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Crystallization and Characterization of Human Chorionic Gonadotropin in Chemically Deglycosylated and Enzymatically Desialylated States†

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ABSTRACT: Crystals suitable for X-ray diffraction studies at moderate resolution have been grown from two forms of human chorionic gonadotropin (hCG): HF-treated hCG and neuraminidase-treated hCG. The enzymatically desialylated form of hCG produced crystals that diffract to 2.8 Å as compared to the HF-treated hCG crystals that diffract to 3.0 Å. Although it was assumed that the high and heterogeneous carbohydrate content of the glycoprotein hormones inhibited their crystallization, this report suggests that it is the negatively charged surface sugars and neither the total carbohydrate content nor its heterogeneity which interferes with crystal formation. Chemical deglycosylation resulted in significantly increased protein degradation during crystal growth. Such peptide bond cleavages were observed to a much lesser extent in the crystals grown from neuraminidase-digested hCG. Sequence analysis of the HF-treated hCG crystals suggested that up to 45% of the molecules within the crystal had an acid-labile peptide bond cleaved. In contrast, the neuraminidase-treated hCG exhibited less than 9% of this type of cleavage. The increase in heterogeneity of the polypeptide chains within both crystals over that existent in the starting proteins was apparently due to changes occurring during crystal growth. The manner in which hCG was treated prior to crystallization was found to be a very important factor in the extent of peptide bond cleavages occurring during crystal growth. HF treatment of glycoproteins may render glycoproteins more susceptible to peptide bond cleavages during crystal growth.

Human chorionic gonadotropin (hCG) is a placental hormone that maintains the steroid secretions of the corpus luteum during early pregnancy. It is a member of a family of glycoprotein hormones that includes three pituitary hormones: follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH). These proteins are dimers formed from the noncovalent association of α subunits common to all members of the family and distinctive β subunits that designate the target specificity of the different hormones (Pierce & Parsons, 1981). Both subunits are heavily glycosylated and bridged by multiple intrachain disulfides. The 92-residue α-chain contains five disulfide bonds and two sites of N-linked glycosylation (Pierce & Parsons, 1981). The β-chain of hCG is 145 residues long and includes six disulfide bridges. It has N-linked carbohydrate at two sites and four sites of O-linked glycosylation (Pierce & Parsons, 1981). Overall, carbohydrate accounts for 30–35% of the 38 900 Da of molecular mass in native hCG (Kessler et al., 1979).

Deglycosylated hormone binds strongly to the receptor, but adenylyl cyclase activation and steroidogenesis are greatly impaired unless the α-chain is glycosylated at its first N-linked site (Matzuk et al., 1989). Recently the receptor has been cloned and its primary structure determined (McFarland et al., 1989; Loosfeit et al., 1989). A lectin-like sequence in the receptor was found, which may be involved in this carbohydrate-mediated signal transduction (McFarland et al., 1989). Neuraminidase-treated hCG retains significant biological activity (Moyle et al., 1975) while HF-treated hCG binds to the receptor but has lost biologic efficacy (Chen et al., 1982;

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Manjunath & Sairam, 1982; Sairam, 1989).

Although the primary structures of these hormones have been known for over 15 years (Pierce & Parsons, 1981), little progress has been made in efforts to determine their three-dimensional structures since they had resisted repeated attempts at crystallization. Carbohydrate heterogeneity was long postulated to be the obstacle to obtaining crystals suitable for X-ray diffraction studies. Recently, under the assumption that it was necessary to remove most of the carbohydrate, Isaacs and colleagues prepared HF-treated hCG and reported obtaining crystals from this preparation that diffract to beyond 3.5-Å Bragg spacings (Harris et al., 1989). However, only partially purified hCG was employed, and no chemical characterization of the starting protein or of the crystals was described. Using conditions similar to those of Harris et al. (1989), we have succeeded in crystallizing two forms of hCG: one with part of the N-linked carbohydrate removed chemically by hydrogen fluoride (HF) treatment and the other with the sialic acid residues removed enzymatically by neuraminidase. We report here on the characterization of the glycoprotein and the crystals obtained after these treatments. We find that half of the carbohydrate content remained even after HF treatment and that crystals of neuraminidase-treated hCG diffract somewhat better than those from HF-treated hCG. Thus, the presence of carbohydrate does not necessarily preclude the crystallization of glycoprotein hormones. However, the charged terminal sialic acid residues, and possible variability in sialylation, can interfere with crystal formation.

Materials and Methods

Materials. hCG was purified from human urine as previously described (Canfield & Morgan, 1973). Ammonium sulfate (reagent grade) was obtained from ICN (Irvine, CA).

Chemical deglycosylation was accomplished by methods similar to those used by Manjunath and Sairam (1982) and by Chen et al. (1982). hCG (100 mg) was dried under vacuum overnight in a desiccator over phosphorus pentoxide and placed in a Teflon reaction vessel; 15 mL of HF was condensed into the vessel and cooled by a dry ice bath. The temperature was raised to 0 °C and maintained at 0 °C for 45 min by immersion in a mixture of water and ice. After 45 min, the HF was slowly evaporated under low vacuum for 20 min, followed by drying under high vacuum for 2 h at room temperature. A total of 10 mL of 0.2 M Tris-HCl at pH 7.5 was then added, and the pH was adjusted to 7.5 with 0.2 M NaOH. The HF-treated hCG was next chromatographed on a Sephadex G-100 column, equilibrated in 0.1 M ammonium bicarbonate, and then lyophilized. The product was dialyzed against water and relyophilized prior to crystallization. Sequence analysis and SDS gel electrophoresis were performed to ascertain that the molecule was intact.

Desialylation. Desialylation of hCG was performed as described earlier with neuraminidase, followed by affinity removal of the enzyme and ion-exchange chromatographic procedures (Rosa et al., 1984). The desialylated material was dialyzed against water and lyophilized. Sequence analysis and gel electrophoresis were employed to assess purity.

Crystallization. The salt-free, dried powders of HF-treated hCG or neuraminidase-treated hCG were dissolved in 30 mM sodium acetate or sodium citrate at pH 4.2. The hanging-drop method was utilized for growing the crystals as described by McPherson (1982) in 24-well tissue culture plates (Flow, Linbro). A 4-μL protein drop was suspended over a 1-mL reservoir solution. The hanging drop was composed of 2 μL of a 40 mg/mL solution of dried protein resuspended in 30 mM sodium acetate or sodium citrate, pH 4.2, and 2 μL of reservoir buffer. The reservoir solution was 32% saturated ammonium sulfate in the acetate or citrate buffer. Lubriseal grease (Fisher) was used to seal the wells. The plates were left at room temperature. Microcrystals grown under identical conditions were used to seed additional wells. This seemed to improve the quality of the crystals produced but did not speed their growth.

Electrophoresis. SDS–polyacrylamide gel electrophoresis was performed according to the procedure described by Laemmli (1970). The sample buffer contained 125 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 0.01% bromophenol blue, and 5% 2-mercaptoethanol. The gel was silver stained according to Wray et al. (1981).

Amino Acid Sequence Analysis. N-Terminal sequence analysis was accomplished on an Applied Biosystems Inc. Model 470A sequencer equipped with an on-line PTH analyzer, Model 120A.

Carbohydrate Analysis. Carbohydrate analyses were performed by F. Perini according to his published procedure (Perini & Peters, 1982).

Reverse-Phase HPLC. The HF-treated hCG crystals (approximately 15 μg) or the neuraminidase-treated hCG crystals (approximately 100 μg) were washed in 100 μL of acetate–sulfate buffer to remove the mother liquor and then diluted with 0.1% TFA to a total volume of approximately 250 μL. This solution was next desalted by reverse-phase HPLC on a Browlee RP-300 column, 2.1 mm × 30 mm (Applied Biosystems Inc., Foster City, CA). The flow rate was 0.5 mL/min; buffer A was 0.1% TFA; buffer B was 0.1 TFA in acetonitrile. The gradient was held at 0% buffer B for 5 min and then increased to 80% buffer B in 20 min. The protein of both crystal species eluted as several incompletely separated peaks, and this entire region was pooled and dried. The pooled desalted protein was subjected to SDS–PAGE and N-terminal sequencing. In order to further document the differences between HF-treated hCG starting material and its crystalline form, portions of each (approximately 20 and 50 μg, respectively) were subjected to reverse-phase HPLC as above but with a more gradual gradient of 1%/min increase in buffer B content. An early eluting peak in the pattern of the HF-treated hCG crystal was analyzed by N-terminal sequencing.

Crystal Characterization. Morphological examination and manipulation of crystals were carried out with a Zeiss stereomicroscope. Crystals were mounted and sealed in thin-walled glass capillaries for diffraction experiments. X-ray diffraction patterns were recorded on CEA Reflex 25 film on a Huber precession camera using radiation from a rotating copper anode in a Rigaku RU-200 X-ray generator operated at 50 kV and 100 mA. Lattice constants were obtained from precession photographs by a least-squares fitting to measurements (29–39) of lattice line positions made on a Supper film-measuring device. The error analysis did not take into account possible systematic errors such as those from possible film shrinkage or instrumental imprecision.

Results and Discussion

Both HF-treated hCG and neuraminidase-treated hCG crystallized under the same conditions (see Materials and Methods), whereas native hCG did not crystallize under these conditions. In wells of identical conditions, 35% of the HF-treated hCG-containing wells produced crystals whereas 12% of the neuraminidase-treated hCG-containing wells developed crystals. While HF-treated material tended to form large crystals within 4–6 weeks, the neuraminidase-treated hormone required 8–12 weeks for growth, but the resulting crystals were of somewhat higher quality than those obtained after HF

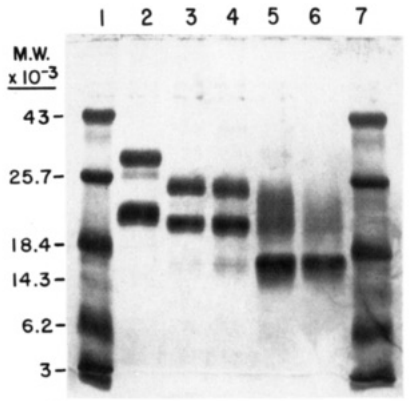

FIGURE 1: A silver-stained 15% reduced SDS–polyacrylamide gel of hCG and its crystallized forms: lane 1, molecular weight markers; lane 2, standard urinary hCG (the upper band is the β subunit while the lower is the α subunit); lane 3, neuraminidase-treated hCG; lane 4, neuraminidase-treated hCG crystal; lane 5, HF-treated hCG; lane 6, HF-treated hCG crystal; lane 7, molecular weight markers.

treatment of the hormone. The X-ray diffraction from still photographs of the HF-hCG crystals extended to Bragg spacings of 3.0 Å whereas the neuraminidase-treated hCG crystals diffracted to 2.8 Å.

Chemical characterization of the two crystallizable forms of hCG produced unexpected findings. SDS gel electrophoretic analysis (Figure 1) of the HF-treated hCG starting material (lane 5) and of HF-treated hCG crystals (lane 6) demonstrated that the β subunit migrated as a much wider band than that observed with the β present in native hCG (lane 2), reflecting an increase in size heterogeneity after the chemical treatment. The gel electrophoretic pattern of the HF-treated hCG in the crystal was very similar to that of the starting material. This absence of selectivity of a particular molecular species by the crystallization process was further substantiated by the results of N-terminal sequence analyses (Table I). The HF-treated crystal displayed the same hCGα amino-terminal heterogeneity (see Table Ia) that was present in the HF-treated hCG starting material and reported in standard urinary hCG (Birken & Canfield, 1980). All standard preparations of urinary hCG also contain 1–3% (molar basis) of β starting at Leu position 45 (Birken, unpublished data). The HF-treated hCG crystal appeared to contain peptide bond cleavages (45% on a molar basis) in the β subunit at the acid-labile Asp–Pro position (112–113) and to a lesser degree at Asp–Ser (117–118) (20% on a molar basis; see Table Ib). These bonds are at the beginning of the unique β C-terminal region of hCG. CD studies suggest that this portion of the molecule is likely to have a high content of β-turns, suggesting some degree of ordered structure (Puett et al., 1982). These cleavages apparently took place during crystal growth since they were not present in the starting material. These cleavages within the HF-treated hCG crystals, although not very apparent on the SDS gel electrophoresis (see Figure 1), were further documented by the isolation of the smaller fragments, β113–145 and β118–145, on reverse-phase HPLC. The yields were consistent with the estimates of peptide bond cleavages derived from sequence analysis of the HF-treated hCG crystals (Table Ib).

The neuraminidase-treated hCG (Table II) within its crystal exhibited only 7% cleavage, on a molar basis, of the acid-labile β 112–113 peptide bond. A cleavage was also observed at the 47–48 bond position. This latter bond is also frequently cleaved, to this extent, in native hCG (Birken, unpublished data). It is not clear as to why this bond cleavage was not detectable in the neuraminidase-treated hCG starting material. On the basis of the relative quantity of peptide bond cleavages, on a molar basis, the neuraminidase-treated hCG crystals were much less heterogeneous than the HF-treated hCG crystals.

Table I

(a) N-Terminal Sequence of HF-hCG (Starting Material)

		1	2	3	4	5
α subunit	27%[a]	Ala-	Pro-	Asp-	Val-	Gln-
		3	4	5	6	7
	6%	Asp-	Val-	Gln-	Asp-	(Cys)-
		4	5	6	7	8
	13%	Val-	Gln-	Asp-	(Cys)-	Pro-
		1	2	3	4	5
β subunit	52%	Ser-	Lys-	Glu-	Pro-	Leu-
		45	46	47	48	49
	2%	Leu-	Gln-	Gly-	Val-	Leu-

(b) N-Terminal Sequence of HF-hCG Crystal

		1	2	3	4	5
α subunit	27%[b]	Ala-	Pro-	Asp-	Val-	Gln-
		3	4	5	6	7
	9%	Asp-	Val-	Gln-	Asp-	(Cys)-
		4	5	6	7	8
	9%	Val-	Gln-	Asp-	(Cys)-	Pro-
		1	2	3	4	5
β subunit	38%	Ser-	Lys-	Glu-	Pro-	Leu-
		113	114	115	116	117
	17%[c]	Pro-	Arg-	Phe-	Gln-	Asp-

[a] Percentage of each sequence is based on yield in picomoles of PTH-amino acids in the first cycle corrected for lag: Ala (476), Asp (111), Val (229), Ser (929), and Leu (30). Estimation of Ser is based on recovery of PTH-Ser and its dehydro adduct. Yields of PTH-amino acids in cycles 2–10 are consistent with sequences shown and with their relative amounts. [b] Percentage of each sequence is based on yield in picomoles of PTH-amino acids in the first cycle corrected for lag: Ala (16), Asp (5), Val (5), Ser (22), and Pro (10). [c] A small amount of cleavage at Asp117–Ser118 can be detected by the presence of Lys at cycle 5 and Ala at cycle 6. The quantities of these PTH-amino acids suggest that, on a molar basis, the amount of the Ser118 peptide is approximately 50% that of the Pro113 peptide.

Both HF-treated hCG and neuraminidase-treated hCG crystallize as stout bipyramids that in each case typically reach a size of 0.4 × 0.4 × 0.6 mm. Faces are usually somewhat rounded, but an underlying hexagonal shape can often be discerned. Diffraction patterns from both forms of hCG can be indexed on a hexagonal lattice, which has unit cell parameters of $a = b = 88.74 \pm 0.02$ Å and $c = 177.10 \pm 0.08$ Å in the case of HF-treated hCG [statistically identical with that of Harris et al. (1989)] and $a = b = 88.57 \pm 0.04$ Å and $c = 177.24 \pm 0.06$ Å in the case of neuraminidase-treated hCG. Precession photographs of the $hk0$ nets show 6-mm symmetry, and the $h0l$ nets (Figure 2) show 2-mm symmetry with absences of $00l$ except at $l = 6n$. Upper levels have mirror symmetry throughout, perpendicular to the c axis. Thus, the space group is $P6_122$ or its enantiomorph $P6_522$, and both crystals appear to be isomorphous with those reported by Harris et al. (1989). The patterns of diffracted intensities from the two forms are not distinguishable to the eye. This suggests that sugars removed by HF treatment might be rather disordered in neuraminidase-treated hCG and that the proteolytic nicks in HF-treated hCG may not have appreciably disrupted the ordered protein structure.

Taking 34.6 kDa as the molecular mass of neuraminidase-treated hCG [10.2-kDa α-chains, 15.5-kDa β-chains, and 8.9-kDa carbohydrate (25.7% w/w, Table III)] and assuming typical partial specific volumes of 0.73 and 0.65 cm^3/g for the polypeptide and carbohydrate components, respectively, the solvent content is 59% with one molecule per asymmetric unit. The solvent content would be impossibly low

Table II

(a) N-Terminal Sequence of Neuraminidase-Treated hCG (Starting Material)

		1	2	3	4	5
α subunit	36%[a]	Ala-	Pro-	Asp-	Val-	Gln-
		3	4	5	6	7
	5%	Asp-	Val-	Gln-	Asp-	(Cys)-
		4	5	6	7	8
	16%	Val-	Gln-	Asp-	(Cys)-	Pro-
		1	2	3	4	5
β subunit	42%	Ser-	Lys-	Glu-	Pro-	Leu-
		45	46	47	48	49
	1%	Leu-	Gln-	Gly-	Val-	Leu-

(b) N-Terminal Sequence of Neuraminidase-Treated hCG Crystal

		1	2	3	4	5
α subunit	31%[b]	Ala-	Pro-	Asp-	Val-	Gln-
		3	4	5	6	7
	6%	Asp-	Val-	Gln-	Asp-	(Cys)-
		4	5	6	7	8
	11%	Val-	Gln-	Asp-	(Cys)-	Pro-
		1	2	3	4	5
β subunit	44%	Ser-	Lys-	Glu-	Pro-	Leu-
		45	46	47	48	49
	1%	Leu-	Gln-	Gly-	Val-	Leu-
		48	49	50	51	52
	4%[c]	Val-	Leu-	Pro-	Ala-	Leu-
		113	114	115	116	117
	3%	Pro-	Arg-	Phe-	Gln-	Asp-

[a] Percentage of each sequence is based on yield in picomoles of PTH-amino acids in the first cycle corrected for lag: Ala (166), Asp (22), Val (76), Ser (195), and Leu (4). Estimation of Ser is based on recovery of PTH-Ser and its dehydro adducts. Yields of PTH-amino acids in cycles 2–10 are consistent with the sequences shown and with their relative amounts. [b] Percentage of each sequence is based on yield in picomoles of PTH-amino acids in the first cycle (with the exception of Val48) corrected for lag: Ala (544), Asp (108), Val (189), Ser (768), Leu (18), and Pro (61). Estimation of Ser is based on recovery of PTH-Ser and its dehydro adduct. Yields of PTH-amino acids in cycles 2–10 are consistent with the sequences shown and with their relative amounts. [c] Percentage of the Val48 peptide is estimated from the picomoles of PTH-Leu in cycle 2 (70). This bond cleavage is frequently observed in standard pregnancy urine hCG.

with two molecules per asymmetric unit as has been suggested by Harris et al. (1989) on the basis of density measurements. An initial 0.5° precession photograph from a neuraminidase-treated hCG crystal recorded for 1 h showed diffraction to beyond 2.8-Å Bragg spacings and a realistic limit of 3.0 Å for measurable data. This crystal survived a total exposure of 40 h, albeit with some eventual decrease in the limit of diffraction. Thus, the neuraminidase-treated hCG crystals are suitable for structure analysis at intermediate resolution.

The HF-treated hCG, which was successfully crystallized, contained approximately 17% (w/w) of carbohydrate as compared to native hCG, which has approximately 35% carbohydrate by weight (Kessler et al., 1979). Carbohydrate analysis (see Table III) showed that at least half of the

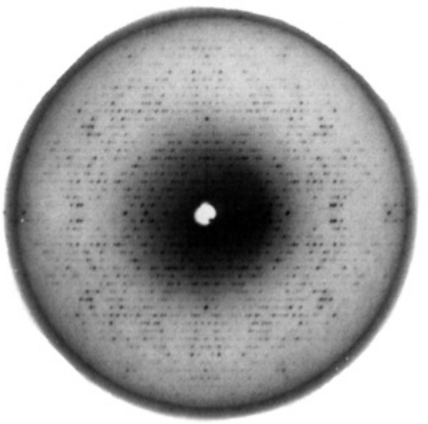

FIGURE 2: An X-ray diffraction pattern produced from the neuraminidase-treated hCG crystals. This pattern is of the *h0l* zone recorded with Ni-filtered Cu Kα radiation during an 18-h exposure from a 50-kV, 100-mA source with a 15° precession angle and a 75-mm crystal-to-film distance. Reflections at the perimeter correspond to 3.0-Å Bragg spacings.

mannose content (N-linked sugars) of untreated hCG was still present as was the full content of O-linked sugars other than sialic acid. Under the conditions employed, less sugar was removed than reported by Manjunath and Sairam (1982) or Chen et al. (1982). This mild treatment may have minimized conformational alterations, a potential problem implied by recent studies of HF-treated glycoproteins using immunological methods (Keutmann et al., 1985).

Recently, Sairam and colleagues (Sairam, 1989) showed that whereas both hCG and neuraminidase-treated hCG are iodinated only in the α subunit, HF-treated hCG could be iodinated in both α and β subunits, suggesting a possible conformational change in the β subunit of HF-treated hCG. Sairam (1989) also found that HF treatment exposes new antigenic determinants not present in untreated native hCG. In addition, recent studies by Merz (1988) showed that deglycosylated hCG was more susceptible to chymotryptic cleavage, adopted a greater degree of ordered structure in helicogenic solvents, and exhibited a much higher subunit dissociation rate at pH 3 as compared to native hCG. All of these findings, especially our observations of the increased peptide bond fragility of HF-treated hCG, discourage use of this harsh chemical treatment.

The high content of the remaining carbohydrate on the HF-treated hCG as well as its heterogeneity (see Table III and Figure 1) prompted us to explore the hypothesis that total carbohydrate content was not the reason for the failure of native hCG to crystallize but that it might be related to the removal of the negatively charged sialic acids from the hormone surface. The enzymatically desialylated hCG was more homogeneous by SDS electrophoresis (Figure 1) and by sequencing analysis (Tables I and II), and it successfully produced crystals that diffract to a higher resolution than those generated from HF-treated hCG. In addition, enzymatically desialylated hCG is known to retain significant biological

Table III: Carbohydrate Analysis[a]

	glycoprotein analyzed[b] (nmol)	$GlcNH_2$[c] (nmol)	$GalNH_2$[d] (nmol)	Gal[e] (nmol)	Man[f] (nmol)	sialic acid[g] (nmol)	% carbohydrate
hCG	1.77	27.3 (15.5)[h]	7.3 (4.1)	17.9 (10.1)	21.2 (12)	27.6	33.9
As-hCG[i]	1.66	25.4 (15.3)	7.9 (4.7)	20.6 (12.4)	19.9 (12)		25.7
HF-hCG	1.65	12.9 (7.8)	8.15 (4.9)	9.1 (5.5)	9.9 (6)	1.96	17.1

[a] Not including fucose. [b] Nanomoles of glycoprotein analyzed was calculated from nanomoles of mannose (12 nmol of mannose/mol of hCG or As-hCG and 6 nmol of mannose/mol of HF-hCG). [c] $GlcNH_2$ = glucosamine. [d] $GalNH_2$ = galactosamine. [e] Gal = galactose. [f] Man = mannose. [g] Sialic acid = *N*-acetylneuraminic acid. [h] Parentheses contain nanomoles normalized to 12 nmol of mannose for hCG and As-hCG and 6 nmol of mannose for HF-hCG. [i] Neuraminidase-treated hCG.

activity, while HF-treated hCG is devoid of such activity (Moyle et al., 1975; Manjunath & Sairam, 1982; Chen et al., 1982). The procedure of removal of the terminal charged sugar should also apply to the crystallization of the other homologous glycoprotein hormones. However, since some of the homologous pituitary glycoprotein hormones are partially sulfated as well as sialylated, utilizing recombinant hormones that are expressed in systems that do not add sulfate or sialic acid may provide the best material for crystal growth. Such materials would require no chemical modifications and have the potential to produce crystals that might diffract X-rays to higher resolution than those described here. Such studies are now in progress.

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Identification of the Site on Calcineurin Phosphorylated by Ca^{2+}/CaM-Dependent Kinase II: Modification of the CaM-Binding Domain

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ABSTRACT: The catalytic subunit of the Ca^{2+}/calmodulin- (CaM) dependent phosphoprotein phosphatase calcineurin (CN) was phosphorylated by an activated form of Ca^{2+}/CaM-dependent protein kinase II (CaM-kinase II) incorporating approximately 1 mol of phosphoryl group/mol of catalytic subunit, in agreement with a value previously reported (Hashimoto et al., 1988). Cyanogen bromide cleavage of radiolabeled CN followed by peptide fractionation using reverse-phase high-performance liquid chromatography yielded a single labeled peptide that contained a phosphoserine residue. Microsequencing of the peptide allowed both the determination of the cleavage cycle that released [^{32}P]phosphoserine and the identity of amino acids adjacent to it. Comparison of this sequence with the sequences of methionyl peptides deduced from the cDNA structure of CN (Kincaid et al., 1988) allowed the phosphorylated serine to be uniquely identified. Interestingly, the phosphoserine exists in the sequence Met-Ala-Arg-Val-Phe-Ser(P)-Val-Leu-Arg-Glu, part of which lies within the putative CaM-binding site. The phosphorylated serine residue was resistant to autocatalytic dephosphorylation, yet the slow rate of hydrolysis could be powerfully stimulated by effectors of CN phosphatase activity. The mechanism of dephosphorylation may be intramolecular since the initial rate was the same at phosphoCN concentrations of 2.5–250 nM.

Protein phosphorylation/dephosphorylation is involved in a variety of neuronal signal transduction mechanisms (Hemmings et al., 1989). When effector molecules interact with a specific cell surface receptor, they, through a direct interaction or second messenger release, alter the activity of multifunctional protein kinases and phosphoprotein phosphatases. These enzymes mediate signal transduction through

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