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A sequence variant in the N-terminal region of the catalytic subunit of the cAMP-dependent protein kinase

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An Asn to Asp exchange in position two is shown to be one sequence difference between the two major isoelectric variants of the catalytic subunit (C) of the cAMP-dependent protein kinase from bovine heart, $C_{7.5}$, representing the main and sequenced form, and $C_{7.1}$, a variant form [(1989), Biochem. Biophys. Res. Commun. 160, 596–601].

Protein kinase; Cyclic AMP; (Bovine)

1. INTRODUCTION

The cAMP-dependent protein kinase, consisting of two regulatory and two catalytic subunits, represents a key element in a number of metabolic cascades which are under hormonal control (for review see [1–3]). It has been shown that more than four stable isoelectric forms of the catalytic subunit (C) exist in bovine cardiac muscle [4]. The variants focusing at pH 7.1 and 7.5 (named $C_{7.1}$ and $C_{7.5}$) represent the predominant part of the peaks C_A and C_B, respectively, as separated from homogeneous enzyme preparations by ion exchange chromatography [5]. The forms C_A and C_B have also been observed in enzyme preparations isolated by other laboratories according to standard procedures [5,6]. The form $C_{7.5}$ appears to represent the enzyme first sequenced [7]. To date two mRNA forms, C_α and C_β, have been discovered [8,9]. In searching for possible sequence differences between $C_{7.1}$ and $C_{7.5}$, cleavage of both variants by Asp-N protease [10] was chosen followed by sequencing of selected peptide fragments. The data indicate a sequence difference between both forms at the N-terminus.

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2. MATERIALS AND METHODS

Immobilines were from Pharmacia/LKB (Freiburg, FRG), endoproteinase Asp-N, sequencing grade, was from Boehringer Mannheim (FRG), trifluoroacetic acid was from Pierce/Faust GmbH (Köln, FRG), acetonitrile was from Zinsser (Frankfurt, FRG), sequencing chemicals were from Applied Biosystems (Weiterstadt, FRG/Foster City, CA, USA).

The separation of the isoforms of the catalytic subunit of the cAMP-dependent protein kinase prepared from bovine heart in high resolution immobiline gels was carried out as described [4]. The bands visible without staining were cut out from the gel and eluted with 1 M tris-(hydroxymethyl)-aminomethane, 0.1% EDTA, 0.12 M 2-mercaptoethanol pH 8.6.

The alkylation of the catalytic subunit isoforms and the cleavage by Asp-N protease were carried out essentially according to [10] with modifications. Nine volumes of the catalytic subunit protein in elution buffer were combined with one volume of 1.2 M iodoacetic acid in the same buffer. After 15 min in the dark the reaction was stopped with 15 μl of 2-mercaptoethanol per 100 μl iodoacetic acid. After dialysis against 0.1 M ammonium bicarbonate pH 7.8, 2 μg Asp-N protease per 200 μg of protein were added. The digestion proceeded for 16 h at 36°C. The peptides were separated by HPLC on a C18-μ-Bondapak column (Waters, 300 mm, ∅ 4 mm). The starting buffer was 0.1% trifluoroacetic acid, buffer B was 60% acetonitrile in 0.1% trifluoroacetic acid and the flow rate was 1 ml/min. The gradient was 0–70% buffer B in 70 min,

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70–100% buffer B in 15 min at 25°C. The peptides were monitored at 206 nm.

Sequencing was done with an Applied Biosystems 470A sequencer equipped with a model 120A PTH online analyzer.

3. RESULTS AND DISCUSSION

The two major isoelectric forms of the catalytic subunit of the cAMP-dependent protein kinase focusing at pH 7.1 and 7.5 ($C_{7.1}$ and $C_{7.5}$) [4] were cleaved by the Asp-N protease and the resulting peptides were separated by reversed-phase chromatography. The elution profiles are shown in fig.1. While most of the peptide peaks have the same position in both profiles, one peak at 43% buffer B appears in $C_{7.1}$ only and one peak at 73% buffer B appears in $C_{7.5}$ only. These different peaks are marked by asterisks. Sequencing of the marked peak in $C_{7.5}$ yielded no detectable phenylthiohydantoin (PTH). The marked peak in $C_{7.1}$ showed an Asp at the first position followed by the amino acids of positions 3–24 of the sequenced bovine enzyme [7]. The regression curve of the yields of the PTH amino acids is shown in fig.2, demonstrating that the peptide was sequenced with an average repetitive yield of more than 90%. The N-terminus of $C_{7.1}$ is blocked, possibly by myristic acid [11]. The yet unknown blocking group and the first amino acid Gly seem to be removed by the Asp-N protease in the case of $C_{7.1}$ because of an exchange from Asn to Asp at position 2. The same result was also obtained in a preliminary experiment with C_A [5] which consists mainly of $C_{7.1}$ [4] (data not shown). The original N-terminal peptide is expected to elute last from the reversed phase column because of the fatty acid. The marked peptide in the $C_{7.5}$ profile represents the original N-terminal peptide which cannot be sequenced because of the blocking group. This was proven by subsequent cleavage of the marked peptide by V8 protease and sequencing of the cleavage products (data not shown). Other peptides from both variants, as listed in table 1, have been sequenced without revealing differences to the published sequence [7]. The isoelectric point of $C_{7.1}$ is 0.4 pH units more acidic than the main form, a difference to which the Asn to Asp mutation contributes; however, in spite of the similarity of the HPLC patterns, there may be additional differences between the two forms.

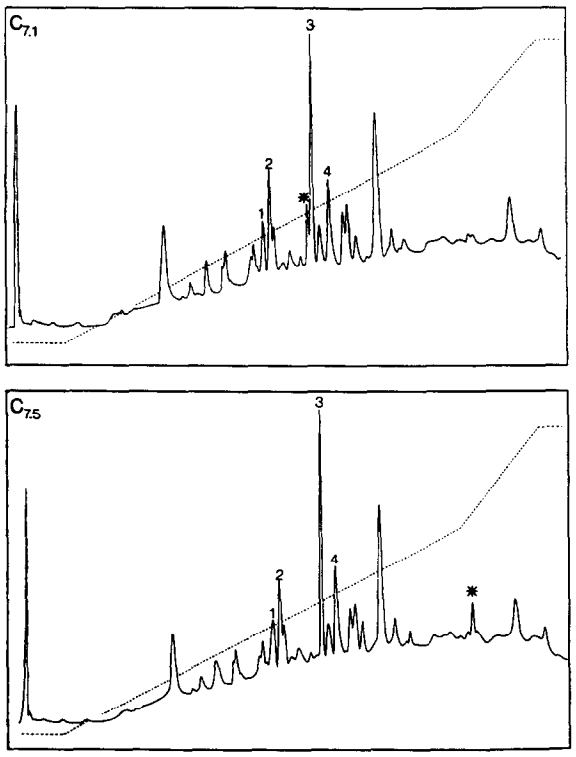

Fig.1. Peptide pattern of $C_{7.1}$ and $C_{7.5}$ cleaved by endoproteinase Asp-N. The dotted line indicates the concentration of buffer B (60% acetonitrile, 0.1% trifluoroacetic acid).

The Asn to Asp exchange does not appear to result from deamidation. Transglutaminases, which can deamidate Gln to Glu in proteins, do not seem to act on Asn [12]. Nonenzymatic deamidation appears to be very unlikely (a) because the change of only one Asn out of seven sequenced is very specific, (b) because of the rather mild conditions of the preparation (pH 6–7), and the facts that (c) various methods including those standardly used in other laboratories yielded the isoenzymes [5,6], (d) the proportions of $C_{7.1}$ to $C_{7.5}$ are reproducible, and (e) the isoelectric points of each form remain the same upon rechromatography [4,5].

On the nucleic acid level this exchange would require an A to G point mutation. The C_α gene is known to consist of several exons and introns [13]. There is no indication of an alternative splicing site responsible for this exchange. Thus, a second C_α gene or an allele has to be postulated, but so far has not been detected.

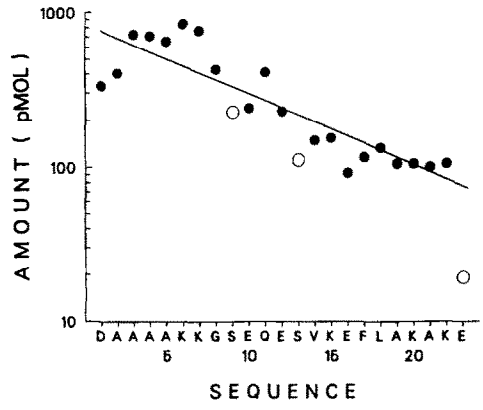

Fig.2. Regression curve of the yields of the PTH amino acids of peak * ($C_{7.1}$). Amino acids are given in the one letter code, yields are in pmol (●). The values for Ser and the C-terminal Glu (○) were omitted in calculating the regression curve.

The exchange in position 2 is not conservative and is therefore likely to cause structural differences in the N-terminal part of the molecule. The function of this part of the catalytic subunit has not been determined. In contrast to the src kinase, the myristic acid and the first amino acids are not necessary for kinase activity as shown in cells overexpressing a catalytic subunit with a modified N-terminus [14]. Therefore $C_{7.1}$ may differ from the main form in its intracellular localization and in nuclear transfer [15] as a physiological consequence of this exchange. This difference would probably influence the capability of $C_{7.1}$ to regulate the transcription of genes.

Table 1

Identified peptides of $C_{7.1}$ and $C_{7.5}$

Peak no.	Sequence[b]	Position in sequence [7]
1	DQPIQIY	241–247
1[a]	DKQKVVKLKQIEHTLN	75– 90
2	DIKNHKWFATT	290–300
*$C_{7.1}$	DAAAAKKGSEQESVKEFLAKAKE	2– 24
3	DFLKKWENPAQNTAHL	25– 40
4	DYEEEEIRVSINEKCGKEFSEF	329–350
4[a]	DWIAIYQRKVEAPFIPKFKGPG	301–322
*$C_{7.5}$	N-terminally blocked	N-terminus

[a] Mixture of two peptides in one peak, sequenced as mixture
[b] Identical to the published sequence [7] except for an Asn to Asp exchange in the first residue of *$C_{7.1}$

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