Influence of Myristoylation, Phosphorylation, and Deamidation on the Structural Behavior of the N-Terminus of the Catalytic Subunit of CAMP-Dependent Protein Kinase†

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ABSTRACT: A number of isoenzymes of the catalytic subunit of cAMP-dependent protein kinase arise through posttranslational modifications of the enzyme outside the catalytic domain; the biological significance of these is not yet fully clear. A clustering of sites for such modification exists at the N-terminus of the protein, where myristoylation (of Gly1), phosphorylation (at Ser10), and deamidation of Asn2 have been observed. As the first two are known to govern membrane binding and thus subcellular compartmentalization in some proteins, it was of interest to see whether the local structure of the N-terminus was being influenced by one or more of these modifications. A series of synthetic peptides mimicing the 16 N-terminal residues of the catalytic subunit Cα was produced covering the full range of possible modifications, singly and in combination, and tested for possible effects on local structure by measuring the circular dichroism under varying polarity. It was found that myristoylation and phosphorylation modify the structure in this region in opposite ways and in a manner designed to amplify the action of a potential myristoyl/electrostatic switch. To what extent deamidation of Asn2 may oppose a potential membrane binding is unknown. Deamidation, however, had no effect on the structure of the peptide either alone or in combination with acylation and/or phosphorylation, suggesting that the change of the nuclear/cytoplasmic disribution in cells caused by deamidation [Pepperkok et al. (2000) *J. Cell Biol. 148*, 715−726] is due to a more complex signaling mechanism. The structural implications of the data are discussed.

The N-terminus of the catalytic subunit major isoenzymes Cα and C*β* (*1*, *2*) of the cyclic AMP-dependent protein kinase (PKA,[1] EC 2.7.1.37) carries co- and posttranslational modifications of as yet poorly understood functional significance. First, Gly1 is acylated by myristic acid (*3*). Second, the encoded Asn2 in both isoforms Cα and C*β* is partly deamidated in vivo leading to Asp2 and iso(*β*)Asp2 (*4*). A third modification close to the N-terminus is the autophosphorylation of Ser10 in vitro (*5*−*7*) in myristylated enzyme apparently in conjunction with deamidation of Asn2.

Any view of the functional significance of the N-terminus of the C-subunit has so far been overshadowed by the observation, among others, that recombinant enzyme lacking the first 14 residues becomes fully assembled— at least in *Escherichia coli*—and does not need the N-terminus for catalysis (*8*, *9*). Therefore, it can be assumed in view of its various in vivo modifications that the N-terminus of the native enzyme may contribute to the fine-tuning of enzyme assembly and/or function in the eukaryotic cell. Although the C-subunit is soluble it possesses several properties of proteins known to interact with membranes through myristoyl- and electrostatically mediated forces (*10*−*12*). A number of basic residues are located around the fatty acid moiety, and there is a potential for electrostatic regulation through introduction of negative charges close to the binding site (*13*)—in the C-subunit by deamidation of Asn2 and phosphorylation of Ser10. The membrane binding potential of the native N-terminus as well as the involvement of the myristyl moiety and of the basic residues Lys7 and Lys8 have in principle been demonstrated with a chimeric src-kinase construct in which the src N-terminus was replaced by that of the PKA C-subunit (*14*). Data by Steinberg (*15*) indicate that the assembly of native C-subunit in the eukaryotic cell starts with a membrane-bound step for which the cotranslational myristylation may be required. Whether partial deamidation of native enzyme at Asn2 requires membrane binding is not known.

In the assembled catalytic subunit the myristyl moiety binds to a hydrophobic pocket on the surface of the large lobe (*16*, *17*). In the case of myristylated proteins, Arf1 and recoverin, the binding of certain ligands appears to mediate allosterically a reorientation of the myristyl moiety leading to an altered membrane binding behavior of these proteins (*18*, *19*). Recently, the binding of myristylated catalytic subunit to liposomes on interaction with regulatory subunit

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[1] Abbreviations: CD, circular dichroism; ES-MS, electrospray mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; LC-MS, liquid chromatography−mass spectrometry; MALDI, matrix-assisted laser desorption ionization; MS/M, standem-mass spectrometry; PKA, cAMP-dependent protein kinase; RP-HPLC, reversed-phase HPLC; TFA, trifluoroacetic acid; TFE, trifluoroethanol.

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Table 1: Peptides Investigated in This Study. the Analytical Data Refer to the Measured Masses and the RP-HPLC as Described under Experimental Procedures

peptide						mass (Da)	retention time on RP-HPLC (min)
N16		G*N*AAA	AKKGS	EQESV	K	1574.6	12.97
N16P		G*N*AAA	AKKG**S$_p$**	EQESV	K	1654.7	12.31
N16M	Myr-	G*N*AAA	AKKGS	EQESV	K	1784.9	36.62
N16MP	Myr-	G*N*AAA	AKKG**S$_p$**	EQESV	K	1864.8	37.24
ID16		G^{iso}*D*AAA	AKKGS	EQESV	K	1575.6	13.22
ID16M	Myr-	G^{iso}*D*AAA	AKKGS	EQESV	K	1785.9	37.22
D16		G*D*AAA	AKKGS	EQESV	K	1575.6	13.21
D16P		G*D*AAA	AKKG**S$_p$**	EQESV	K	1655.5	12.48
D16M	Myr-	G*D*AAA	AKKGS	EQESV	K	1785.9	37.38
D16MP	Myr-	G*D*AAA	AKKG**S$_p$**	EQESV	K	1865.8	37.68
C2	Ac-	G*D*AAA	AKKGS	EQESV	K	1617.9	14.11
C2P	Ac-	G*D*AAA	AKKG**S$_p$**	EQESV	K	1697.8	13.32
C6	C_6-	G*D*AAA	AKKGS	EQESV	K	1673.9	19.30
C6P	C_6-	G*D*AAA	AKKG**S$_p$**	EQESV	K	1753.8	18.95
C10	C_{10}-	G*D*AAA	AKKGS	EQESV	K	1729.9	28.61
C10P	C_{10}-	G*D*AAA	AKKG**S$_p$**	EQESV	K	1809.8	28.49

type II was described and proposed as occurring through a similar mechanism (*20*). Recent data show that the N-terminus helps to fine-tune the enzyme distribution within the cell in vivo. Upon microinjection into the cytoplasm of cultured cells in the presence of cAMP the relative nuclear concentration of the deamidated form of native C-subunit was decreased when compared with the encoded (Asn2) form (*21*), thus indicating that a cell can discriminate by an unknown mechanism between the forms of the free C-subunit on the basis of specific properties of the N-terminus. These considerations indicate that the structural features of the C-subunit N-terminus must be viewed under at least two aspects: its role in the enzyme structure and its membrane binding capacity.

It is not yet possible to isolate the individual subpopulations of the enzyme modified in various combinations at the N-terminus for study; nor is it possible to obtain the myristylated deamidated forms either as recombinant protein or by targeted deamidation of Asn2. To learn more about the mutual influence of various modifications, myristoylation, deamidation, and phosphorylation, a set of hexadecapeptides based on the Cα gene product was synthesized with different combinations of these modifications including variations of the chain length of the fatty acid. As deamidation in vivo could lead to the formation of iso(β)Asp, peptides with this residue were added to the set. The structural behavior of these peptides under the influence of solvent polarity (which mimics possible interactions with other proteins or membranes) was studied by CD-spectroscopy. The data show that the combination of the different modifications influences the peptide structure in specific ways potentially relevant for the in vivo assembly and/or fine-tuning of the enzyme.

EXPERIMENTAL PROCEDURES

Peptide Synthesis and Purification. The peptides were synthesized by standard Fmoc-methodology on an Applied Biosystems ABI 433 automated synthesizer (Applied Biosystems, Weiterstadt, Germany) employing HBTU [2-(1H−benzotriazole-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate] activation. For peptide acid synthesis preloaded Fmoc-Lys(Boc)-TCP-resin (amino-methylpolystyrol with trityl-linker, 0.55 mmol/g, PepChem, Tübingen, Germany) was used.

Fmoc-Ser(^{t}Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Glu(O^{t}Bu)-OH, Fmoc-Ala-OH, Fmoc-Val-OH, and Fmoc-Gly-OH were used as amino acid building blocks. Fmoc-Asn(Trt)-OH, Fmoc-Asp(O^{t}Bu)-OH, or Fmoc-Asp-(OH)-O^{t}Bu (for the synthesis of isoAsp-peptides) were used as building blocks in position 2. Fmoc-Ser[PO(OBzl)OH]-OH (*22*), was used as a building block for phosphopeptide synthesis. We observed no significant β-elimination of the phosphoryl group upon exposure to base during peptide synthesis. Ac-Gly-OH was used to introduce N-terminal acetyl group, *N*-decanoyl-glycine and Myr-Gly-OH (Nova-Biochem, Bad Soden, Germany) were used to introduce the N-terminal C_{10}-fatty acid and the N-terminal myristic acid, respectively. The synthesis of C_6-Gly-OH is described below.

Cleavage of the peptides was performed with trifluoro acetic acid (TFA)/triisopropylsilane/water (90:8:2, v:v:v) for 2 h at room temperature. Peptides were purified by quantitative reversed-phase HPLC (Kromasil, C_{18}, 5 μm, 100 Å) and analyzed by reversed-phase HPLC (Kromasil, C_{18}, 5 μm, 100 Å, 0.75 mL/min, 1 min, isocratic 0.1% TFA in water, then in 40 min linear gradient to 60% acetonitrile/0.085% TFA in water), MALDI-MS, and electrospray MS (ES-MS). Prior to the preparation of stock solutions, the peptides were lyophilized from water several times.

Sequences, abbreviations and analytical data of the peptides investigated in this study are shown in Table 1.

Synthesis of C_6-Gly-OH. Fmoc-Gly-TCP-resin was deprotected following standard procedures. Ten equivalents (calculated on the base of the amount of glycine on the resin) of capronic acid was solved in DMF, and 10.5 equiv of diisopropylcarbodiimide and 10 equiv of 1-hydroxy-benzotriazole (HOBt) were added, and the mixture was stirred for 1 h at room temperature. The preactivated capronic acid was added to the resin. After gently shaking for 2 h at room temperature, the resin was washed with DMF, chloroform, and diethyl ether and dried by air suction. The C_6-glycine was cleaved from the resin with 95% TFA/5% water (1 h). After removal of the solvent under reduced pressure, the oily residue was dissolved in water and lyophylized several times. The resulting white powder (M = 173.1 g/mol) was readily soluble in DMF and was used for solid-phase peptide synthesis as described above.

	G	(D) N	A	A	A	A	K	K	G	S	E	Q	E	S	V	K
P_α	.89 (1.0)	1.02 (1.10)	1.10 (1.17)	1.27 (1.34)	1.37	1.32	1.15	1.01	1.03	1.02	1.10	1.14	1.20	1.12	1.13	1.0
P_β	.82	.82	.82	.84	.81	.79	.77	.76	.66	.74	.66	.66	.85	.93	.88	1.0

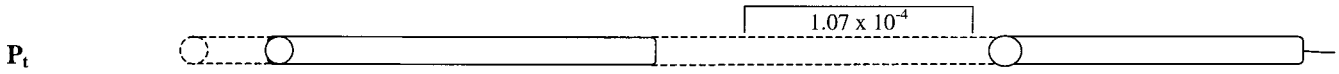

FIGURE 1: Secondary structure propensity for residues 1−16 of the PKA catalytic subunit Cα in the encoded (Asn2) and the deamidated (Asp2) form [according to Chou and Fasman (*24*)]. Cylinders denote areas with significant helical propensity. The region G9-Q12 has a high turn potential that could interrupt the helix. Deamidation would serve to extend the helix in the N-terminal direction,

Cicular Dichroism Measurements. Stock solutions were prepared (500 μg/mL for myristoylated peptides, 5 mg/mL for all other derivatives) by dissolving the peptides in doubly distilled water. Exact concentrations of the peptide stock solutions were estimated by quantitative amino acid analysis. Far-UV (190−240 nm) circular dichroism spectra were obtained using a Jasco J-710 automatic recording spectropolarimeter calibrated with 0.05% (w:v) β-androsterone in spectral grade dioxane as a standard. Samples were measured in a 1 mm dichroically neutral quartz cuvette at a sensitivity of 10 mdeg, a time constant of 2 s, and a scan speed of 5 nm/min. Spectra presented are a 4-fold signal average with identically measured and signal-averaged baseline subtraction, converted from millidegrees to mean residue elipticity [Θ_{MR}] for use in curve fitting and secondary structure analysis.

Peptide concentrations were 100 μg/mL. To show the absence of aggregation, measurements were also done before and after ultracentifugation and at higher and lower peptide concentrations (0.5 mg/mL with 0.2 mm path length and 10 μg/mL with 1 cm path length).

Estimation of Secondary Structure Contents. The content of secondary structure of the peptides was calculated from the far UV CD spectra using the program PEPFIT (*23*).

RESULTS

An analysis was first carried out on the potential for forming secondary structure elements of the N-terminal peptide (residues 1−16) of the PKA catalytic subunit Cα in the encoded (Asn2) and the deamidated (Asp2) form with the use of folding algorithms according to Chou and Fasman (*24*). With this method the influence of myristoylation and phosphorylation cannot, of course, be factored in. The region corresponding to Ala3 to Lys 7 has a high helix forming potential in the encoded ($P_\alpha = 1.24$ on average) and the deamidated ($P_\alpha = 1.27$ on average) form (Figure 1). The part starting with Glu11 through the following residues has a helix forming potential as well ($P_\alpha = 1.14$ on average). A turn forming potential is observed from Gly9 to Gln12 ($P_\tau = 1.07 \times 10^{-4}$) i.e., at the potential helix capping motif (residues 11 and 12) near Ser10, the site of in vitro autophosphorylation (*5*). If instead of Ser10 a negatively charged residue such as a Glu10 was calculated in order to mimic the negative charge of phosphorylation the turn forming potential was absent.

The actual content of ordered secondary structure of the different peptides was investigated by CD spectroscopy

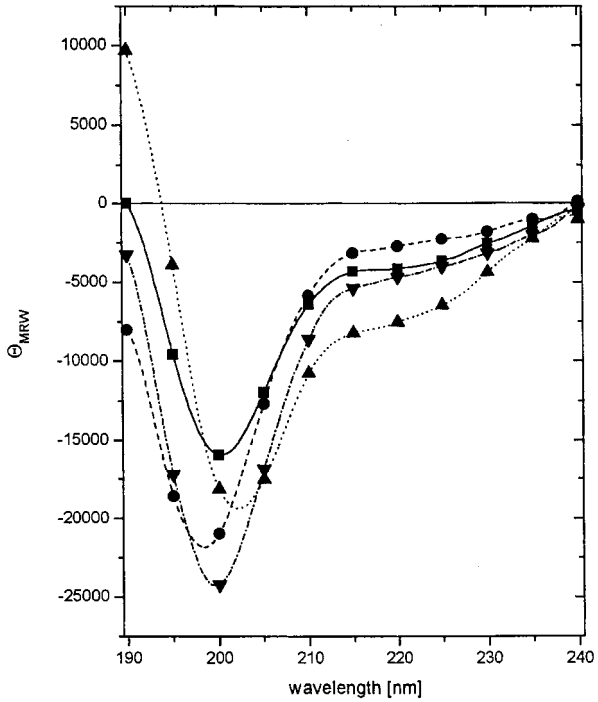

FIGURE 2: Changes in the far UV CD spectrum of the 16-residue N-terminal PKA peptide (N16) due to various posttranslational modifications occurring in vivo. A representative set (40% TFE) is shown. N16 (■); N16P (●); N16M (▲); N16MP (▼).

including a determination of the solvent polarity dependent behavior of the structures. In earlier experiments on the truncated sequence a-Gly-Xaa-Ala-Ala-Ala-Ala-Lys-OH we could observe aggregation of the peptides in solution, especially in the case of a = myristic acid and Xaa = Asn (unpublished data). The 16mer peptides investigated in this study exhibited no aggregation at all. The peptides were readily soluble in distilled water up to a concentration of 1 mg/mL independent of the modifications. All CD-spectra were independent of concentration in the range between 0.1 and 0.5 mM.

Asn2-Peptides. The CD spectra of the unmodified Asn2-peptide (N16, Table 1) in water was characteristic of a peptide consisting of a combination of coil and turn structures (*23*). The small negative shoulder between 215 and 225 nm, the minimum at 197 nm and the negative sign of the band below 195 nm are typical for such peptides (Figure 2). Estimation of the contents of secondary structure by comparison with model peptides confirmed this impression (Table 2). By decreasing the solvent polarity (with an increasing

Table 2: Contents of Secondary Structures of the Different Peptides in Dependence of Solvent Polarity as Estimated with the Program PEPFIT (*23*)[a]

%-TFE	helical	coil	turn-	%-TFE	helical	coil	turn-
N16				D16			
0	0	65	35	0	0	63	37
20	4	59	37	20	2	55	43
40	10	50	40	40	9	48	43
60	15	40	45	60	15	35	50
80	26	38	36	80	27	30	43
100	50	34	16	100	60	25	15
N16P				D16P			
0	3	75	22	0	3	75	22
20	4	72	24	20	4	68	28
40	8	68	24	40	6	65	29
60	10	61	29	60	8	58	34
80	18	50	32	80	18	47	35
100	38	40	22	100	34	35	31
N16M				D16M			
0	4	55	41	0	5	50	45
20	16	55	29	20	18	50	32
40	23	51	26	40	25	47	28
60	33	50	17	60	35	45	20
80	46	35	19	80	48	33	19
N16MP				D16MP			
0	7	81	12	0	8	84	8
20	11	71	18	20	11	71	18
40	11	62	27	40	12	58	30
60	19	58	23	60	21	54	25
80	38	42	20	80	40	40	20
ID16				ID16M			
0	4	57	39	0	5	50	45
20	8	52	40	20	16	55	29
40	11	42	47	40	24	46	28
60	15	34	51	60	34	47	19
80	26	38	36	80	48	33	19
100	60	25	15	100			

[a] (−) Not estimated.

concentration of TFE), an increasing content of helical structure could be induced in this peptide.

Phosphorylation of Ser10 (N16P) led to a more unordered structure of the peptide compared to the unmodified derivative, as seen by the increased amplitude of the negative band below 200 nm (Figure 2). On titration with TFE, higher contents of helical structure could still be induced but these were always lower than those observed for the unmodified peptide (Table 2).

In contrast to the peptides with a free amino terminus, peptides with an N-myristoylated terminus had a higher content of ordered secondary structure, especially of helical structure, which could be observed both by a qualitative inspection of the spectra (Figure 2) and by estimation of the secondary structure content (Table 2).

Where both modifications were present, e.g., phosphorylation and N-myristoylation, over the whole range of solvent polarity the peptide structure was intermediate between the structures observed when only one of these modifications was present (Table 2).

Asp2-Peptides. The Asp2 peptides showed, independent from the other modifications, the same structural behavior as described for the Asn2-peptides. There was no significant difference in conformation between the Asn2 and the Asp2-peptides (Table 2).

IsoAsp2-Peptides. The isoAsp2-peptides were investigated either with free (ID16) or myristoylated N-terminus (ID16M). The nonmyristoylated peptide formed a slightly higher content of ordered secondary structure in aqueous solution compared to the Asn2 or Asp2 peptides. This difference could be observed up to a concentration of about 60% TFE. Further decrease of solvent polarity negated this effect completely (Table 2). When the N-terminus was myristoylated no significant difference between the isoAsp containing peptide and the corresponding Asn2 and Asp2-peptides could be seen over the whole range of solvent polarity.

Influence of the Chain Length of the N-Acyl Group. To investigate the influence of the chain length of the N-terminal acyl group we compared the CD-spectra of peptides D16 (free N-terminus), C2 (C2:0-fatty acid), C6 (C6:0), C10 (C10:0) and D16M (myristic acid, C14:0-fatty acid) in both the unphosphorylated and the phosphorylated form.

Acylation of the N-terminus of the unphosphorylated peptides led to an increased content of helical structure over the whole range of solvent polarity (Figure 3). This behavior could be observed even with an N-terminal acetyl group but increased linearily with growing chain length of the acyl groups. With myristic acid as the blocking group, the content of random coil structure decreased more slowly with decreasing solvent polarity than for the other acyl end groups. On the other hand, the content of turn structures did fall more rapidly than for the other derivatives.

Acylation of the phosphorylated peptides led to similar results (Figure 4). In these peptides as well, a longer fatty acid led to a higher content of helical structure, but again this was always lower than for the unphosphorylated peptides. The content of random coil structure decreased with decreasing solvent polarity independent of the chain length.

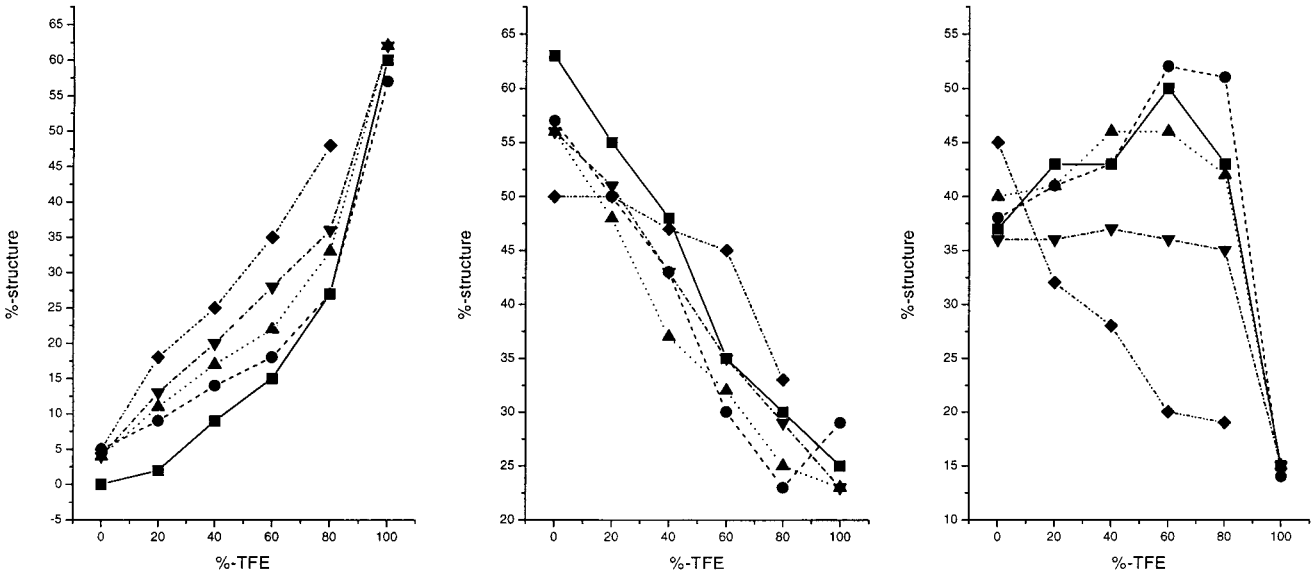

FIGURE 3: Secondary structure response to medium polarity as a function of acyl group chain length. D16 (■); C2 (●); C6 (▲); C10 (◆); D16M (◆). Left, helix; center, coil; right, reverse turn.

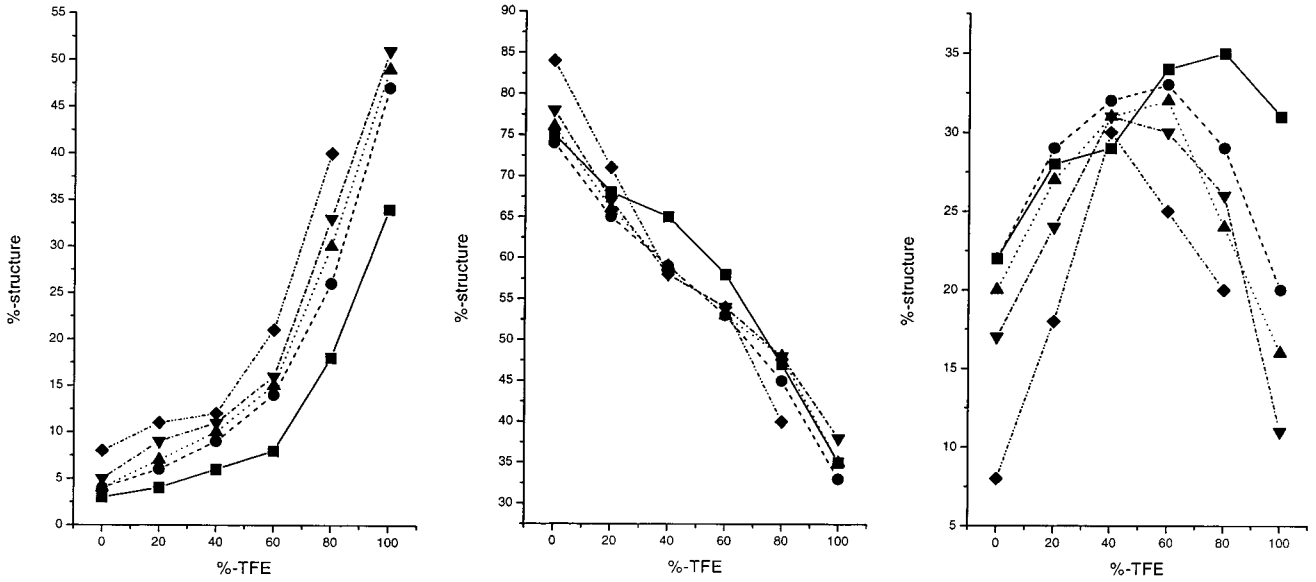

FIGURE 4: Secondary structure content response of phosphorylated peptides as a function of acyl group chain length. D16P (■); C2P (●); C6P (▲); C10P (▼); D16MP (◆). Left, helix; center, coil; right, reverse turn.

In the range between 0 and 40−60% TFE for all phosphorylated peptides, an increase in the content of turn structures could be estimated. At higher concentrations of TFE, these turn structures decreased at the same rate as a rise in helical structure. This phenomenon was weaker for peptides with longer fatty acids. Additionally, longer fatty acids stimulated the decrease of turn structures described above.

DISCUSSION

We have examined the potential of posttranslational modifications of the N-terminus of PKA catalytic subunit in various combinations to elicit local conformational changes in the peptide. This has been done using circular dichroism spectroscopy to determine the secondary structure content in solution of synthetic peptides emulating the sequence of the Cα gene product in this region. The structure content as calculated from CD spectra, of course, represents the weighted average of an equilibrium distribution among different allowed structures. A shift in observable secondary structure content thus indicates a significant change in the energetically most favorable conformer. The structural impact of a possible membrane environment for the N-terminus or of its hydrophobic surrounding within the enzyme structure may to a certain extent be mimiced by changes of the solvent polarity through variations of the TFE concentration.

The results can be summarized as follows: Any acylation at Gly1 promotes a higher proportion of helix in the conformational ensemble, i.e., is helix stabilizing. The naturally occurring fatty acid moiety myristic acid, however, seems to differ from the others in promoting helix at the expense of turn rather than causing a coil to helix transition. This effect is independent of the presence of other modifying groups. The presence of a phosphoryl group at Ser10 has the effect of destabilizing turn structure (in particular under polar conditions) and helix (under apolar conditions). The effect is similar to that observed by comparison of artificially

generated pairs of phospho-dephospho-peptides (*25*). Deamidation, i.e., the presence of isoAsp2 (in combination with N-myristylation) or of Asp2 instead of Asn2, has no effect on the local structure.

In most crystal structures of the C-subunit the aminoterminal region is poorly defined, probably indicating high mobility. The only structure to date which shows the complete aminoterminal region is solved at low resolution from porcine enzyme (*17*). In this structure, the long amphipathic A-helix appears to begin at residue 6, whereas in a high-resolution structure of the myristylated deamidated enzyme the A-helix starts at residue 10; however, the first seven residues and the proximal half of the myristic acid are invisible (*16*). Residues 1−5 fold back in a widened turn, and the distal half of the myristic acid occupies a hydrophobic pocket formed from residues both of the A-helix and the large lobe (*16*, *17*). In recombinant enzyme lacking the myristic acid, resolution of the A-helix extends no further than to residue 15 (*26*). This can be improved by cocrystallizing the detergent Mega 8, which replaces the fatty acid in the hydrophobic pocket (*27*), and extends the resolved part to residue 10 (*27*, *28*). The better definition of electron density might in part be due to the helix stabilizing effect of the myristoyl moiety seen here in the CD measurements.

Indications of the probable location of secondary structure elements within the N-terminal sequence (Figure 1) obtained by the application of folding algorithms (*24*) identified two regions with high helix propensity and one stretch with high turn probability. The helical region starting at Ser10 correlates with the proposed helix capping motif beginning with Ser 10 (*29*) and the higher resolution of this region in the myristylated or detergent bound structures. The helical tendency from residues 3−7 predicted by the folding algorithms actually overlaps with the beginning of the A-helix in the porcine enzyme structure (*17*). Mobility measurements at the N terminus of the enzyme employing residue 16 modified to carry a fluorescence marker indicated a high surface mobility in the nonmyristylated enzyme which decreases in the presence of myristic acid (*20*). Myristylation has been shown to increase the structural stability of the enzyme (*8*).

The observation that in contrast to encoded myristylated enzyme the more acidic form exhibits autophosphorylation (*5*), i.e., the deamidated form, may hint at a specific structural property elicited by deamidation which makes the otherwise masked Ser10 recognizable for phosphorylation by PKA in vitro. The peptide structure data presented here, however, do not show a difference at this level, nor does the crystal structure of deamidated enzyme indicate any significant difference around the phosphorylation site (*16*). The crystal structure, however, lacks resolution of the preceding region, which may become recognizable for the enzyme by the negative charge of Asp2.

The part of the N-terminus with the high turn probability, Gly9 to Gln12 (Figure 1), overlaps with the proposed helix capping motif S-*E*-*Q*-E which includes Ser10 (*30*). The measured destabilization of the turn structure by a phosphoryl group at Ser10 under polar conditions and the decrease of the helix content especially under apolar conditions is suggestive of a negative influence on helix capping. The consequences of the phosphorylation of a serine residue within a helix capping motif have been analyzed in the case of phosducin (*31*), where it destabilized a 20-residue helix.

Iso-aspartate is formed as a major product of the β-aspartyl shift mechanism for the deamidation of asparagine residues via a succinimide intermediate (for review, see ref *32*). Although the yield of isoAsp is about 3:1, in the cell its proportions are reversed to the aspartate product, suggesting that it is "repaired". This can occur by repeated cycles of protein carboxymethylation involving protein-isoaspartyl-methyl transferase (PIMT).

The additional methylene group in the peptide backbone in the case of isoAsp2 might be expected to destabilize the local protein conformation around isoAsp (*33*). An unstructured nature of the N-terminal region of the catalytic subunit would render it more susceptible to proteolytic degradation. It is thus interesting that in the absence of myristic acid the iso-aspartate derivative shows a slightly higher tendency to helix formation.

The Asp2 isoenzyme constitutes a good 25% of the catalytic subunit isolated from a wide variety of animal tissues, and would thus appear to serve an important function in the cell (*34*, *4*). In point of fact, deamidation has been seen to affect the level of catalytic subunit in the nucleus (*21*). This and the fact that the Asp2 isoenzyme possesses an additional negative charge, led to the expectation that deamidation would directly affect the backbone conformation. The observation that it has absolutely no effect on the local structure, either alone or in combination with the other possible N-terminal modifications, is rather remarkable and points to its involvement in a more complex, possibly nonlocal, type of signaling mechanism in the context of other cellular features.

On the basis of the work by Silverman and Resh (*14*), a theoretical analysis of the observed structural effects of the various PKA N-terminal modifications on a potential membrane association of the enzyme appeared justified, considering the striking similarity of the PKA N-terminus with other myristoyl switch proteins. If potential membrane binding via the myristoyl group is supported by electrostatic interaction with the positively charged N-terminal lysines (residues 7, 8, 16), partial unfolding of the N-terminal helix could interfere with the membrane contact by causing an unfavorable position of certain lysine residues, especially Lys7, which is closest to the myristate. In view of this, it is interesting that a modification that should in itself promote membrane affinity (acylation) also promotes helix formation, the amphipathicity of the helix fold acting as a further promoter. Conversely, a modification that should in itself reduce membrane affinity, (phosphorylation, due to electrostatic repulsion from phospholipid headgroups), also destabilizes helix and so further reduces membrane affinity. The dual effect of these two modifications means that they can work quite sharply to alter the membrane binding potential of the calatytic subunit. Their ability to influence the structure of the protein as well as altering its physiochemical properties enhance the potential of such posttranslational alterations to promote partitioning of the enzyme.

In conclusion, a multiplicity of PKA isoenzymes is due to the broad potential for posttranslational modifications offered within a relatively small sequence at the N-terminus of the catalytic subunit. In an attempt to obtain insight into the function each of these may fulfill, we have examined a simplified system of synthetic peptides comprising the full

spectrum of possible modifications to observe their effect on local structure. The results indicate that the two most commonly understood modifications, phosphorylation and myristylation, exert their influence by changing the preferred conformation of this region as well as by altering its physical properties. The possibility that deamidation may work through a localized structural mechanism is unlikely.

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