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ORIGINAL ARTICLE

Expedient chemical synthesis of 75mer DNA binding domain of MafA: an insight on its binding to insulin enhancer

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Abstract An expedient chemical synthesis of a 75mer peptide corresponding to the DNA binding domain (DBD, 227–301) of the human MafA leucine zipper transcription factor is reported. The application of microwave-assisted solid phase peptide synthesis (MW-SPPS) with a protocol modified respect to the standard one allowed obtaining the desired 75mer peptide in a short time with high quantity and optimal purity. MW-SPPS methodology was thus demonstrated as a valuable alternative to recombinant methods to obtain protein domains. Considering that recent findings suggest an involvement of MafA in the pathogenesis of diabetes mellitus, we also performed circular dichroism studies both on DBD folding and its interaction with MafA recognition element (MARE) on insulin enhancer. From our results, it was evicted that a disorder to order transition occurs after DBD interaction with insulin MARE which is mediated by specific structural elements on the N-terminus of the DBD.

Keywords MafA · Leucine zipper · Insulin enhancer · Microwave-assisted solid phase peptide synthesis · Circular dichroism

Abbreviations

ACN	Acetonitrile
b-LZ	Basic leucine zipper
BOC	Tert-butoxycarbonyl
BR	Basic region
CD	Circular dichroism
DBD	DNA binding domain
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethylsulfoxide
EDT	1,2-Ethanedithiol
EHR	Extended homology region
ESI–MS	Electrospray ionization mass spectrometry
Fmoc	9-Fluorenylmethyloxycarbonyl
HBTU	*O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate
HCCA	*O*-cyano-4-hydroxy cinnamic acid
HOBT	1-Hydroxybenzotriazole
LZ	Leucine zipper
MARE	Maf recognition element
MBHA	4-Methylbenzhydrylamine
MW-SPPS	Microwave-assisted solid phase peptide synthesis
NMP	1-Methyl-2-pyrrolidinone
Pbf	2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl
RP-HPLC	Reverse phase high performance liquid chromatography
SPPS	Solid phase peptide synthesis
TAN	Thioanisole
TFA	Trifluoroacetic acid
TFE	2,2,2-Trifluoroethanol
TIS	Triisopropylsilane
Trt	Trityl

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Introduction

In recent years, great attention has been paid to structural and functional intrinsic disorder inside the cell. In particular, it has been recognized that many eukaryotic proteins require a considerable level of conformational flexibility for their function (Dyson and Wrigth 2005; Liu et al. 2006). This observation has particular importance to the basic leucine zipper (b-LZ) class of transcription factors, whose activity relies on disorder–order transitions of their DNA binding domains (DBD) for specific dimerization and DNA binding (Miller 2009). Within b-LZs, Maf proteins represent a new paradigm for DNA binding specificity as they recognize a long palindromic DNA sequence (known as Maf recognition element, MARE) which is composed by a core and a flanking region (Blank and Andrews 1997). The core, usually, corresponds to the TRE AP-1 recognition site (T-MARE: TGCTGACTCAGCA) or the CRE c-AMP responsive element (C-MARE: TGCTGACGT CAGCA), but can be more degenerated (Eychène et al. 2008); the flanking region (TGC and GCA) is instead unique to Mafs (Kataoka 2007). The DBD of Maf is also peculiar as it harbors a b-LZ combined to a unique and highly conserved N-terminal motif called extended homology region (EHR) (Kusunoki et al. 2002; Blank 2008). From the crystal structure of the MafG/MARE25 complex available in the literature (Kurokawa et al. 2009), it is evicted that Maf DBD is composed by three α-helices (H1, H2 and H3): H1, H2 and the N-terminal region of H3 form the EHR, while the inner part of H3 forms the basic region (BR) and the C-terminal part of H3 the LZ dimerization domain.

Seven members of the Maf family have been reported and were subdivided into two groups depending on their molecular weight: large Mafs (MafA, MafB, C-Maf and NRL, 26–39 kDa), and small Mafs (MafF, MafG and MafK, 17–18 kDa). Mafs are involved in different biological events such as embryogenesis and cell differentiation during development (Motohashi et al. 1997; Kataoka 2007; Blank 2008). They are also directly implicated in carcinogenesis, and in several diseases (Eychène et al. 2008). MafA is the principal pancreatic transcription factor required for β-cell formation and function (Aramata et al. 2007). MafA knockout mice display age-dependent pancreatic islet abnormalities, glucose intolerance and develop diabetes mellitus (Kaneto et al. 2009); furthermore, it was recently reported that MafA induces insulin expression in non-β cells (Kaneto et al. 2005). All these findings make the detailed understanding of MafA-mediated activation valuable for diagnosing and treating β-cell compromised diabetic patients. MafA recognizes a peculiar MARE (TGCAGCCTCAGCC) on insulin enhancer as, with respect to consensus MARE, this sequence is not palindromic, i.e., it contains a degenerated core (neither TRE nor CRE) and furthermore it does not contain the classical flanking region (TGC/GCC instead of TGC/GCA) (Matsouka et al. 2004).

To our knowledge, Mafs are nowadays available only by recombinant techniques. Stepwise solid phase synthesis (SPPS) of small- and medium-size proteins is an attractive alternative to biotechnological methods, as it permits backbone modification, conformational constraints, incorporation of non-natural and/or labeled amino acids. Nevertheless, its use for the preparation of long peptides is hampered by several limitations, such as slow reactions, degradation of resin, and accumulation of by-products (Kent 1988). The use of microwave irradiation, anyway, was recently demonstrated to be effective for peptides up to 50 residues (Friligou et al. 2011). In this work, we present the first example of chemical synthesis of a 75mer peptide from MafA corresponding to the highly conserved EHR, the basic region and the N-terminal part of the LZ responsible for the dimerization. In particular, we demonstrate the effectiveness of stepwise microwave-assisted SPPS (MW-SPPS) also for peptides with a chain length longer than 50 residues. Finally, starting from the obtained 75mer peptide and fragments corresponding to its different structural domains, we perform circular dichroism (CD) studies both on folding and interaction with MafA MARE on insulin enhancer.

Materials and methods

Fmoc protected amino acids, Rink amide PEG MBHA resin, HBTU and DIPEA were purchased from Iris Biotech Gmbh. HOBT, piperidine, acetic anhydride, triisopropylsilane (TIS), thioanisole (TAN), phenol, TFA, and HPLC-grade solvents were from Sigma Aldrich. 1-Methyl-2-pyrrolidinone (NMP) was obtained from BDH/VWR/PROLABO and 1,2-ethanedithiol (EDT) from Fluka. The DNA sequences were purchased at Primm Srl.

All peptides were prepared by MW-SPPS using a Liberty Microwave Peptide Synthesizer (CEM Corporation) and purified by preparative RP-HPLC using Jasco BS-997-01 equipment and a DENALI C-18 column from GRACE VYDAC (10 μm, 250 × 22 mm). Two mobile phases were used: A = 95 % water, 5 % ACN, 0.1 % TFA; B = 95 % ACN, 5 % Water, 0.1 % TFA. The peptides were analyzed by analytical HPLC using a Jasco BS-997-01 equipment and detector series Star 800 from VARIAN. The mass spectra were recorded on a LCQ Advantage spectrometer from Thermo Finnigan for ESI–MS analysis and on Autoflex 3 from Bruker Daltonics for MALDI-TOF analysis. The CD spectra were recorded at room temperature using a Jasco J-810 spectropolarimeter and a 0.01 cm quartz cell (Hellma Suprasil).

MW-SPPS

Peptides were synthesized C-terminally amidated and N-terminally acetylated using Rink amide PEG MBHA resin with a loading of 0.35 mmol g^{-1} and on a 0.1 mM scale. Amino acid side-chain protection was as follows: tBu for Asp, Glu, Tyr, Thr, Ser; Pbf for Arg; Boc for Lys; Trt for Asn, Gln and His.

Protocol A (for peptide up to 28 residues): single couplings were performed with Fmoc-amino acid (5 equiv.) activated in situ with HBTU (5 equiv), HOBt (5 equiv.) dissolved in DMF, and DIPEA 1 M in NMP (10 equiv.); each coupling was achieved using MW irradiation (5 min, 75 °C, 20 W; for His and Cys 50 °C, 2 min at 0 W and 4 min at 25 W). Fmoc cleavage was accomplished by treating the peptidyl-resin with 20 % piperidine in DMF (3 min, 75 °C, 40 W).

Protocol B (for Arg and, starting from the 35th residue, for all amino acids): double couplings were performed with Fmoc-amino acid (5 equiv.) activated in situ with HBTU (5 equiv.), HOBt (5 equiv.) dissolved in DMF, and DIPEA 1 M in NMP (10 equiv.). The first coupling was performed at room temperature (1 h and without MW irradiation); the second one was achieved using MW energy (5 min, 75 °C, 25 W). Fmoc cleavage was accomplished by treating the peptidyl-resin with 20 % piperidine in DMF (3 min, 75 °C, 40 W).

N-terminal acetylation

Synthesized peptides were manually acetylated at the N-terminus on resin using acetic anhydride (10 equiv.) and DIPEA (10 equiv.) in DCM for 30 min at room temperature.

Cleavage from the resin

The peptides were manually cleaved from the resin with a mixture of TFA/water/TIS (90:5:5 v/v) for 6 h at room temperature or, when Cys and Met were present with the mixture TFA/water/TIS/TAN/EDT (90:3:5:5:3 v/v). The crude peptides were precipitated from ice-cold diethyl ether and recovered by centrifugation at 4 °C for 5 min (4,500 rpm). Three diethyl ether washes/centrifugation cycles were carried out to efficiently remove the scavengers. If Cys was present, a mixture of *t*-butyl-methyl ether/petroleum ether (1:1 v/v) was used to avoid oxidation.

Peptide purification and characterization

The crude peptides were dissolved in DMSO and diluted in ACN/water (50:50 v/v) solution. The purification was performed by preparative HPLC and injecting 1 mL of peptide solution at a flow rate of 20 mL/min. The gradient was 95 % A for 5 min and 95–50 % A over 50 min. UV detection was made at 220 nm. The purified peptides were freeze-dried and analyzed using analytical HPLC (95 % A for 5 min; 95–30 % A over 20 min) and MALDI-TOF (sinapinic acid or 4-HCCA matrix) and ESI mass spectrometry.

General procedure for DNA annealing

The two complementary DNA oligomers were diluted in HPLC-quality water, heated at 95 °C and gradually cooled (10 °C every 10 min) to room temperature. The obtained duplex was stored at −20 °C before using.

Circular dichroism

Peptide stock solutions were prepared in HPLC-quality water (100 μM, 1.5 mL). Spectra were obtained from 195 to 250 nm with a 0.1 nm step and 1 s collection time per step, taking three averages. The spectrum of the solvent was subtracted to eliminate interference from cell, solvent, and optical equipment. The CD spectra were plotted as mean residue ellipticity θ (degree × cm^2 × $dmol^{-1}$) versus wave length λ (nm). Noise-reduction was obtained using a Fourier-transform filter program from Jasco.

Results and discussion

Synthesis of 75mer DBD and its fragments

The chemical synthesis of the 75mer peptide from MafA DBD represented a tough challenge, not being available previous reports on the preparation of such domain using conventional SPPS protocols. Furthermore, this sequence is particularly rich in Arg residues, which are somewhat difficult to couple. As the usefulness of microwave irradiation was recently demonstrated for the preparation of long peptides and, particularly, for difficult sequences, (Friligou et al. 2011) we planned to use automated MW-SPPS for obtaining 75mer MafA DBD and its different domains (i.e., the EHR, the basic region BR, and the dimerization domain; see Fig. 1). We also planned the synthesis of shorter fragments corresponding to H1–H2, to the N-terminus of H3 (named H3 short) and to the sequence H2-H3 short in order to perform circular dichroism studies (see below and Table 1 in "Materials and methods").

Rink amide PEG MBHA resin (0.35 mmol/g loading) was chosen for its higher swelling efficiency with respect to Rink amide polystyrene. All the peptides were prepared using Fmoc/tBu methodology and entirely assembled using automatic MW-SPPS, applying different microwave

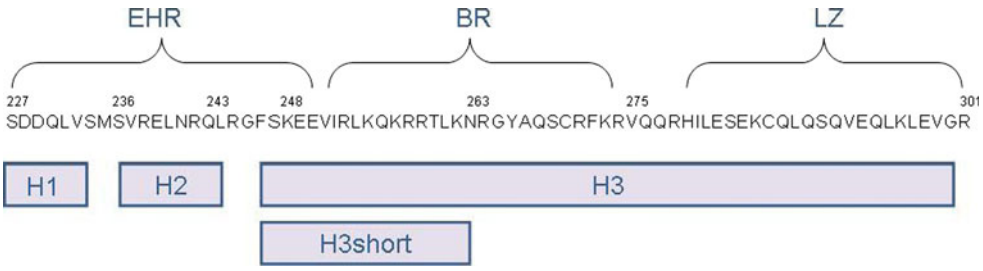

Fig. 1 MafA DBD

Table 1 Synthesized peptides

Peptide	Length	Protocol[a]	MW_{calc}(Da)	MW_{found}(Da)[b]	t_r (min)[c]	Total yield (%)[d]
248–263 (H3short)[e]	16	A	2,039.49	2,038.18	14.08	60
227–243 (H1H2)	17	A	2,032.28	2,031.20[f]	18.19	50
277–301 (LZ)	25	A	3,020.44	3,019.65	16.91	20
248–275 (BR)	28	A	3,531.19	3,532.30	15.27	18
236–263 (H2H3short)	28	A	3,537.16	3,536.12	16.54	17
227–263 (EHR-H3short)	37	B	4,500.18	4,497.00	18.80	15
227–278 (EHR–BR)	52	B	6,365.33	6,360.50	19.23	10
248–301 H3	54	B	6,622.71	6,621.30	16.39	10
227–301 DBD	75	B	9,041.48	9,034.70	17.25	6.5

[a] A: standard MW assisted single coupling; B: standard MW-assisted single coupling up to 35 residue, then double coupling (see text); [b]MALDI-TOF analysis (see Supporting Information); [c]see text for analytical HPLC gradient; [d]purified peptides; [e]not N-terminally acetylated; [f]ESI–MS analysis (see Supporting Information)

protocols to the coupling steps depending on the amino acid type and the chain length (see Table 1). In the standard protocol, which was applied to peptides up to 28 residues, couplings were performed using a fivefold excess of Fmoc-amino acids, HOBT/HBTU (5 equiv., 0.45 M in DMF) as activators and DIPEA (10 equiv., 1 M in NMP) as base and with a 5 min microwave irradiation at 75 °C. Fmoc deprotection was achieved using piperidine (20 % in DMF) with a 3 min microwave irradiation at 75 °C. In order to avoid cysteine and hystidine racemization, their couplings were performed at 50 °C. For arginine, which possesses a low reactivity toward peptide couplings, we adopted a double coupling approach (first coupling: 30 min at 75 °C without microwave energy; second coupling: 5 min at 75 °C with microwave energy). This last protocol was used in the elongation of longer peptides starting from the 35th residue, regardless of the amino acid type. All the peptides were N-terminally acetylated (tenfold excess Ac_2O and DIPEA in DCM) and cleaved from the resin using TFA and scavengers. The crude HPLC traces indicated a good homogeneity for all compounds (Fig. 2), which were then purified with preparative HPLC and identified using mass spectrometry. It should be underlined that in a relatively short time (70 h) it was possible to obtain 50 mg of pure 75mer DBD, thus demonstrating the effectiveness of stepwise MW-SPPS technique also for peptides with a chain length higher than 50 residues.

Circular dichroism experiments

The CD technique is particularly useful for examining macromolecular structures which in solution lack a well-ordered tertiary structure, as well as for determining dynamic protein/DNA interactions (Greenfield 2006; Garbett et al. 2007). In phosphate buffer (0.1 M, pH 7.5), the entire 75mer peptide showed a negative band close to 200 nm (amide $\pi \rightarrow \pi^*$ transition) and a negative shoulder near 222 nm (amide $n \rightarrow \pi^*$ transition) (Fig. 3). The absence of a positive band below 200 nm and the blue shift of the $\pi \rightarrow \pi^*$ one indicated the presence of different conformations. This behavior was independent from sample concentration (data recorded from 5 to 50 μM, while at higher concentration, the DBD was not soluble). Accordingly, secondary structure analysis of the CD spectrum with the CONTIN algorithm (Provencher and Glockner 1981; Whitmore and Fallace 2008) indicated the presence of 46 % unordered fraction together with α helix and, surprisingly, β sheet structure (Table 2). By analyzing the CD spectra of different DBD fragments (Fig. 3), it was found that the LZ fragment contained lesser β sheet contribution (α/β ratio, 2:1) than the other peptides. The entire DBD and the EHR-H3short (227–263) sequence possessed a α/β ratio of 1:1, while for other fragments this ratio was in favor of β sheet. These findings suggest that the presence of both the EHR and LZ is crucial for the stabilization of α

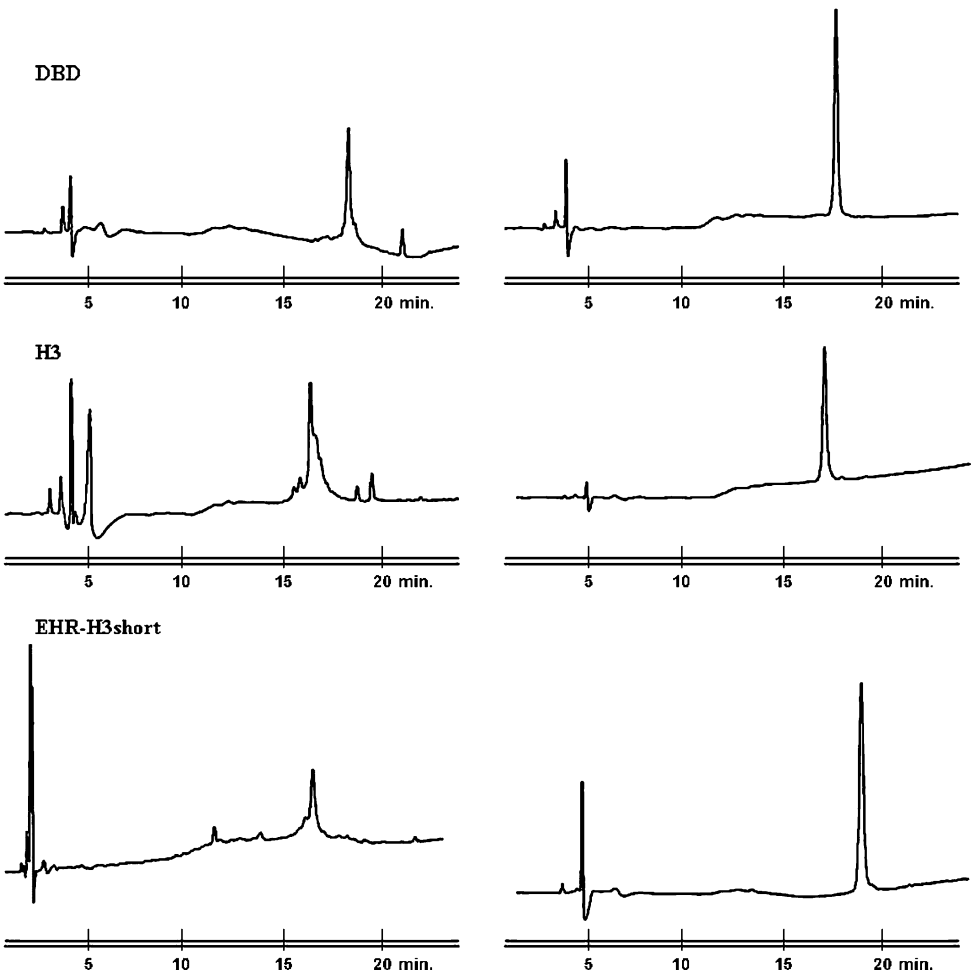

Fig. 2 Representative HPLC traces of crude (*left*) and purified (*right*) peptides

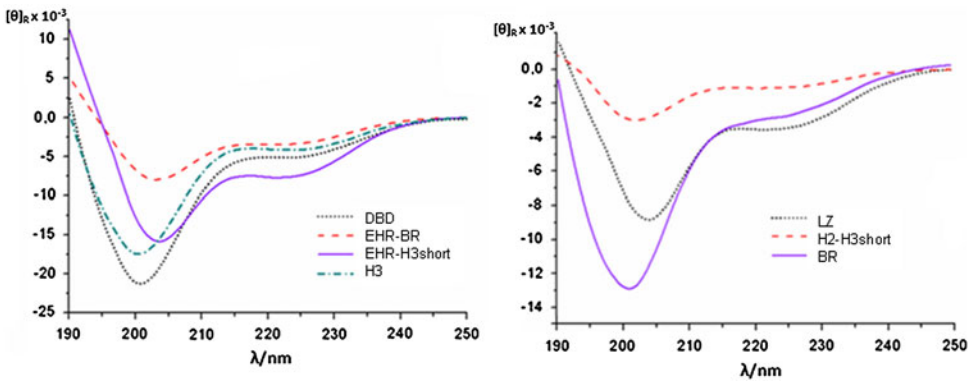

Fig. 3 CD spectra of synthesized peptides in phosphate buffer (20 μM solution)

helix structure. Nevertheless, in all cases the addition of 50 % TFE led to the increase of the helix conformation indicating that β sheet structure is favored by a hydrophilic environment (Table 2 below).

EHR domain is a peculiarity of Maf proteins and, from the literature data on MafG, hydrophobic interactions between residues from H1 and N-terminus of H3 (H3short) are evicted (Kurokawa et al. 2009). In order to ascertain if they are fundamental for the folding, we performed interaction studies between EHR-H3short (227–263) peptide and H1H2 (227–243) and H3short (248–263) fragments. In order to minimize the contribution of β sheet conformation, the experiments were carried out in 20 % TFE. Under these conditions, all the three peptides were found almost helical (EHR-H3short: 61 %, H1H2: 48 %, H3short: 76 %; helix percentage was estimated using the procedure of Chen et al. 1974; see Fig. 4).

As shown in Fig. 5a, the CD spectrum of the mixture containing EHR-H3short and H3short peptide was not superimposable to the arithmetic sum of the CD spectra of

Table 2 Secondary structure properties of synthesized peptides

Peptide	Buffer				50 % TFE			
	% Helix	% Strand	% Turns	% Unordered	% Helix	% Strand	% Turns	% Unordered
DBD	20	20	14	46	100	–	–	–
H3	15	29	15	41	100	–	–	–
EHR–BR	14	31	15	40	100	–	–	–
EHR-H3short	25	21	14	40	85	–	7	8
H2H3short	6	30	24	40	67	–	14	19
BR	11	30	15	44	70	–	10	20
LZ	20	12	22	46	71	1	13	15
H1H2	9	36	14	41	55	5	11	29
H3short	12	30	8	50	73	–	12	15

The CD spectra were analyzed using the CONTIN algorithm

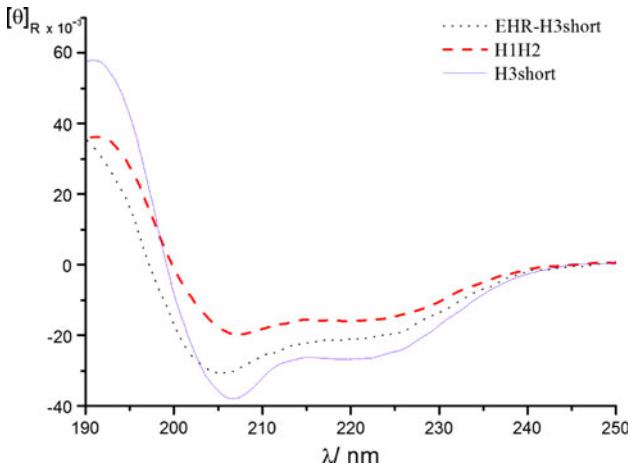

Fig. 4 CD spectra in 20 % TFE (20 μM solution)

the single components. The same behavior was observed also for the mixture of EHR-H3short and H1H2 (Fig. 5b), indicating that in both cases an interaction was effective. In particular, a blue shift of the $\pi \rightarrow \pi^*$ band was observed, suggesting that an increase of unordered fraction, probably, occurs for all peptides. From these findings, we postulated that, when single H1H2 or H3short fragments were added to EHR-H3short, they could act as folding disrupter interfering in the formation of intramolecular interactions within EHR-H3short.

Finally, the interaction of MafA DBD with a DNA sequence of 26 bp from human insulin enhancer containing MafA MARE (CGGAAATTGCAGCCTCAGCCCCCAGC; Matsouka et al. 2004) was studied. First, the CD spectrum of the DNA duplex was recorded in phosphate buffer (0.1 M, pH 7.5; Fig. 6), showing a B-DNA conformation characterized by the presence of a positive peak at 270 nm and two negative ones, at 250 and 210 nm, respectively (Baase and Johnson 1979).

The CD spectrum of 75mer DBD alone was compared with the spectrum derived from a mixture with human insulin

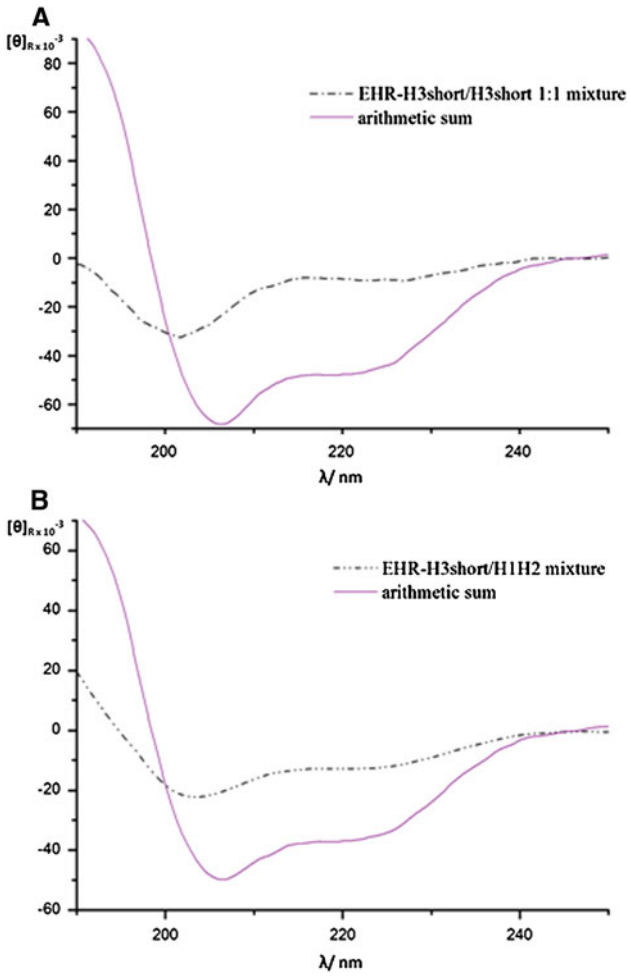

Fig. 5 Interaction between EHR-H3short with H3short (**a**) and H1H2 (**b**)

MARE (DBD/DNA, 2:1) by subtracting DNA contribution: an increase of α-helical content was observed (62 % helix content found), indicating that an interaction was effective (Fig. 6). In order to investigate if the binding to DNA was

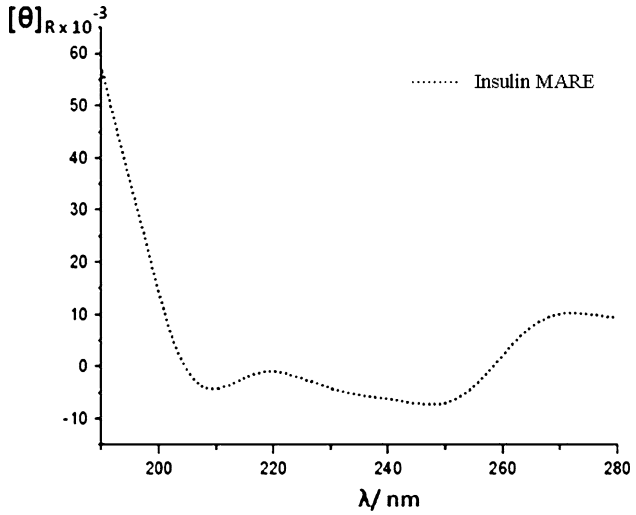

Fig. 6 CD spectrum of 26 bp DNA sequence from human insulin enhancer

sequence specific, we also studied the interaction of DBD with a scrambled DNA sequence (GATCAGTACCACGCGCTC CACGCCGA). As expected, we obtained no specific conformational changes in the CD spectrum of DBD (Fig. 7).

In order to gain an insight in the binding of MafA to insulin MARE which, as reported above, is uncommon with respect to standard ones (Matsouka et al. 2004), CD studies were also performed using shorter sequences from DBD. In the presence of DNA, a structural change (20 % helix structure in CD spectrum) was only observed for the peptide corresponding to EHR–BR sequence (227–278) (Fig. 8). On the contrary, we did not observe any interaction with either the EHR-H3short fragment (227–263) or, interestingly, the entire BR (248–275) and the entire H3 (248–301) (data not shown).

From these results, we can postulate that under physiological conditions and in the absence of specific DNA, MafA is in a dynamic equilibrium between folded and unfolded states. In particular, important β sheet and unordered contributions were found for 75mer DBD and its fragments in an hydrophilic environment, while an hydrophobic medium stabilized the α helix structure. This conformation is also dependent on two domains of the DBD: the LZ and EHR. This last region is characteristic and highly conserved within the Maf family. This region is rich in hydrophobic residues forming a hydrophobic cluster which is surrounded by hydrophilic amino acids (Kurokawa et al. 2009). Our CD results demonstrated that, in the absence of DNA, these intramolecular hydrophobic interactions are effective in partially stabilizing the conformation of DBD. H1H2 (227–243) and H3short (248–263) fragments, in particular, could act as folding disrupters (see Fig. 5).

The interaction with insulin MARE induces conformational changes by stabilizing the three-dimensional conformation of MafA domain in a similar manner as reported for other family members. Early CD studies on c-Maf and palindromic consensus MARE reported DNA sequence-dependent folding. In particular, the flanking region of MARE was found to be dramatically important for the interaction with DBD, thus stabilizing the α helix structure, while the core seemed to have a scarcer relevance (Dlakic et al. 2001). The binding of MafA to insulin enhancer then represents a case in this paradigm, the MARE recognition sequence being not palindromic, and possessing degenerated core and flanking regions (Matsouka et al. 2004). Our CD results, anyway, indicate that also in the case of a degenerated MARE, the interaction with DNA induces an increase of the helical content in MafA DBD (62 % helical content found; Fig. 7).

Further conclusions can be drawn starting from the interaction studies between MafA shorter fragments and insulin MARE. From the crystal structure of the MafG/MARE25 complex (PDB ID 3A5T; Kurokawa et al. 2009), it was evicted that conserved residues from BR are involved in DNA binding. In particular, Arg57 and Asn61 (corresponding to Arg259 and Asn263 in MafA) were shown to directly interact with DNA, while residues Arg62, Tyr64, Ala65, Arg69, and Lys71 (corresponding to Arg264, Tyr266, Ala267, Arg272, and Lys274) are fundamental for the correct spatial orientation of Arg57 and

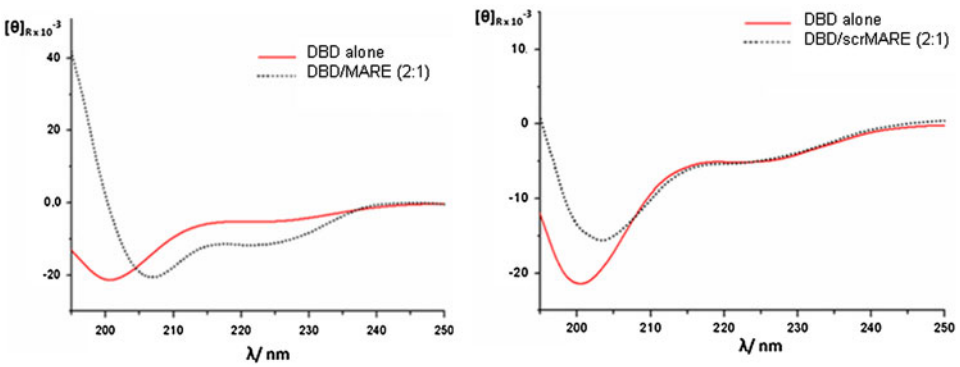

Fig. 7 Comparison between DBD spectrum alone and the spectrum derived from the 2:1 mixture with insulin MARE (on the *left*) and scrambled DNA (on the *right*)

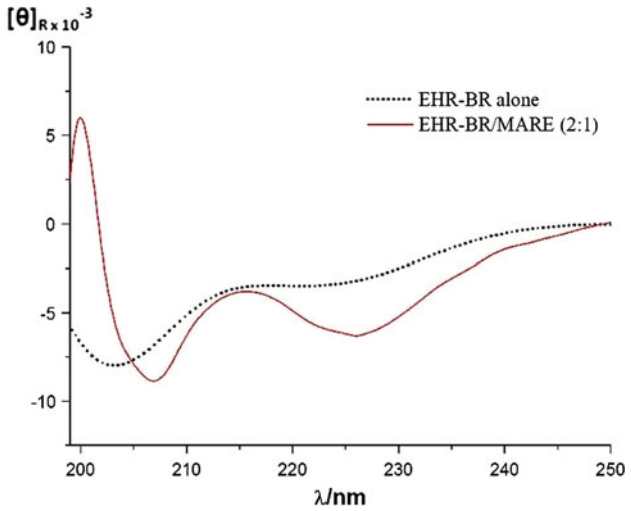

Fig. 8 Comparison between EHR–BR spectrum alone and the one derived form the 2:1 mixture with insulin MARE

Asn61 side chains (see Figure 7a in Kurokawa et al. 2009). Our CD studies on BR, anyway, showed that both peptides H3short (248–263) and EHR-H3short (227–263), containing the key residues Arg 259 and Asn 263, were found unable to interact with DNA, as well as the entire BR (248–275). Curiously, also the H3 peptide (248–301, corresponding to BR and LZ regions) was also unable to bind insulin MARE, whereas the EHR–BR (227–278) peptide interacted (20 % helical content found; Fig. 8). From these data, we can assume that the highly conserved EHR is not only important for DBD folding but also for binding to insulin enhancer. Our hypothesis is that, since from the crystal data on MafG/MARE25 this part of the DBD seemed not to be directly involved in DNA interaction, its role probably lies in a correct folding inducer. In this context, the hydrophobic cluster within the EHR (in particular Leu 231 and Met 234) could then act as a local structural element that triggers the folding of the entire DBD in the presence of DNA. Considering that, from CD data, the LZ region seems not to be necessary for DNA binding, the dimerization of the DBD is an event that, probably, occurs after DNA recognition. Indeed, we can postulate that MafA undergoes a disorder-to-order transition through a coupled binding and folding mechanism similarly to that reported for other b-LZ domains (Seldeen et al. 2008).

Conclusion

In this work, we presented the first example of a chemical synthesis of the entire DNA binding domain of MafA protein, the principal transcription factor responsible of β-cell function. The use of automated MW-SPPS led to the achievement of the corresponding 75mer peptide synthesis in high amount and short time. Considering the advantages of this technique, e.g., the insertion of unnatural amino acids, its use could be considered a valuable alternative to biotechnology methods.

Circular dichroism experiments were performed in order to gain insight on MafA interaction with insulin enhancer MARE. In the absence of DNA, this domain was found intrinsically disordered, while its conformation became more stable in the presence of DNA, suggesting a coupled folding and binding mechanism. EHR domain and the N-terminal part of the basic region, although not directly involved in the binding, are fundamental for the interaction with DNA, probably acting as local structural elements responsible of folding stabilization and inducing the correct spatial of the amino acids involved in DNA binding.

Conflict of interest The authors declare that they have no conflict of interest.

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