Structural analysis of the major ribosomal P epitope C22

[1]Kai Kessenbrock, [2]Marvin J. Fritzler and [3]Michael Mahler

[1]Max Planck Institute of Neuroimmunology, Martinsried, Germany, [2]University of Calgary, Calgary, AB, Canada, [3]Dr. Fooke Laboratorien GmbH, Neuss, Germany

INTRODUCTION

Autoantibodies to the three ribosomal phospho (-P) proteins (P0, P1 and P2 summarized as Rib-P) are found in 10-40% patients with systemic lupus erythematosus (SLE). The Rib-P antigen consists of three components of the 60S ribosomal subunit designated P0(38 kD), P1(19 kD), and P2(17 kD). A pentameric complex of one copy of P0 and two copies each of P1 and P2 interacts with the 28S rRNA molecule forming a GTPase domain, which is active during the elongation step of protein translation. A common major epitope (C22) of the Rib P antigens has been localized to the C-terminus. In a more precise study the epitope-core has been identified as GFGLFD, wherein the phenylalanine residues Phe^{111} and Phe^{114} (of human P2) represent the key residues for antibody recognition.

MATERIALS AND METHODS

Membrane bound peptides prepared by SPOT technology were used to confirm the key amino acid residues of the C22 peptide recognized by anti-ribosomal P protein antibodies in larger group of anti-Rib-P samples (n=20) all from SLE patients. Structural analysis of the C22 peptide was done by Nuclear magnetic resonance (NMR) on a Bruker DRX600 spectrometer (Bruker, Billerica, MA, USA). NMR data was used to generate an approximated molecular model of the six C-terminal amino acids using a molecular visualization and analysis program for the display and manipulation of the surfaces of molecules and their electrostatic properties (GRASP).

RESULTS

SPOT technology showed that all 20 of the anti-Rib-P positive sera recognized an epitope clustered around the amino acids *Phe^{111}* and *Phe^{114}*. Based on the distance of the key amino acid residues identified by mutational analysis we suggested an alpha-helical structure of the epitope. Using NMR analysis we found that the C-terminal six amino acids of the C22 peptide show a helical tendency. Based on the NMR data we propose a 3D-model of the Rib-P major epitope showing an alpha-helical structure.

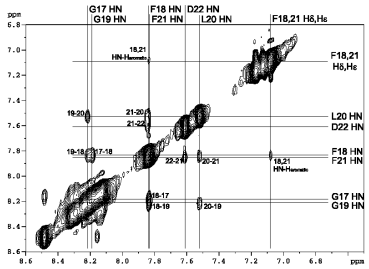


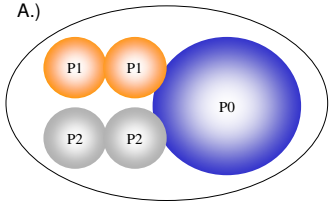
Figure 1

NOESY (Nuclear Overhauser Effect SpectroscopY): ^{1}H-^{1}H homonuclear NOESY experiment of the C22 peptide recorded in the presence of 40% Trifluoroethanol, showing cross peaks corresponding to short range HN-HN distances, as typically found in an αlpha-helical conformation. The assignment of the chemical shifts is indicated.

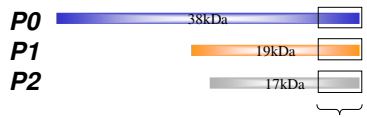
Figure 2

Identification of the key amino acids for antibody binding to C22 by Alanine walk mutational analysis and structure prediction of the epitope based on the NMR data. The structure of the heterodimer P0(P1/P2)$_2$ of the ribosomal subunit a.) is based on two copies of each P1 and P2 and of one copy of P0 b.). The shared C-terminus (C22) c.) contains two phosphorylated serine residues and the epitope-core GFGLFD. Mutational analysis revealed that the two Phe residues represent the key amino acids for antibody binding d.). The approximated structure of the epitope-core based on NMR data is shown in e.).

A.)



B.)



C.)

K V E A K K E E S* E E S* D D D M G F G L F D

D.) SPOT analysis

No	ID	Sequence
	WT	DDDMGFGLFD
1	ΔD^{106}	**A**DDMGFGLFD
2	ΔD^{107}	D**A**DMGFGLFD
3	ΔD^{108}	DD**A**MGFGLFD
4	ΔM^{109}	DDD**A**GFGLFD
5	ΔG^{110}	DDDM**A**FGLFD
6	ΔF^{111}	DDDMG**A**LFD
7	ΔG^{112}	DDDMGF**A**LFD
8	ΔL^{113}	DDDMGFG**A**FD
9	ΔF^{114}	DDDMGFGL**A**D
10	ΔD^{115}	DDDMGFGLF**A**

E.)

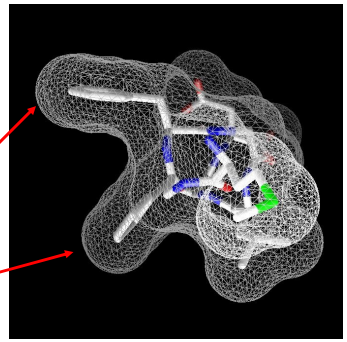
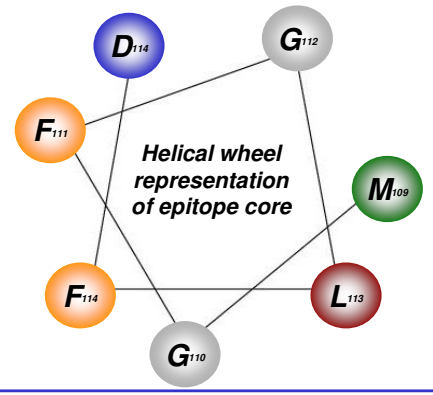


Figure 3

Helical wheel representation of the C22 epitope. Binding relevant Phe residues (Phe^{111} and Phe ^{114} of human P2) are exposed on one side of the alpha-helix.



CONCLUSION

Based on mutational analysis and NMR analysis, we propose an alpha-helical structure of the major ribosomal P protein epitope. Furthermore, we conclude that the key amino acids of the epitope core are located on the same side of the alpha-helix.

REFERENCES

Sturgill BC, Carpenter RR. Antibodies to ribosomes in systemic lupus erythematosus. Arthritis Rheum 1965; 8:213-218.

Elkon KB, Parnassa AP, Foster CL. Lupus autoantibodies target ribosomal P proteins. J Exp Med 1985; 162:459-471.

Bonfa E, Golombek SJ, Kaufman LD, Skelly S, Weissbach H, Brot N, Elkon KB. Association between lupus psychosis and anti-ribosomal P protein antibodies. N Engl J Med 1987; 317:265-271.

Mahler M, Kessenbrock K, Raats J, Williams R, Fritzler MJ, Blüthner M. Characterization of the human autoimmune response to the major C-terminal epitope of the ribosomal P proteins. J Mol Med 2003; 81:194-204.

Gerli R, Caponi L. Anti-ribosomal P protein antibodies. Autoimmunity 2005; 38:85-92. Review

Soares MR, Bisch PM, Campos de Carvalho AC, Valente AP, Almeida FC. Correlation between conformation and antibody binding: NMR structure of cross-reactive peptides from T. cruzi, human and L. braziliensis. FEBS Lett. 2004 Feb 27;560(1-3):134-40.