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Letter to the Editor: Sequence-specific resonance assignments of Q83, a lipocalin highly expressed in v-*myc*-transformed avian fibroblasts

Georg Kontaxis[a,*,§], Theresia Matt[b,*], Wolfgang Schüler[a], Bernhard Kräutler[a], Klaus Bister[b] & Robert Konrat[a,**]

[a]*Institute of Organic Chemistry and* [b]*Institute of Biochemistry, University of Innsbruck, A-6020 Innsbruck, Austria*

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Biological context

The protein product (c-Myc) of the protooncogene c-*myc* is a transcriptional regulator playing a key role in cellular growth control and differentiation. Deregulation of c-*myc* leads to oncogenic activation and cell transformation. However, the cellular targets mediating the biological effects of Myc are largely unknown (Bister and Jansen, 1986; Grandori and Eisenman, 1997). We have isolated a cDNA clone (Q83) derived from a highly abundant mRNA in v-*myc*-transformed quail embryo fibroblasts. The deduced 178-amino acid protein product of Q83 contains an N-terminal signal sequence and a lipocalin sequence motif, the hallmark of a family of secretory proteins binding small hydrophobic molecules (Flower, 1996). The quail Q83 protein displays 87% sequence identity with a developmentally regulated chicken protein, termed p20K or Ch21 (Bedard et al., 1989; Cancedda et al., 1990). Here we report the sequence-specific assignments for a 157-amino acid recombinant protein representing the mature form of Q83.

Methods and results

Applying subtractive hybridization techniques (Bister et al., 1993; Weiskirchen and Bister, 1993) to the identification of genes that are overexpressed in v-*myc*-transformed quail embryo fibroblasts, cDNA clone Q83 was isolated. A polymerase chain reaction (PCR) was performed using Q83 cDNA as a template and oligonucleotides 5′-d(CATAGTACTGTGCCGGACAGGAGCGAGATTG)-3′ and 5′-d(TGGATCCATCCTATACTTCATCAACGGTGC)-3′ as 5′ and 3′ primers, respectively. The 5′ primer corresponds to nucleotides 66–96 of the Q83 cDNA sequence (GenBank accession no. AF229030), with substitutions (underlined) introducing a novel *Sca*I site. The 3′ primer is complementary to nucleotides 523–552 of the cDNA sequence with substitutions introducing a novel *Bam*HI site. The PCR product was digested with *Sca*I and *Bam*HI, and the 475-nt fragment was ligated into plasmid pET3d that had been cut by *Nco*I, filled in by Klenow DNA polymerase, and then digested by *Bam*HI. The expression plasmid pET3d-Q83 encodes a 157-amino acid protein corresponding to the mature Q83 protein. Uniformly $^{13}C/^{15}N$- or ^{15}N-labeled Q83 or unlabeled protein was obtained by growing BL21(DE3)pLysS bacteria transformed by pET3d-Q83 in minimal medium containing 2 g [^{13}C]-D-glucose (CIL) and/or $^{15}NH_4Cl$ (CIL) per liter, or the unlabeled components, respectively. Mass spectrometry and amino-terminal sequencing confirmed the identity of the purified recombinant protein and revealed that 40% of the protein sample lacked the N-terminal methionine. The final yield of labeled or unlabeled Q83 was 6.5–7.0 mg per liter of bacterial culture. For NMR analysis, protein samples were concentrated to 1.8–3.0 mM.

NMR experiments were recorded at 26 °C on a Varian Unity Plus 500 MHz spectrometer. The data were processed using NMRPipe (Delaglio et al., 1995) and analyzed using ANSIG (Kraulis, 1989).

*Both authors contributed equally.
§Present address: Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, U.S.A.
**To whom correspondence should be addressed at the Institute of Organic Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria. E-mail: robert.konrat@uibk.ac.at

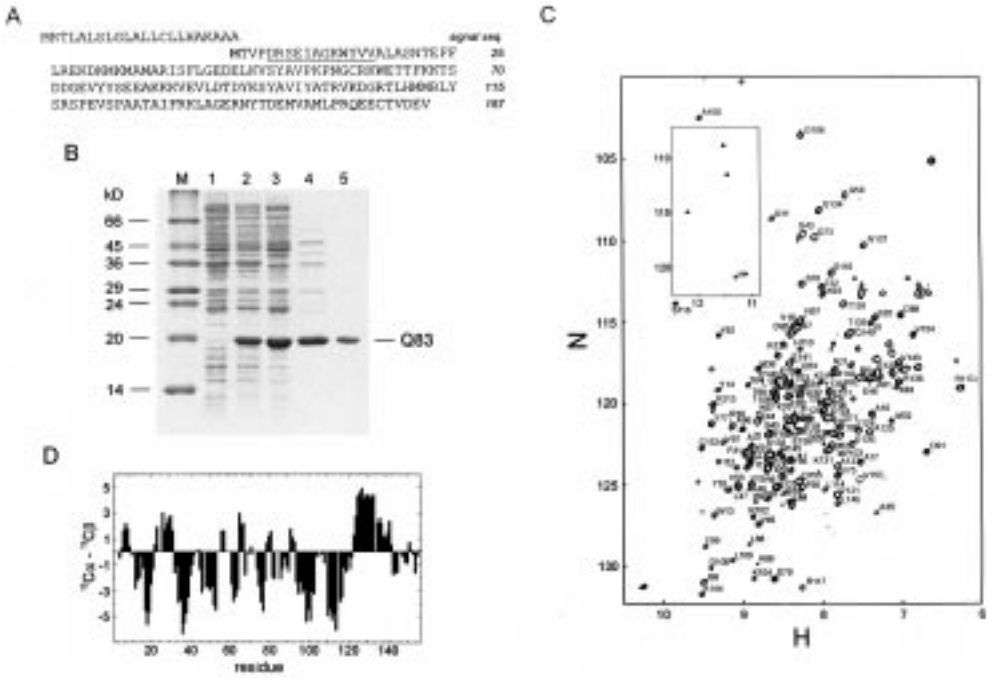

Figure 1. (A) Amino acid sequence of the 157-amino acid recombinant protein representing the mature Q83 protein. The initiating methionine specified by vector sequences is shown in bold type, and the lipocalin sequence motif is underlined. The 22-amino acid signal sequence present in the 178-amino acid primary translational product of Q83 mRNA is also shown. (B) SDS-polyacrylamide (15%, w/v) gel electrophoresis of total proteins from the bacterial culture before (1) and 4.5 h after (2) induction of Q83 synthesis, from the soluble fraction of a freeze–thaw lysate (3), from the supernatant of a salt fractionation step (4), and from the pooled Q83-containing fractions of gel filtration chromatography (5). M = molecular mass markers. Proteins were stained with Coomassie Brilliant Blue. (C) Sensitivity-enhanced 2D ^{1}H-^{15}N HSQC spectrum of 3 mM ^{13}C-,^{15}N-labeled Q83 at 26 °C and pH 6.4. Unassigned peaks shown in the boxed region displayed only intra-residue connectivities. (D) Smoothed $^{13}C^{\alpha}$-$^{13}C^{\beta}$ secondary chemical shifts are plotted as a function of residue position.

Main-chain $^{1}H^{N}$, ^{15}N, $^{13}C'$, $^{13}C^{\alpha}$, $^{1}H^{\alpha}$ and side-chain $^{13}C^{\beta}$, $^{1}H^{\beta}$ resonances were assigned using HN-CACB, CBCA(CO)NH, HNCA, HNCO, HNCACO, C(CO)NH, HCCH-TOCSY, ^{15}N NOESY-HSQC, and ^{15}N TOCSY-HSQC (Cavanagh et al., 1996).

Extent of assignments and data deposition

Sequence-specific assignments ($^{1}H^{N}$, ^{15}N, $^{13}C'$, $^{13}C^{\alpha}$, $^{1}H^{\alpha}$, $^{13}C^{\beta}$, $^{1}H^{\beta}$) for recombinant Q83 have been deposited in the BioMagResBank under accession number 4664. Resonance assignments have been made for amino acid residues 4 through 157 (except for residues 24, 25, 54, 55, 59, 60, 84 and 85).

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