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ORIGINAL ARTICLE

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Identification of an α-helical epitope region on the PM/Scl-100 autoantigen with structural homology to a region on the heterochromatin p25β autoantigen using immobilized overlapping synthetic peptides

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Abstract The polymyositis-scleroderma overlap syndrome (PM/Scl) autoantigen is a nucleolar multiprotein particle, presumably participating in the maturation of 5.8S rRNAs. The major target antigens of this particle are two polypeptides with apparent molecular masses of 100 and 75 kDa. In this study we identified the major linear epitopes along the PM/Scl-100 protein sequence by probing overlapping oligopeptides with anti-PM/Scl autoantisera. A major epitope region was identified between amino acids 231 and 245 of the PM/Scl-100 polypeptide. Mutational analysis of the corresponding peptide LDVPPALADFIHQQR by glycine-walk followed by immunodetection of the resulting peptides indicated that amino acids 234, 237, 240, and 241 of the PM/Scl-100 autoantigen are essential for binding of the corresponding antibodies. These results allow us to propose a local α-helical secondary structure for the PM/Scl-100 major epitope region. A homology search with the peptide LDVPPALADFIHQQR against the Swiss-Model three-dimensional database reveals some topological homology of the PM/Scl-100 major epitope region with the heterochromatin modifier protein p25β, a known autoantigen recognized by antibodies from a subset of scleroderma patients.

Key words Polymyositis-scleroderma overlap syndrome · Autoantigen · Epitope-mapping · Secondary structure · Heterochromatin protein p25β

Abbreviations *PM/Scl:* Polymyositis-scleroderma overlap syndrome · *TBS:* Tris-buffered saline

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Introduction

A hallmark of systemic autoimmune diseases is the occurrence of circulating antibodies immunoreactive with defined intracellular components. It is thought that this autoimmune response is antigen driven. A hypothesis forwarded by Tan [1] suggests that autoantibodies are directed against components of functionally important subcellular particles and that the target epitopes are closely associated with active sites of the targeted subcellular particle. The autoimmune response is thought to be ini-

tially directed against a defined structure on the target antigen and subsequently to spread to other epitopes on the same molecule or a molecule in close contact. Based on these observations the precise determination of epitope sequences and their secondary structures becomes an important step in the elucidation of both the cellular function of the targeted antigen and the events ultimately leading to a pathogenic autoimmune response.

Sera from patients suffering from polymyositis-scleroderma overlap syndrome are frequently reported to contain anti-nucleolar antibodies targeting a particle termed PM/Scl [2, 3, 4]. Anti-PM/Scl antibodies are also found in sera from patients with scleroderma (3%) or polymyositis (8%) [5]. The PM/Scl antigen is localized predominantly in the granular component of the nucleolus, but an additional weak nuclear staining pattern in indirect immunofluorescence studies suggests also its presence in the nucleoplasm [3, 4, 6]. The antigen itself is a particle consisting of 11–16 proteins with molecular masses ranging from 20–100 kDa, as has been shown by immunoprecipitation studies [4, 6].

The cellular function of the complete particle has long remained obscure. However, very recently, following the identification of yeast homologues of the PM/Scl-complex it was shown that in both human and yeast, where it is termed exosome, the PM/Scl-particle is involved in splicing of 5.8 S rRNA and several U-snRNAs [7, 8, 9]. The yeast homologue of the PM/Scl-100 protein is termed Rrp6p, with amino acids 256–597 of the PM/Scl-100 protein sharing some homology with the RNase D family and amino acids 290–554 encoding an exonuclease domain [7, 10].

In previous studies we and others [11, 12, 13] have shown that PM/Scl sera react with two polypeptides of apparent molecular masses of 100 and 75 kDa, termed PM/Scl-100 and PM/Scl-75, respectively. The cDNAs for both major PM/Scl antigens have been cloned with the cDNA sequence of the PM/Scl-100 autoantigen displaying a deletion/insertion polymorphism of 25 amino acids starting at position 695 [11, 12, 13]. Antigenic regions on the PM/Scl-100 antigen have been mapped to some extent [11, 14], and a major epitope core has been identified between amino acids 232 and 241 [15, 16]. In the study presented here we focused on the distribution of epitopes and fine-mapping of the major PM/Scl-100 epitope.

Materials and methods

Human sera

Anti-PM/Scl autoantisera, all previously tested for anti-PM/Scl-100 specificity, were provided by Drs. E. Genth, Rheumaklinik Aachen, Aachen, Germany, and E.M. Tan, W.M. Keck Autoimmune Disease Center, La Jolla, Calif., USA. Some clinical data were available for ten sera. Of these ten patients five (A2, A4, A7, Lhs, Knl) had systemic sclerosis (sSc), two (A3, A10) had dermatomyositis/systemic sclerosis overlap (DM-sSc), one (A9) had polymyositis/systemic sclerosis overlap (PM/Scl), and two (A6, Zmr) had polymyositis (PM). No antibody specificities other than anti-PM/Scl were detected except in patient A7, who was positive for rheumatoid factor.

Human control sera from healthy donors were obtained from the Blood Center of Heidelberg Medical School, Heidelberg, Germany.

Miscellaneous antibodies

Horseradish peroxidase coupled goat anti-human IgG antibodies were purchased from Dianova (Hamburg, Germany).

Synthesis of immobilized oligopeptides and immunodetection

Peptides were either synthesized manually on activated membranes containing a di-β-alanine spacer (SPOT system, Genosys, UK) or with a ASP222 machine on activated membranes containing a PEG 600 amino spacer (Abimed, Germany) using fMoc protected amino acid derivatives (SPOT) according to the procedure described by Frank [17]. Following completion of the synthesis membranes were washed three times with Tris-buffered saline (TBS; 10 mM Tris/Cl pH 7.6, 150 mM NaCl) for 10 min per wash. Nonspecific binding sites were blocked by overnight incubation of the membranes in blocking buffer [TBS containing 50% horse serum, 1× commercial blocking buffer (supplied with the SPOTs synthesis kit), 5% sucrose (w/v), 0.2% Tween 20 (v/v)] at 4°C followed by one wash with TBS/0.2% Tween 20. The membranes were then incubated twice for 2 h with serum antibodies at dilutions of 1/100 in antibody buffer [TBS containing 3% horse serum, 1× commercial blocking buffer (supplied with the SPOTs synthesis kit), 5% sucrose (w/v), 0.05% Tween 20 (v/v)]). Unbound antibodies were removed by washing the membranes 3× in TBS/0.2% Tween 20 for 10 min per wash, and 3× in TBS for 10 min per wash. Bound antibodies were visualized using horseradish peroxidase coupled goat anti-human IgG antibodies at a dilution of 1/5000 in antibody buffer for 75 min followed by detection with ECL western blotting detection reagents (Amersham International, UK).

Mutational analysis of the major epitope

The major epitope-containing peptide LDVPPALADFIHQQR was analyzed by glycine-walk. For a complete mutational analysis every amino acid of the major epitope-containing peptide LDVPPALADFIHQQR was replaced by any one of the 20 naturally occurring amino acids. The resulting peptides were probed with selected anti-PM/Scl autoantisera.

Computer assisted analysis of the PM/Scl-100 major epitope

Secondary structure prediction was carried out with the program PEPTIDESTRUCTURE from the/HUSAR program package (version 5.0) of German Cancer Research Center, Heidelberg, Germany. Search for 3-D homology models was performed using the search option of the Swiss-Model automated comparative protein modeling server (http://www.expasy.ch/swissmod/swiss-model.html) with the PM/Scl-100 major epitope peptide LDVPPALADFIHQQR as template. Homology models were visualized with the program RASMOL (version 2.6, http://www.umass.edu/microbio/rasmol/). A helical wheel representation was calculated using the program HELWHEEL of the PC-Gene program package (version 6.0).

Results

Epitope distribution on the PM/Scl-100 autoantigen

Peptides of 15 amino acids in length with overlaps of ten amino acids were synthesized on activated membranes

Table 1 Reactivities of anti-PM/Scl autoantisera with PM/Scl-100 15-mer peptides

Peptide		Amino acid position	Serum												
No.	Sequence		A6[a]	A7[a]	Ctr[a]	A2	A3	A4	A9	A10	Lhs	Zmr	Le	Hts	Knl	Nes
2	TREPRVLSATSATKS	6–20	+	+	–	–	+	–	+	–	+	+	+	–	+	+
34	KAKSETFRLLHAKNI	166–180	+	–	+	+++	++	–	–	–	+	+	+	++	+	++
35	TFRLLHAKNIIRPQL	171–185	+	–	+	+++	–	–	–	–	–	–	+	–	+	+
39	IDNSNTPFLPKIFIK	191–205	+	–	+	++	–	–	–	–	–	+	–	–	–	–
40	TPFLPKIFIKPNAQK	196–210	+	+	+	+++	–	–	–	–	–	+	–	–	+	+
43	PLPQALSKERRERPQ	211–225	+	+	–	+	++	–	–	–	++	–	–	+	++	++
47	**LDVPPALADFIHQQR**	**231–245**	**+**	**+**	**+**	**+++**	**+++**	**+**	**+++**	**+++**	**+++**	**+++**	**+**	**+++**	**++**	**+++**
100	KQKKHLNTQQLTAFQ	497–511	–	+	+	–	–	–	–	+	+	–	–	–	–	–
108	MLKIAEELPKEPQGI	537–551	+	+	–	–	–	–	–	–	–	–	–	–	–	–
114	NEMHLLIQQAREMPL	567–581	+	+	–	–	–	–	–	–	–	–	–	–	–	–
115	LIQQAREMPLLKSEV	572–586	+	+	–	++	–	–	–	–	–	–	–	–	–	–
135	GPLTVAQKKAQNIME	672–686	+	+	+	–	–	–	–	–	–	+	–	–	–	–
140	PSLGHRAPVSQAAKF	697–711	–	+	–	–	–	–	–	–	+	–	+	–	+	–
141	RAPVSQAAKFDPSTK	702–716	–	+	–	–	–	–	–	–	+	+	+	+	+	–
149	KAAEQTAAREQAKEA	742–756	+	+	–	–	–	+	–	–	–	–	–	–	–	+
150	TAAREQAKEACKAAA	747–761	+	+	–	–	–	–	–	–	–	–	–	–	–	–
154	VRQQVVLENAAKKRE	767–781	+	+	–	–	–	–	–	+	–	–	+	–	–	–
159	QKQEKKRLKISKKPK	792–806	+	+	+	+	–	–	–	++	–	–	–	–	+	–
160	KRLKISKKPKDPEPP	797–811	+	+	+	+	++	–	–	++	–	–	–	–	+	+
168	SSQFDPNKQTPSGKK	837–851	–	+	+	+++	+	–	–	++	++	++	–	+	+++	+++
169	PNKQTPSGKKCIAAK	842–856	–	+	+	+++	–	–	–	+	–	–	–	–	+++	++
170	PSGKKCIAAKKIKQS	847–861	+	+	+	+	–	–	+	++	–	–	+	–	+	++
171	CIAAKKIKQSVGNKS	852–866	+	+	+	+	+	–	+	+	+	–	++	++	+	++

[a] Anti-PM/Scl-100 autoantisera used for the initial scan of the complete PM/Scl-100 autoantigen with 15-mer peptides overlapping by ten amino acids

as described above, and covered the complete amino acid sequence of the PM/Scl-100 autoantigen (accession number JH0796) [11]. Upon scanning of the membranes with three anti-PM/Scl sera (A6, A7, and Ctr) several epitopes could be identified on the PM/Scl-100 autoantigen (data not shown). Peptides recognized by at least two of the three sera used in the initial immunoscan were probed with more anti-PM/Scl autoantisera (Table 1). Peptides 140 and 141 (amino acids 697–716) were included in this second synthesis since they are located in the deletion/insertion polymorphism region (amino acids 695–720) [11, 12, 14]. Most sera react with epitopes located between amino acids 166 and 245 (Table 1). A second cluster of epitopes is located between amino acids 672 and 866. Epitopes between amino acids 246 and 671 are only infrequently recognized by the PM/Scl-sera. An epitope located between amino acids 231 and 245 (Table 1, peptide 47) is recognized by all anti-PM/Scl-sera. As for the individual sera, no association was observed between the epitope recognition pattern and the underlying disease.

Fine-mapping of the PM/Scl-100 major epitope

For fine-mapping of the PM/Scl-100 major epitope core, amino acids 226–255 were synthesized again in 15-mers offset by one amino acid and probed with two representative patient sera. The results show that the major epitopes are centered around the core sequence PALADFI but are of different lengths with respect to the serum used for the

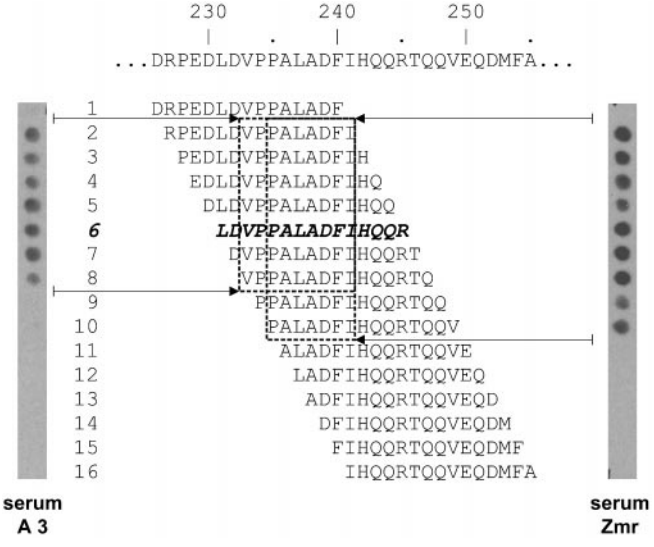

Fig. 1 Fine-mapping of the PM/Scl-100 major epitope. For fine mapping of the PM/Scl-100 major epitope amino acids 226–255 were synthesized as 15-mer peptides offset by one amino acid and probed with two anti-PM/Scl autoantisera (A3, Zmr) at dilutions of 1/100. The results of the peptide scans are aligned with the corresponding peptides. The minimal epitope recognized by a given serum is the consensus sequence of all reactive peptides (*boxed sequences*). The minimal epitope recognized by serum A3 is the nonamer VPPALADFI. The minimal epitope recognized by serum Zmr is the heptamer PALADFI

assay (Fig. 1, Table 2). The consensus epitope sequence for serum A3 is the 9-mer VPPALADFI whereas the consensus epitope for serum Zmr is the 7-mer PALADFI. Altogether the length of the major epitope as recognized by different sera varies between 5 and 11 amino acids (Table 2). When the peptides around the major epitope core sequence were synthesized in decreasing lengths and probed with serum A3, the above 9-mer was also detected, however, with different strengths of binding. This result is in reasonable agreement with the fine-mapping studies (Fig. 2).

Table 2 Serum-specific minimal length of the PM/Scl-100 major epitope

Serum	Minimal amino acid sequence recognized	Amino acid position	Peptide length
A3	VPPALADFI	233–241	9-mer
A6	DLDVPPALADF	230–240	11-mer
A10	ALADF	236–240	5-mer
Knl	LDVPPALADF	231–240	10-mer
Nes	LDVPPALADF	231–240	10-mer
Zmr	PALADFI	235–241	7-mer

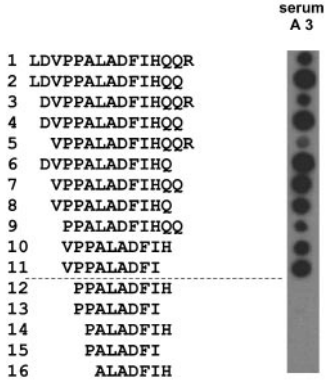

Fig. 2 Minimal peptide recognized by anti-PM/Scl autoantiserum A3. For determination of the minimal amino acid sequence recognized by serum A3 peptides of decreasing lengths from 15 to 7 amino acids were synthesized centering around leucine at position 237 of the PM/Scl-100 sequence. The peptides were probed with serum A3 at a dilution of 1/100. The minimal immunoreactive peptide (peptide no. 11, VPPALADFI) recognized by serum A3 corresponds well with the results from epitope fine-mapping

Mutational analysis of the major epitope region

The initial 15-mer major epitope region of the PM/Scl-100 antigen was subjected to a mutational analysis in order to determine the amino acids relevant for the binding of the corresponding antibodies. For this purpose every single amino acid in the antigenic peptide LDVPPALADFIHQQR (amino acid 231–245) was successively replaced by glycine, and the resulting mutant peptides were probed with different sera (Fig. 3, Table 3). The effect of a single mutation on antibody binding was most clearly visible when serum Lhs was used for the immunodetection but was also observed when other anti-PM/Scl sera were used (Fig. 3). The results show that Leu-237 of the original PM/Scl-100 sequence is the most essential amino acid for binding of the correspond-

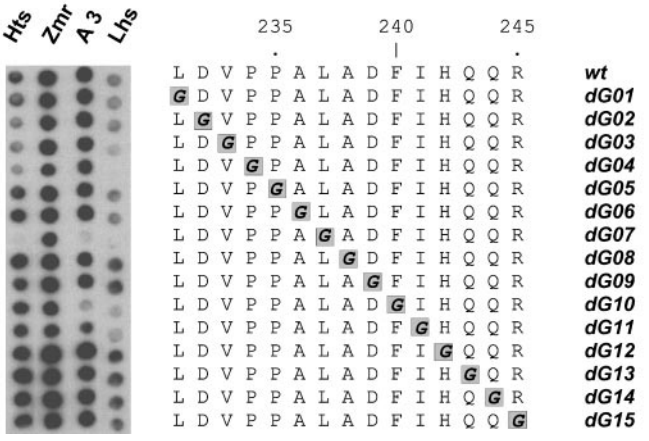

Fig. 3 Mutational analysis of the PM/Scl-100 major epitope by glycine replacement. To investigate the degree of contribution of a given amino acid to binding of anti-PM/Scl-100 autoantibodies all amino acids in the sequence LDVPPALADFIHQQR (amino acids 231–245) were successively replaced by glycine, and the resulting peptides were probed with four PM/Scl sera (Hts, Zmr, A3, Lhs). The results of the peptide scan were aligned with the corresponding peptide sequences. A comparable pattern of reactivity can be seen with all four sera. However, when using serum Lhs the pattern of immunoreactivity is most pronounced. It is evident that the central Leu-237 has an essential role in binding of the corresponding antibodies (dG07). Additional contributions to antibody binding come from Pro-234, Phe-240, and Ile-241 (dG04, dG10, dG11). *dG* Mutant peptides; *wt* wild type

Table 3 Inhibitory effect of successive glycine replacement on antigenicity [(+) weak inhibition, + inhibition, ++ strong inhibition, +++ very strong inhibition]

Serum	Amino acid replaced by G														
	L→G	D→G	V→G	P→G	P→G	A→G	L→G	A→G	D→G	F→G	I→G	H→G	Q→G	Q→G	R→G
Ctr				+			+			+					
A3				(+)			+++			++	+				
A 10							+			++	++				
Lhs				+++			+++			++	++				
Zmr				+			+			+	+				
Le							+++			+	+				
Hts				+			+++				+				

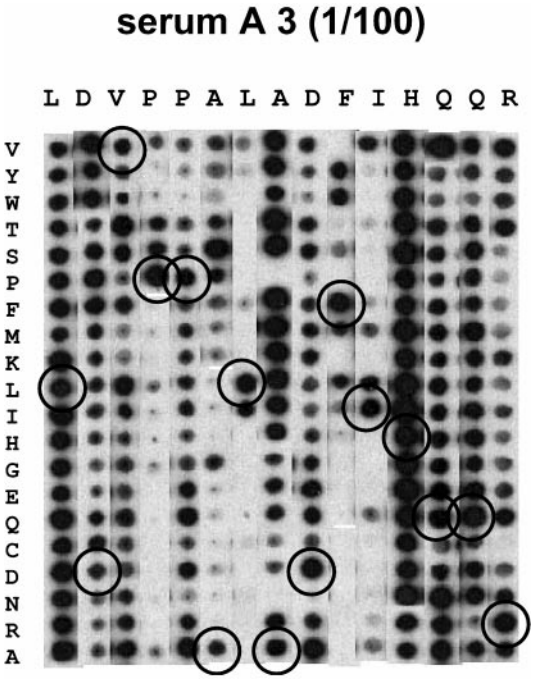

Fig. 4 Mutational analysis of the PM/Scl-100 major epitope by complete amino acid exchange. To further establish the contribution of the single amino acids of the PM/Scl-100 major epitope to antibody binding all amino acids in the epitope sequence LDVPPALADFIHQQR (amino acids 231–245) were successively mutated to each of the 20 naturally occurring amino acids. The resulting peptides were probed with serum A3 at a dilution of 1/100. *Circled* Wild-type peptides

Table 4 Secondary structure prediction for the PM/Scl-100 major epitope region[a] (*T* turn, *H* α-helix)

Position	Amino acid	CF-Pred[b]	GOR-Pred[c]
1	L	–	–
2	D	–	–
3	V	–	–
4	P	–	–
5	P	T	H
6	A	T	H
7	L	H	H
8	A	H	H
9	D	H	H
10	F	H	H
11	I	H	H
12	H	H	H
13	Q	H	H
14	Q	H	T
15	R	H	T

[a] As calculated by the program Peptidestructure from the HUSAR program package (version 6) of the German Cancer Research Center

[b] Secondary structure prediction according to the Chou-Fasman method

[c] Secondary structure prediction according to the Garnier-Osguthorpe-Robson method

ing autoantibodies. Additional essential amino acids are Pro-234, Phe-240, and Ile-241.

To verify this result, a complete mutational analysis using serum A3 was performed as described (Fig. 4). The central role of Leu-237 was further established showing that it can be replaced only either by Ile or to a lesser extend by Val, two amino acids similar in size and charge. All other amino acid replacements in this position inhibit binding of the antibodies. Pro-234 can only be replaced by Val, Thr, or Ser, Phe-240 by Tyr, Trp, Met, or Leu, and Ile-241 by Val, Met, or Leu. In addition, Ala-236 also was shown to contribute significantly to autoantibody binding since it can only be replaced by Val, Tyr, Thr, Ser, Pro, or Gly.

Amino acids Leu-231, Asp-232, Val-233, His-242, Gln-243, and Gln-244 obviously do not contribute to antibody binding, since they can be replaced by any other amino acid without affecting antigen-antibody complex formation. The apparent increase in intensity of the immunoreaction compared to the immunoreactivity with the wild-type peptide when mutating the C-terminal amino acids His, Gln, or Arg probably rather reflects a low coupling efficiency of those amino acids during the peptide synthesis procedure than a true increase in immunoreactivity (Figs. 3, 4) [17].

Theoretical structure predictions of the PM/Scl-100 major epitope

The distribution of amino acids contributing to antibody-binding suggests a local alpha-helical secondary structure for the PM/Scl-100 major epitope. Indeed, secondary structure prediction for the 15-mer LDVPPALADFIHQQR with the program PEPTIDESTRUCTURE from the/HUSAR program package supports this notion (Table 4).

A homology search with the antigenic peptide of the PM/Scl-100 antigen (LDVPPALADFIHQQR) against the Swiss-Model 3-D database revealed topological homology with 15 amino acids (58–72) of the human heterochromatin protein p25β (accession number P23197) whose 3-D structure is reported to be determined by magnetic resonance analysis (Swiss-Model 3-D database with the number 1AP0; Fig. 5a) [18, 19]. According to these data, those amino acids of the p25β protein corresponding to the antigenic peptide of the PM/Scl-100 major epitope form a local α-helix (Fig. 5a). A helical wheel representation of amino acids 231–243 of the PM/Scl-100 antigenic peptide shows that the amino acids relevant for binding of the autoantibodies in serum Lhs are in close proximity and are located on one side of the α-helix thus rendering them accessible to an antibody (Fig. 5b).

No direct immunological relationship was observed, however, between the PM/Scl-100 major epitope and the structurally related corresponding peptide of the human heterochromatin protein p25β since none of our patient sera tested reacted with the peptide LDCPDLIAEFLQSQK (amino acids 58–72 of p25β, data not shown).

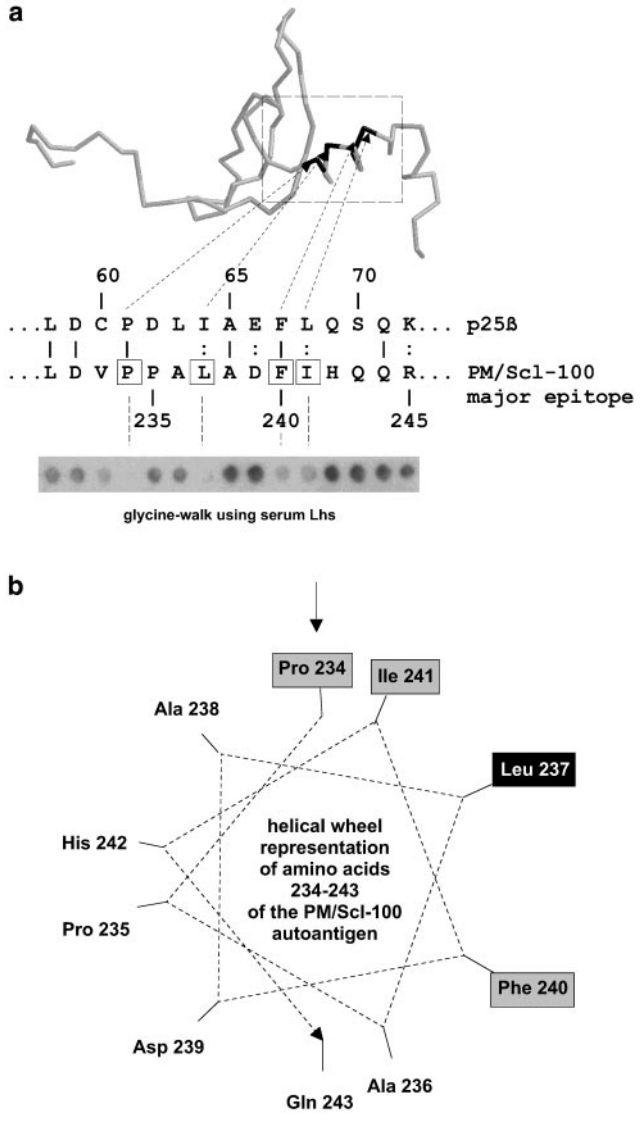

Fig. 5a,b Proposed structural model for the PM/Scl-100 major epitope region. **a** A homology search with the amino acid sequence LDVPPALADFIHQQR (amino acids 231–245) containing the PM/Scl-100 major epitope against the 3-D Swiss-Model database revealed some structural homology to amino acids 58–72 of the human heterochromatin protein p25β (accession number P23197). The structure of amino acids 10–80 of p25β was determined by magnetic resonance and deposited in the Swiss-Model database (accession number 1AP0). An alignment of the relevant amino acids of the two proteins together with the results of the mutational analysis of the PM/Scl-100 major epitope using serum Lhs are displayed (see Fig. 3). *Boxed* Amino acids of the PM/Scl-100 major epitope responsible for antibody binding. The amino acids of the p25β peptide corresponding to the amino acids of the PM/Scl-100 major epitope relevant for antibody binding are in close contact when assuming an α-helical structure, implying that the PM/Scl-100 major epitope also forms an α-helix. **b** A helical wheel representation of amino acids 234–243 demonstrates that the amino acids relevant for binding of the antibodies in serum Lhs are located on the same side of the α-helix making them accessible to a single antibody. *Boxed, shaded* Amino acids relevant for binding of the antibody; *white on black background* the central leucin (Leu-237)

Discussion

By systematically scanning the PM/Scl-100 autoantigen with anti-PM/Scl patient sera for epitopes we demonstrated that a cluster of epitopes is located between amino acids 166 and 245. Within this first cluster of epitopes a major epitope was detected between amino acids 231 and 245, a finding that is consistent with previous results [15, 16]. A second cluster of epitopes is located between amino acids 672 and 866. With the exception of the major epitope, no other epitope emerged which was unequivocally recognized by all anti-PM/Scl-sera tested. Thus one must discriminate between a general major epitope, recognized by all anti-PM/Scl autoantisera, and several individual epitopes, recognized by individual sera.

Epitopes between amino acids 246 and 671, in contrast, are infrequently recognized by anti-PM/Scl autoantisera. These results are consistent with those of previous studies that determined immunoreactive areas in amino acids 153–324 and 492–703 [11] or 156–312, 507–749, and 750–882 [14]. In view of these results it is notable that the region of amino acids 246–671, where only few epitopes are detected, covers the area of highest homology of the PM/Scl-100 antigen to its yeast homologue [7]. A homology search using hidden-Markow models identified the PM/Scl-100 antigen as a member of the RNase D family [10]. The region of highest homology of the PM/Scl-100 antigen to other members of this family is also located between amino acids 291 and 554. In addition, this region is reported to contain an exonuclease domain (amino acids 291–378) [20].

An additional interesting finding is the localization of epitopes between amino acids 697 and 716 that are recognized by 6 of 14 anti-PM/Scl autoantisera. The published PM/Scl-100 cDNA sequences differ in the presence or absence of a stretch of 25 consecutive amino acids starting from amino acid 695 [11, 12, 14]. It is unclear whether this difference is based on differential splicing or reflects a polymorphism. It is also unclear whether the difference in length affects the cellular function of the PM/Scl-100 protein. Thus antibodies reacting with epitopes in this area may aid in the elucidation of the cellular distribution or function of these two different forms of the PM/Scl-100 antigen.

Fine-mapping of the major epitope region reveals that with respect to the individual serum tested the length of the major epitope varies between 5 and 11 amino acids These results imply that with respect to the antigen one must discriminate between an “epitope,” which is recognized by an individual serum, and an “epitope core,” which is a minimal sequence within an antigen common to all individual epitopes within the region under investigation. The different intensities of immunoreactive peptides of decreasing length probably reflect structural differences in the peptides due to the presence or absence of specific amino acids rather than true differences in immunoreactivity. One must keep in mind that epitopes are seen within the context of a polypeptide chain whereas

the approach presented here focuses on isolated peptides whose accessibility by an antibody can be influenced directly by the peptides' secondary structure.

Further characterization of the PM/Scl-100 major epitope suggests a local α-helical structure for several reasons: (a) Mutational analysis reveals that about every third amino acid of the peptide is relevant for binding of the corresponding antibodies. Assuming an average of 3.6 amino acids per helix turn, an α-helical structure of the peptide would bring this relevant amino acids in close contact with each other and thus form a consecutive amino acid sequence easily recognized by an antibody, as was demonstrated by a helical wheel representation of the major epitope region. (b) A computer-based structure analysis predicts a local α-helical structure for the peptide LDVPPALADFIHQQR. (c) A homology search in the Swiss-Model database shows some structural homology with an experimentally determined α-helical secondary structure from the heterochromatin protein beta (p25β).

The proposed structural homology of the PM/Scl-100 major epitope to a stretch of amino acids in the heterochromatin protein p25β is of high interest. Heterochromatin protein p25β is a known autoantigen recognized by anti-chromo autoantibodies from a subset of patients having also anti-centromere autoantibodies [21, 22, 23]. The chromodomain, which is responsible for binding of p25β to heterochromatin is highly conserved among species [24]. Constitutive heterochromatin itself is an integral part of the centromeres and nucleolar organizers [25]. The stretch of amino acids (amino acids 58–72) homologous to the PM/Scl-100 major epitope is located at the C-terminal borderline of the p25β chromo-domain (accession number P23197). In this context it is of interest to note that a subset of anti-chromo autoantibodies are reported to recognize a recombinant fragment covering amino acids 1–69 of the heterochromatin protein p25β [18]. However, since none of our anti-PM/Scl sera reacted with the corresponding peptide LDCPDLIAEFLQSQK of the heterochromatin protein p25β, a direct immunological relationship appears improbable. Nevertheless, we do not exclude a more complex immunological relationship between the major epitope of the PM/Scl-100 autoantigen and its corresponding peptide in the heterochromatin protein p25β since structural features inherent to our assay might have some influence on the immunoreactivity of a serum with a peptide.

In summary, we analyzed a major antigenic peptide of the PM/Scl-100 autoantigen. Based on mutational analysis and theoretical structure prediction we propose an α-helical structure for this peptide. The results of a search for a 3-D homology hints at some structural relationship with the heterochromatin protein p25β. From the data presented here, however, it is unclear whether there is a common mechanism leading to an autoimmune response against both the PM/Scl-100 autoantigen and the heterochromatin protein p25β. Since both antigens are associated with scleroderma-related autoimmune diseases, a possible immunological correlation between these two antigenic entities should prove highly interesting.

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