells. In Fig. 6 *A* and *B*, the peptide concentration was varied at a constant concentration of ATP, while in *C* and *D*, the ATP concentration was varied at a constant concentration of peptide. As seen in *A*, EGF treatment had no effect on the apparent K_m (0.83 mM) of the kinase for its peptide phosphate acceptor. In contrast, the apparent V_{max} of hormone-stimulated casein kinase II was increased ≈4-fold, from 41 to 166 pmol/min per mg of protein. A similar kinetic effect has been reported for insulin-stimulated casein kinase II from mouse 3T3-L1 cells (12).

As seen in Fig. 6*B*, treatment with insolubilized alkaline phosphatase had no significant effect on the reaction kinetics of casein kinase II in EGF-naive cytosol (apparent K_m = 0.77 mM; apparent V_{max} = 38 pmol/min per mg of protein). Thus, basal levels of phosphorylation (i.e., levels found in hormone-naive cells) do not appear to affect the enzyme's activity. This result is not surprising, since basal levels of phosphorylation likely result from the enzyme's autophosphorylation activity (1, 2). A number of previous studies have shown that autophosphorylation does not affect the activity of casein kinase II (1, 2, 4, 5). In contrast, phosphatase treatment of hormone-stimulated casein kinase II greatly reduced its apparent V_{max}. The resulting value of 43 pmol/min per mg of protein was similar to that of the naive kinase. As expected, phosphatase did not affect the apparent K_m of the enzyme. In addition, no effect of alkaline phosphatase on enzyme activity was observed when inhibitors such as vanadate or EDTA were present at the time of phosphatase incubation (not shown).

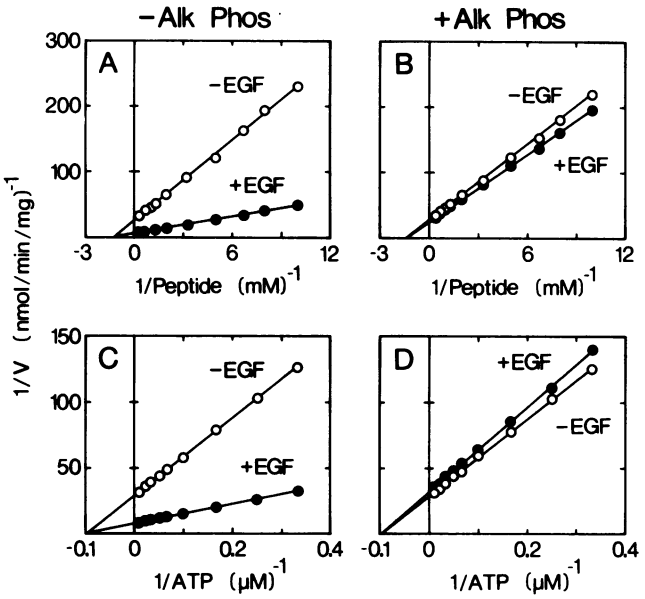

FIG. 6. Effect of alkaline phosphatase on the reaction kinetics of casein kinase II. Double-reciprocal plots are shown. Activity of the kinase in cytosolic extracts from hormone-naive (−EGF, ○) and EGF-treated (+EGF, ●) A-431 cells was determined by the ability to phosphorylate the specific peptide substrate Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu. Cytosolic extracts were incubated in the absence (*A* and *C*) or presence (*B* and *D*) of insolubilized alkaline phosphatase prior to kinase assays. In *A* and *B*, peptide was varied from 0.1 to 5 mM at a constant level of 15 μM ATP. In *C* and *D*, ATP was varied from 3 to 60 μM at a constant level of 0.15 mM peptide.

Similar kinetic results were found for the interaction of casein kinase II with ATP (Fig. 6 *C* and *D*). EGF treatment of cells had no effect on the enzyme's affinity for its phosphate donor (apparent K_m = 10 μM) but increased its apparent V_{max} ≈4-fold. After phosphatase treatment, enzyme kinetics of EGF-stimulated casein kinase II were similar to those of the hormone-naive enzyme.

Taken together with the correlation between enzyme activity and [^{32}P]phosphate content presented in Figs. 1 and 2, these phosphatase studies strongly suggest that the hormonal activation of casein kinase II results from a hyperphosphorylation of its β subunit.

DISCUSSION

EGF elicits a number of biological responses in a wide variety of eukaryotic cells (26, 28–30). Among its many effects is the transient stimulation of cytosolic casein kinase II activity (12, 14). A previous study (14) on the physiological regulation of casein kinase II concluded that hormonal activation of the enzyme required the phosphorylation of either the kinase or a terminal stimulatory factor. The data presented in the present investigation indicate that the stimulation of casein

kinase II by EGF in human A-431 carcinoma cells results from an increase in the kinase's phosphoserine/phosphothreonine content. Sites of hyperphosphorylation are located predominantly (≥97%) in the β subunit of the kinase. These findings strongly suggest that the β subunit plays an important role in the physiological regulation of casein kinase II.

EGF-induced hyperphosphorylation of casein kinase II stimulates catalytic activity by increasing the kinase's apparent V_{max} for both its phosphate donor and acceptor. The enzyme's K_m values are not affected. A similar hormonal effect on the kinetics of insulin-stimulated casein kinase II has been reported by Sommercorn *et al.* (12). Thus, diverse polypeptide hormones such as EGF and insulin may regulate the cellular activity of casein kinase II by a common biochemical mechanism.

Considering that (*i*) sites of EGF-induced hyperphosphorylation are located in the β subunit of casein kinase II, (*ii*) the β subunit contains the primary site(s) of autophosphorylation (4, 5, 8, 11, 31), and (*iii*) the site(s) of normal cellular (i.e., basal) modification [which presumably represents at least in part the site(s) of autophosphorylation] and hyperphosphorylation are located in the same peptide(s), it is possible that hyper- and autophosphorylation are interrelated. Since the proteolytic mapping experiments of Fig. 5 produced peptides considerably smaller than 2 kDa, sites of hyper- and autophosphorylation must be no further than 5 to 10 amino acids apart. Despite the fact that autophosphorylation in itself does not appear to alter enzyme activity (1, 2, 4, 5), it will be interesting to determine whether sites of hyper- and autophosphorylation are filled or alter enzyme function in a synergistic fashion.

The cellular activities of many protein kinases are modulated by phosphorylation (2, 32, 33). In some cases, modification is catalyzed by a different kinase, while in others, it is mediated by an autophosphorylation event. Although the mechanism by which EGF induces the hyperphosphorylation of casein kinase II is not yet known, it is clear that this enzyme must now be added to the growing list of kinases whose cellular functions are regulated by phosphorylation.

We thank Brenda Crews for preparing EGF and maintaining the A-431 cells used in the above study and Susan Heaver for her conscientious preparation of this manuscript. This research was supported by National Institutes of Health Grants CA-43720 (to N.O.), GM-33944 (to N.O.), and GM-33237 (to C.V.C.G.) and Basic Research Grant 1-1074 (to N.O.) from the March of Dimes Birth Defects Foundation.

1. Hathaway, G. M. & Traugh, J. A. (1982) *Curr. Top. Cell. Regul.* **21,** 101–127.
2. Edelman, A. M., Blumenthal, D. K. & Krebs, E. G. (1987) *Annu. Rev. Biochem.* **56,** 567–613.
3. Krebs, E. G., Eisenman, R. N., Kuenzel, E. A., Litchfield, D. W., Lozeman, F. J., Lüscher, B. & Sommercorn, J. (1988) *Cold Spring Harbor Symp. Quant. Biol.* **53,** 77–84.
4. Hathaway, G. M. & Traugh, J. A. (1979) *J. Biol. Chem.* **254,** 762–768.
5. Dahmus, M. E. (1981) *J. Biol. Chem.* **256,** 3319–3325.
6. Hathaway, G. M., Zoller, M. J. & Traugh, J. A. (1981) *J. Biol. Chem.* **256,** 11442–11446.
7. Cochet, C. & Chambaz, E. M. (1983) *J. Biol. Chem.* **258,** 1403–1406.
8. Meggio, F. & Pinna, L. A. (1984) *Eur. J. Biochem.* **145,** 593–599.
9. Saxena, A., Padmanabha, R. & Glover, C. V. C. (1987) *Mol. Cell. Biol.* **7,** 3409–3417.
10. Takio, K., Kuenzel, E. A., Walsh, K. A. & Krebs, E. G. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4851–4855.
11. Glover, C. V. C., Shelton, E. R. & Brutlag, D. L. (1983) *J. Biol. Chem.* **258,** 3258–3265.
12. Sommercorn, J., Mulligan, J. A., Lozeman, F. J. & Krebs, E. G. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8834–8838.
13. Klarlund, J. K. & Czech, M. P. (1988) *J. Biol. Chem.* **263,** 15872–15875.
14. Ackerman, P. & Osheroff, N. (1989) *J. Biol. Chem.* **264,** 11958–11965.
15. Carroll, D. & Marshak, D. R. (1989) *J. Biol. Chem.* **264,** 7345–7348.
16. Savage, C. R., Jr., & Cohen, S. (1972) *J. Biol. Chem.* **247,** 7609–7611.
17. Dahmus, G. K., Glover, C. V. C., Brutlag, D. L. & Dahmus, M. E. (1984) *J. Biol. Chem.* **259,** 9001–9006.
18. Kuenzel, E. A. & Krebs, E. G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 737–741.
19. Kuenzel, E. A., Mulligan, J. A., Sommercorn, J. & Krebs, E. G. (1987) *J. Biol. Chem.* **262,** 9136–9140.
20. Sommercorn, J. & Krebs, E. G. (1987) *J. Biol. Chem.* **262,** 3839–3843.
21. Ackerman, P., Glover, C. V. C. & Osheroff, N. (1988) *J. Biol. Chem.* **263,** 12653–12660.
22. Soderquist, A. M. & Carpenter, G. (1987) *Methods Enzymol.* **146,** 49–63.
23. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
24. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1311–1315.
25. Cleveland, D., Fischer, S., Kirschner, M. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252,** 1102–1106.
26. Stoscheck, C. M. & Carpenter, G. (1983) *J. Cell. Biochem.* **23,** 191–202.
27. Segel, I. H. (1975) *Enzyme Kinetics* (Wiley, New York), pp. 100–160.
28. Carpenter, G. & Cohen, S. (1979) *Annu. Rev. Biochem.* **48,** 193–216.
29. Staros, J. V., Cohen, S. & Russo, M. W. (1985) in *Molecular Mechanisms of Transmembrane Signaling*, eds. Cohen, P. & Houslay, M. D. (Elsevier, New York), pp. 253–277.
30. Carpenter, G., Goodman, L. & Shaver, L. (1986) in *Oncogenes and Growth Control*, eds. Kahn, P. & Graf, T. (Springer, Heidelberg), pp. 65–69.
31. Meggio, F., Brunati, A. M. & Pinna, L. A. (1983) *FEBS Lett.* **160,** 203–208.
32. Flockhart, D. A. & Corbin, J. D. (1982) *CRC Crit. Rev. Biochem.* **12,** 133–186.
33. Krebs, E. G. (1986) in *The Enzymes*, ed. Boyer, P. (Academic, New York), 3rd Ed., Vol. 17, pp. 3–18.