Journal of Biomolecular NMR **29:** 553–554, 2004.
© 2004 *Kluwer Academic Publishers. Printed in the Netherlands.*

Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments of the apo Sm14-M20(C62V) protein, a mutant of *Schistosoma mansoni* Sm14

Thelma A. Pertinhez[a], Maurício L. Sforça[a], Adriana C. Alves[a], Celso R.R. Ramos[b], Paulo L. Ho[b,c,d], Miriam Tendler[e], Nilson I.T. Zanchin[a] & Alberto Spisni[a,f,*]
[a]*Center for Structural Molecular Biology, LNLS, P.O. Box 6192, Campinas – SP, 13084-971, Brazil;* [b]*Biotechnology Center, Butantan Institute, São Paulo, Brazil;* [c]*Chemistry Institute and* [d]*Bioscience Institute, University of São Paulo, São Paulo, Brazil;* [e]*Helmintology Department, FIOCRUZ, Rio de Janeiro, Brazil;* [f]*Department of Experimental Medicine, Sect., University of Parma, 43100 Parma, Italy*

Received 2 April 2004; Accepted 26 April 2004

Key words: FABP, *Schistosoma mansoni*, Sm14 protein, vaccine

Biological context

Schistosomiasis is a public health problem in many developing countries and is considered the most important parasitic disease after malaria. Schistosomiasis treatment relies heavily on prazinquantel, and concerns are being raised about emerging drug-resistant worms (Doenhoff et al., 2002). Vaccine development against *S. mansoni* is thought to be a better way to control the disease, along with establishment of proper sewerage systems and education. While new potential vaccines will arise from genomic information (Verjovski-Almeida et al., 2003), several vaccine candidates are under investigation. One of these is the antigen Sm14, a 14 kDa protein identified in an immunoscreening using antibodies raised against an extract from adult *S. mansoni* worms (Moser et al., 1991). Sm14 belongs to the fatty acid binding protein family (FABP) and it shows 47% of sequence similarity with the human brain type FABP (PDB 1JJX). Since schistosomes cannot synthesize sterols and fatty acids 'de novo', they are heavily host-dependent for these molecules and Sm14 is expected to play a vital role in the uptake, transport and compartmentalization of host-derived fatty acids. Sm14 was shown to reduce the worm burden of vaccinated mice challenged with cercariae larvae of *S. mansoni* and to protect against the meta-cercariae challenge of *Fasciola hepatica*, thus indeed resulting in a potential candidate for an anti-helminth vaccine (Almeida et al., 2003). The polymorphism of the protein is low, the most common being at the amino acid position 20 (Met ↔ Thr). Interestingly, the methionine isoform is more stable than the threonine isoform, while it exhibits a higher affinity for the long-chain fatty acids, no differences are detected for the short-chain ones (Ramos et al., 2003). The instability of the recombinant isoforms has been one of the main concerns during Sm14 production, transport and storage as it hampers its use as a vaccine. Therefore, studies to determine the structure and molecular flexibility of these isoforms are necessary to devise mutants showing both higher stability and protective efficiency against these parasitic diseases. The only cysteine residue at position 62 was identified as responsible for Sm14 time dependent dimerization and aggregation (C.R.R. Ramos et al., unpublished results). A mutant protein, in which Cys62 was replaced by Val, Sm14-M20(C62V), was over-expressed in *E. coli*. Since it showed good protective effect as well as a folding and a structural stability comparable to the native protein, as judged by CD analysis, it has been selected for further structural studies.

*To whom correspondence should be addressed. E-mail: alberto@lnls.br or aspin@unipr.it

Methods and experiments

The Sm14-M20(C62V) was expressed in *E. coli* strain BL21(DE3) using the pAE vector (Ramos et al., 2003). For ^{15}N and ^{13}C isotopic enrichment, *E. coli* cells were incubated in M9 minimal medium containing $^{15}NH_4Cl$ and $^{13}C_6H_{12}O_6$ as nitrogen and carbon sources, respectively. The protein, which sediments with cell debris following centrifugation of the

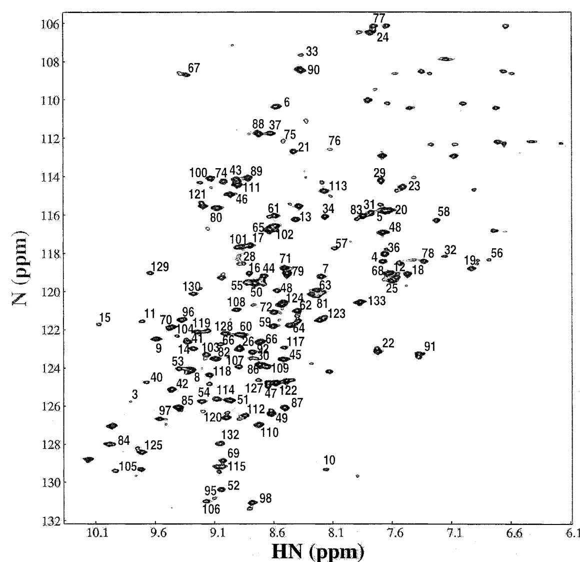

Figure 1. ^{15}N-HSQC spectrum of 0.7 mM apo Sm14-M20(C62V) protein in phosphate buffer pH 6.3 at 293 K, collected at 600 MHz. Only the backbone cross peaks are indicated with the residue numbers. The extra NH peaks should be related to conformational exchange.

E. coli lysates, was extracted with deoxycholate 0.5% and fractionated by anion exchange chromatography. A final chromatographic step on a Lipidex column (Sigma) was used to convert the protein to the apo form [apo-Sm14-M20 (C62V)].

Samples for NMR analysis were prepared dissolving the protein in 50 mM NaH_2PO_4 pH 6.3, 0.05% NaN_3 in 5% or 100% D_2O to a final concentration of 0.7 mM. NMR spectra were collected at 293 K on either a Varian Inova® 500AS or 600AS spectrometer at the LNLS, Brazil, or on a Bruker Avance® 600 MHz spectrometer, at the Porto Conte Laboratory, Alghero, Italy.

For the backbone assignments ^{15}N edited HSQC spectrum, the HNCA, HN(CO)CA, HNCO, HNCACB, CBCACONH 3D triple resonance experiments and a TOCSY-NHSQC were obtained using the 5% D_2O sample. For the side-chain assignments we carried out ^{13}C edited HSQC and HCCH-TOCSY, HCCH-COSY, (H)CCH-TOCSY and TOCSY-CHSQC experiments using the 100% D_2O sample. Spectra were processed and analysed using the NMRPipe/NMRView softwares (Delaglio et al., 1995; Johnson and Blevins, 1994).

Extent of assignments and data deposition

Approximately 95% of the backbone has been assigned. Figure 1 shows the 124 assigned peaks from a total of 131 (133 amino acids minus 2 prolines). Briefly, the CO, Cα, Cβ and Hα assigned atoms corresponded to 100%, 100%, 96% and 100% of the total resonances of each nucleus. From the unassigned amide residues, two are located at the N-terminus (M1 and S2). The remaining five unassigned residues correspond to T39, T93, Q94, T116 and R131.

Approximately 90% of the total side-chain signals have been completely assigned (120 from 133 residues). Some aromatic protons remain unassigned for F42, F57, F70 and F66, as well as some aliphatic protons of N15 and S75. The forthcoming NOE analysis is expected to allow their determination.

The global chemical shift analysis, based on the available data and carried out using the CSI method (Wishart et al., 1992), provided a preliminary description of the protein secondary structure confirming the expected presence of 10 β-strands and two helical segments (data not shown).

Resonance assignments have been deposited in the BioMagRes Bank under accession number 6150.

Acknowledgements

Supported by FAPESP grants (NMR project 99/11030-9, CEPID-CBME 98/14138-2, PLH 98/14961-0, TAP 00/02026-7); CNPq (PLH, 521036/96); Fundação Butantan (PLH). CRRR – CAPES fellowship; MLS is recipient of a FAPESP post-doc fellowship.

References

Almeida, M.S., Torloni, H., Ho, P.L., Vilar, M.M., Thaumaturgo, N., Simpson, A.J.G. and Tendler, M. (2003) *Parasite Immunol.*, **25**, 135–137.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Doenhoff, M.J., Kusel, J.R., Coles, G.C. and Cioli, D. (2002) *Trans. Roy. Soc. Trop. Med. Hyg.*, **96**, 465–469.

Johnson, B. and Blevins, R.A. (1994) *J. Biomol. NMR*, **4**, 603–614.

Moser, D., Tendler, M., Griffiths, G. and Klinkert, M.Q. (1991) *J. Biol. Chem.*, **266**, 8447–8454.

Ramos, C.R.R., Figueredo, R.C.R., Pertinhez, T.A, Vilar, M.M., Nascimento, A.L.T.O., Tendler, M., Raw, I., Spisni, A. and Ho, P.L. (2003) *J. Biol. Chem.*, **278**, 12745–12751.

Verjovski-Almeida, S. et al. (2003) *Nat. Genet.*, **35**, 148–157.

Wishart, D.S., Sykes, B. and Richards, F.M. (1992) *Biochemistry*, **31**, 1647–1651.