2009 International Nuclear Atlantic Conference - INAC 2009
Rio de Janeiro,RJ, Brazil, September27 to October 2, 2009
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: **978-85-99141-03-8**

APPLICATION OF PERTURBED ANGULAR CORRELATION SPECTROSCOPY IN IgG IMMUNOGLOBULINS

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ABSTRACT

In the present work, the technique of perturbed angular correlation (PAC) spectroscopy was used to measure the electric hyperfine field at IgG immunoglobulins using $^{111}In \rightarrow ^{111}Cd$ and $^{181}Hf \rightarrow ^{181}Ta$ probe nuclei. The biological materials studied in this work were originating from the immunological response of different mice lineages infected by the Y strain of *T. cruzi.* The samples were measured at room temperature (295K) and at liquid nitrogen temperature (77K). The PAC results showed that, samples measured with ^{111}In obtained better results when they were compared with samples measured with ^{181}Hf.

1. INTRODUCTION

When our organism is stimulated by antigens, it answers with a specific immunological response that can be cellular, humoral or both, depending on the antigen. Immunoglobulin IgG1 induces a pattern of response type Th2 that corresponds to an allergic immunological response, whereas immunoglobulin IgG2 induces a pattern of response Th1 that corresponds to a cellular immunological response, suited for response against parasites [1].

Chagas' disease is a disease of the tissues and circulating, of endemic character in extensive areas of the American continent. It generally has chronicle evolution and is caused by a flagellated protozoan, Trypanosoma cruzi, transmitted to the man and several sensitive mammals by *Triatominae* insect [2].

Chagas' disease is among the main health problems in South America, affecting millions of people [3]. Absence of vaccines or readiness of specific treatment leads the researchers to follow different approaches to study how to find a treatment for this disease. In the present work, an investigation of interactions in molecular level has been done trying to understand the metabolic dynamics of Immunoglobulin IgG1 and IgG2 molecules.

The technique of perturbed angular correlation (PAC) has been used to study the microscopy behavior of biomolecules by measuring electric quadrupole hyperfine interactions (EQI) in probe nuclei added to them. EQI are interactions between the electric nuclear quadrupole moment of probe nuclei and the electric field gradient (EFG) from the charge distribution surrounding them [4, 5]. It is therefore possible to microscopically investigate the biomolecule behavior and improve the understanding of biological phenomena related to the metabolic dynamics of those systems.

2. EXPERIMENTAL

In order to carry out the experiments, IgG immunoglobulins samples originating from different mice lineages infected by the Y-strain of *T. cruzi* were prepared. Biomolecules was marked in direct way, where the probe nuclei ^{111}In and ^{181}Hf are bonded in specific sites of the biomolecule. These samples were diluted in distilled water, in which the $^{111}InCl_3$ or $^{181}HfF_4$ was added (48µl of H_2Od, 1 µl of IgG and 1 µl of $^{111}InCl_3$ or $^{181}HfF_4$). Samples were measured at both room temperature (295K) and at the liquid nitrogen temperature (77K). A detailed description of perturbed angular correlations is given in the review article by Bauer [6].

PAC measurements were carried out using a BaF_2 four-detector spectrometer, which yields twelve coincidence spectra W(θ,t) that were analyzed by means of TDPAC [7] software. TDPAC output is the $A_{22}G_{22}(t)$ function given by the combination of W(θ,t) spectra:

$$A_{22}G_{22}(t) = 2[C(180^o,t) - C(90^o,t)]/[C(180^o,t) + 2C(90^o,t)] \quad (1)$$

where,

$$C(180°,t) = \sqrt[8]{\prod_{i=1}^{8} W_i(180°,t)} \quad \text{e} \quad C(90°,t) = \sqrt[4]{\prod_{i=1}^{4} W_i(90°,t)} \quad (2)$$

The coincidence spectra $W_i(\theta,t)$ are produced from signals of combinations of two detectors with angles θ = 90° or 180° between them, after subtracting the effects of unwanted accidental coincidences (t): $W_i(\theta, t) = W_i(\theta,t) - W_A(t)$. From $A_{22}G_{22}(t)$ function was possible to obtain ω_i transition frequencies corresponding to the splitting of intermediate energy level from probe nucleus gamma cascade due to the presence of an Electric Field Gradient originated from the electronic neighborhood [5, 8]. PAC measurements were carried out with biomolecules in solution, in which the rotational diffusion effect is present. This effect is represented by the rotational correlation time τ_{CR}, which describes the mobility of a molecule in the solution. τ_{CR} depends on the viscosity (ξ), the temperature (T) and the volume of the molecule (V): $\tau_{CR} = V.\ \xi/(k_BT)$, where k_B is Boltzmann's constant. The influence of the dynamic interaction is stronger when $\omega_0\tau_{CR}\approx1$, and as a consequence the effect on the PAC spectrum is a fast damping of the anisotropy. There are two possible situations: (1) when the quadrupole interaction fluctuation is fast, $\omega_0\tau_{CR}<<1$, since the fluctuation time is small when compared with time scale of the quadrupole interaction characterized by ω_0, hence, the nucleus loses the phase coherence and the perturbation function becomes an exponential decay; (2) and the quadrupole interaction fluctuation is slow, $\omega_0\tau_{CR}>>1$, the fluctuation time is long when compared with time scale of the quadrupole interaction, and the effect is a slow damping of the anisotropy. In the limit when $\tau_{CR}\rightarrow\infty$, the interaction is pure static. Only in this case it is possible to determine simultaneously the quadrupole frequency ω_0 and the asymmetry parameter η, the hyperfine parameters related to the local structure around the probe nucleus into the biomolecule.

3. RESULTS AND DISCUSSION

Figures 1, 2 and 3 show PAC spectra at 295 K and 77 K measured with ^{111}In and ^{181}Hf for IgG1, IgG2a and IgG2b, respectively. Results presented in tables 1, 2 and 3 show that all samples measured with ^{111}In at 295 K presented dynamic interactions with single frequency highly distributed each. Results for IgG1 and IgG2b with ^{111}In at 295K show λ = 19.2 and 11.3 respectively, indicating the existence of mobility of the molecules in solution, whereas for IgG2a measurements with ^{111}In at 295K resulted in λ = 1.3, indicating less mobility of the molecules in solution. This kind of interaction might be associated with the molecular weight of each immunoglobulin, because the amino acid sequence that composes them present different molecular weights, resulting in differences of mobility when they are in solution. At 77K all samples presented static interactions, which are expected for measurements at low temperatures, with single fraction each one also with highly distributed frequencies.

The mobility of biomolecules in solution at 295 K might affect the electric field gradient around the probe nucleus make it fluctuate. On the other hand, at 77K, the electric field gradient around the probe nucleus might be altered as the structure of biomolecule changes with the contraction of the molecule when it is frozen.

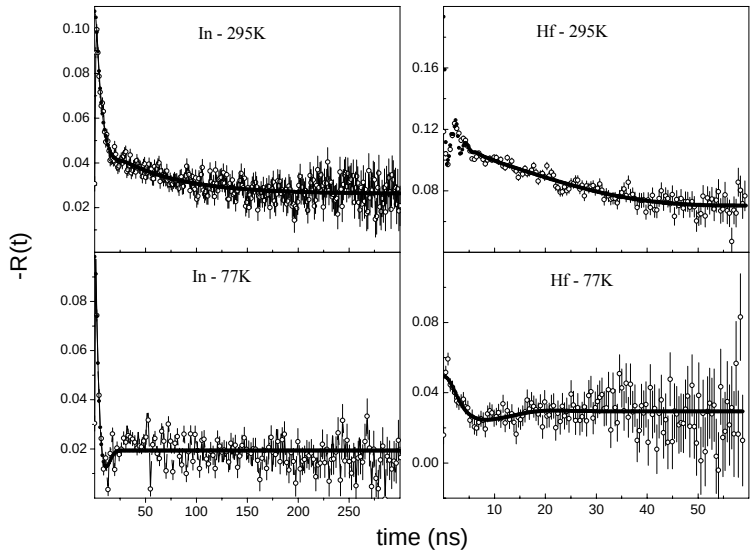

Figure 1. TDPAC spectra of ^{111}In and ^{181}Hf for Immunoglobulins IgG1 measured at room temperature and liquid nitrogen temperature

Tabela 1. Hyperfine parameter showed for IgG1 immunoglobulin after measured of PAC.

	^{111}In	
Parameter	**295K**	**77K**
Fraction	1	1
ν_Q (MHz)	125.4 (302)	173.2 (133)
η	0	0.37 (1)
Delta	0.62 (1)	0.71 (0)
λ (MHz)	19.2 (24)	0

	^{181}Hf		
Parameter	**295K**		**77K**
Fraction	0.57	0.39	1
ν_Q (MHz)	1228.4	2454.1	310,0
η	0.65	0	0
Delta	0.19	0.05	0.90
λ (MHz)	46.4	0	0

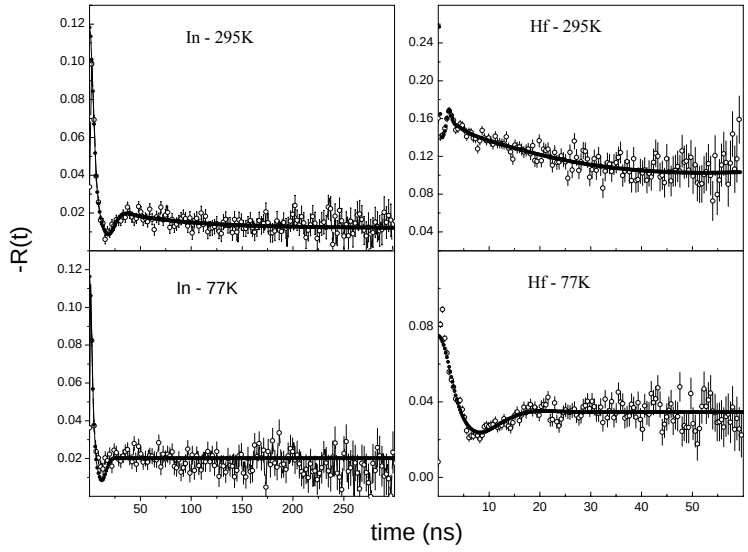

Figure 2. TDPAC spectra of ^{111}In and ^{181}Hf for Immunoglobulins IgG2a measured at room temperature and liquid nitrogen temperature

Tabela 2. Hyperfine parameter showed for IgG2a immunoglobulin after measured of PAC.

	^{111}In	
Parameter	**295K**	**77K**
Fraction	1	1
ν_Q (MHz)	119.7 (195)	168.2 (179)
η	0.35 (1)	0
Delta	0.66 (0)	0.64 (0)
λ (MHz)	1.29 (5)	0

	^{181}Hf		
Parameter	**295K**		**77K**
Fraction	0.38	0.61	1
ν_Q (MHz)	35.9	2890.3	231.1
η	0	0.20	0.47
Delta	0.64	0.14	0.44
λ (MHz)	0	122.4	0

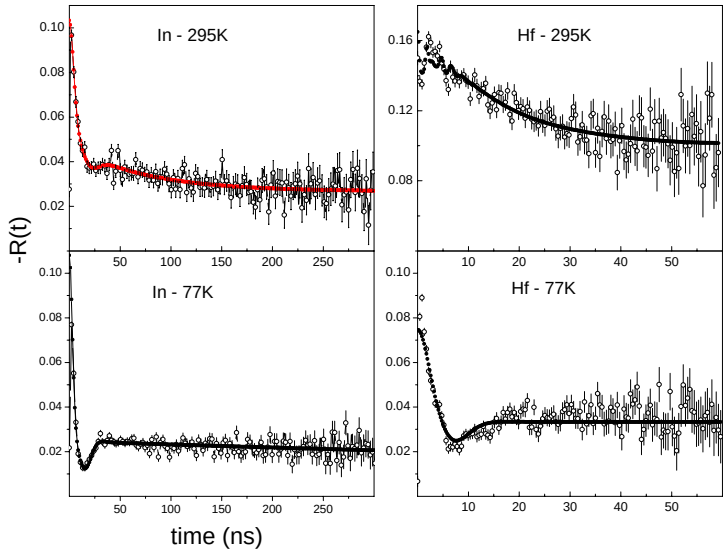

Figure 3. TDPAC spectra of ^{111}In and ^{181}Hf for Immunoglobulins IgG2b measured at room temperature and liquid nitrogen temperature

Tabela 3. Hyperfine parameter showed for IgG2b immunoglobulin after measured of PAC.

	^{111}In		
Parameter	**295K**		**77K**
Fraction	1		1
ν_Q (MHz)	75.3 (41)		148.2 (515)
η	0.57 (1)		0.28 (0)
Delta	0.71 (13)		0.57 (0)
λ (MHz)	11.33 (17)		0
	^{181}Hf		
Parameter	**295K**		**77K**
Fraction	0.31	0.68	1
ν_Q (MHz)	17.0	2771.5	211.4
η	0	0.34	0.76
Delta	0.62	0.22	0.60
λ (MHz)	28.7	95.6	0

Results of hyperfine parameters for the samples measured with ^{181}Hf also presented in tables 1, 2 and 3, show two fractions ate 295 K whereas at 77 K only one fraction was observed.
The two fractions observed show that probe nuclei are bonded to different sites of the biomolecules. This occurs because the chemical composition of molecules is complex and might offer several sites where probe nuclei can be bonded to. The differences observed for measurements with ^{181}Hf at 295 K and 77 K are not totally understood yet and indicate that more experiments must be carried out in order to reach unambiguous conclusions

4. CONCLUSION

It was observed that for samples measured with ^{111}In, the values of quadrupole frequency and the asymmetry parameter show considerable differences that depend on the temperatures at what the samples were submitted. The difference in the electric field gradient at 295 K and 77 K can be explained considering that the structure around the probe nucleus probably changes with the contraction of the molecule when it is frozen. Results showed that, samples measured with ^{111}In obtained better results when they were compared with samples measured with ^{181}Hf.These results might contribute to better explain the different patterns of immunological response presented by the different mice lineages when infected by Y strain of *T. cruzi.*

ACKNOWLEDGMENTS

Financial support for this research was provided by the Fundação de Amparo para Pesquisa do Estado de São Paulo (FAPESP). AWC thankfully acknowledge the support provided by CNPq in the form of research fellowship.

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