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Thermal behavior and signature patterns of human cytokine and soluble cytokine receptors investigated using dielectric thermal analysis and thermogravimetry

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Abstract Cytokines are small regulatory proteins secreted mostly by cells of the immune system. Cytokines participate in anti-inflammatory and pro-inflammatory processes in the body and in responses to host exposure to pathogens. In this study, the thermal behavior of human recombinant cytokines and soluble cytokine receptors; IFNγ, TNFα, IL-1 receptor antagonist, soluble TNF-receptor types 1 and 2, and sIL-2 receptor α were analyzed by dielectric thermal analysis at 37 °C and by thermogravimetry. Measurements were performed at a frequency range of 0.1–300,000 Hz. Permittivity and loss factor measurements were used to calculate mobility of charges (tan δ values) in the proteins from Debye plots. Peak frequencies and polarization times were used to determine dielectric signatures for each cytokine and receptor. Peak frequencies and polarization times were obtained for each cytokine and receptor analyzed. Detection of unique dielectric signatures of the proteins will aid development of sensitive dielectric sensors capable of detecting cytokines and soluble cytokine receptors in various human samples for malaria diagnosis.

Keywords Dielectric thermal analysis · Thermogravimatric analysis · Cytokines · Malaria · *Plasmodium falciparum* · Polarization times

Introduction

Cytokines are secreted mostly by cells of the immune system. They are small regulatory proteins that participate in anti-inflammatory and pro-inflammatory processes in the body. Cytokines and their soluble receptors are implicated in the pathogenesis of malaria, a disease which continues to be a major global health concern [1]. Over 515 million clinical cases and 1–2 million deaths occur in children under the age of 5 years [2]. Malaria severity is associated with a dysfunction in the regulation of pro-inflammatory and anti-inflammatory mediators [1, 2]. High levels of angiopoietin (ANG) 2, Interleukin (IL)-1β, IL-1Ra (receptor antagonist), IL-12, IFNγ, neopterin, TNFα, soluble (s) TNFR1, sTNFR2 are among the different molecules associated with severe malaria and cerebral malaria [3–6]. High ratios of ANG-2:ANG-1 predict poor prognosis in malaria and discriminate between uncomplicated malaria and cerebral malaria [6]. In addition, malaria symptomatic children had higher ratios of IFNγ:IL-10 and IFNγ:IL-12 compared to asymptomatic controls [3]. Children with respiratory distress (RD) as a result of malaria had significantly higher TNFα:IL-10 ratios compared to those without RD [5]. Circulating disease mediators can serve as biomarkers for malaria diagnosis.

The most widely used rapid diagnostic tests (RDTs) for the diagnosis of malaria detect *Plasmodium falciparum* histidine rich protein 2 (PfHRP2), expressed immediately after red cell invasion. PfHRP2 persists in the blood

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following parasite clearance [7]. Some *P. falciparum* strains from different geographic areas do not express PfHRP2 [8, 9] leading to false negative test results. Inclusion of human biomarkers associated with disease is necessary to aid in the accurate diagnosis of malaria. No human proteins are currently used as biomarkers in commercial malaria RDTs. Cytokines and other inflammatory host mediators have not been analyzed by thermoanalytical methods such as dielectric thermal analysis (DETA) and thermogravimetry (TG), as the basis for diagnostic test fabrication.

DETA is a thermoanalytical technique that measures changes in electrical properties of a sample, liquid or solid, as a function of temperature, time, and frequency [10]. The electrical properties are the loss factor (ε'') which is related to the energy to align dipoles and move ions and is converted into the ionic conductivity (pS/cm). The permittivity (ε') is another physical property and is related to the dipole content. Tan δ is a ratio of loss factor divided by the permittivity ($\varepsilon''/\varepsilon'$). A sample of known quantity is heated in a special DETA instrument at a given rate (°C/min). The sample can be characterized in the scanning DETA temperature mode for measuring the log conductivity vs. temperature which reveals the changes in conductivity as a function of frequency for the solid state and liquid state properties [10]. In the isothermal DETA (isoDETA) a plot of tan δ vs. log frequency at a given temperature can determine the polarization time (in µs or ms) which is a measure of charge mobility or transport that is calculated from a peak frequency. DETA has been used to study structural properties of various proteins including melanin, keratin, lysozyme and the effects of hydration on proteins [11–14]. Thermal analysis techniques can detect and characterize phase transitions in diverse materials and polymers including proteins. In DETA, a sample is subjected to an oscillating sinusoidal electric field. The sample becomes polarized under an applied voltage of 10 V/mm [15, 16].

TG is a thermoanalytical technique that measures changes in mass of a sample as a function of temperature or time (isothermal TG) [17, 18]. A sample of exact mass is heated in a special instrument at a given rate (°C/min) through a measured mass loss attributed to either of the following: dehydration, solvent or solute desorption, a gaseous product formation from a reaction, e.g., loss of carbon dioxide from calcium carbonate with a residue of calcium oxide or decomposition of the sample. The first derivative of the mass vs. temperature curve (DTG) highlights key transitions or changes in the sample more definitively for enhanced interpretation. TG is typically used to characterize chemicals, polymers, biological entities or pharmaceuticals [17].

Ionic conductivity of proteins in this study, were measured at a frequency (f) range of 0.1–300,000 Hz.

$$\text{AC conductivity} = \varepsilon'' \times f \times 2\pi \,(\text{pS/cm}) \quad (1)$$

DETA measures frequency modulated conductivity and tan δ (ionic mobility) as a function of temperature [15]. A Debye plot of tan δ versus log frequency was used for identifying the critical frequency ranges of charge mobility in the proteins.

$$\text{Tan } \delta = \varepsilon''/\varepsilon' \quad (2)$$

$$\text{Polarization time } (\tau) \text{ ms} = 1000/2\pi f_c (\text{Hz} = 1/\text{s}) \quad (3)$$

where f_c is the critical peak frequency (in ms and $\times 1000$ for µs).

Dielectric signatures of cytokines obtained can be used to establish unique "barcodes" for the proteins based on the polarization times (response times), tan δ (charge mobility) values, ionic conductivity, permittivity, hydration status of the proteins, and types of bound water interacting with proteins.

The focus of this study is to identify unique dielectric signature differences, among cytokines and soluble cytokine receptors using DETA and TG. Unique dielectric signatures will serve as the basis for discrimination of native cytokines and soluble cytokine receptors in human samples. In this study, cytokines and soluble cytokine receptors associated with severe malaria were investigated. Recombinant human proteins; IFNγ, TNFα, sTNFR1, sTNFR2, sIL-2Rα, and IL-1Ra were analyzed by DETA and TG. Reproducible differences in peak frequencies, polarization times, and frequency-dependent ionic conductivity were obtained for each protein. It is our hypothesis that charging values for amino acids such as the hydroponics index will be related to the tan δ and conductivity values. Potential applications of the data obtained include fabrication of cost-effective and field deployable point-of-care (POC) diagnostic devices that will greatly improve diagnosis of malaria, by simultaneously detecting parasite and human biomarkers in malaria patient samples.

Methods

Recombinant proteins

Lyophilized recombinant human cytokines and soluble cytokine receptors were obtained from Peprotech (Rocky Hill, NJ). Individual cytokines were reconstituted to 0.1–1 mg/mL according to manufacturer's instructions in the following solvents; water (IFNγ, TNFα, TNFR2, and IL-1Ra), 5 mM Tris pH 8.0 (sTNFR1), and 1× PBS pH 7.2 (sIL-2Rα). Three different concentrations of cytokines

were analyzed for each protein. Protein volumes of 10, 20, and 40 μL were used for DETA measurements. Individual solvents were also analyzed using DETA as controls. Amino acid sequences (Peprotech) were analyzed for the frequency of occurrence in cytokines and soluble cytokine receptors using the Protein Information Resources search window (http://pir.georgetown.edu/cgi-bin/comp_mw.pl).

Dielectric thermal analysis

Single surface gold ceramic electrode dielectric analysis was performed using a TAI 2970 DEA. 10, 20, and 40 μL of control solvents were placed on a single surface electrode and heated to 37 °C. Measurements were taken through a frequency range of 0.1–300,000 Hz. All samples were analyzed using TA Instruments universal analysis software and after sensor calibration all samples were run at 25 °C and isothermal for 60 min. Samples were ramped at 5 °C/min to 37 °C in an inert atmosphere with a flow rate of 50 mL in nitrogen. DETA was also performed by placing protein samples on the surface of a single gold ceramic interdigitated electrode in an isolated nitrogen rich dry atmosphere. The samples were ramped at a rate of 10 °C/min from room temperature (25 °C) to 250 °C. Conductivity measurements were recorded at controlled interval frequencies ranging from 0.1 to 300,000 Hz for all temperatures [14]. Dielectric properties were determined as a function of time, temperature, and frequency in order to obtain the dielectric constant or permittivity [ε'], the dielectric loss [ε''] and the loss tangent [tan $\delta = \varepsilon''/\varepsilon'$]. A Debye plot of tan δ versus log frequency was plotted from the values obtained.

Thermogravimetric analysis

Thermogravimetric (TG) analysis was performed using a TAI 2950 TGA. It was calibrated with calcium oxalate hydrate (water loss 12.2 wt% ±0.1%). TG was used to measure the percent mass loss of proteins heated to temperatures below the melting point, but above the boiling point of water [15, 16]. Samples were loaded into aluminum pans and heated in an isothermal nitrogen environment to 150 °C. Isothermal conditions were maintained to ensure reaction completion. The TG curves were collected from 25 to 37 °C at a heating rate of 5 °C/min then held isothermally at 37 °C for 60 min. The stability of water in an aqueous solution of 5 μL of protein was determined as a function of time. TAI Universal Software was used to calculate the mass loss as a function of time. Heat flow (heat of fusion) and temperature were calibrated with respect to the melt endotherm of Indium as a standard. Differential Thermal Gravimetric Analysis DTG curves were taken as the first derivative of the TG curves.

Results and discussion

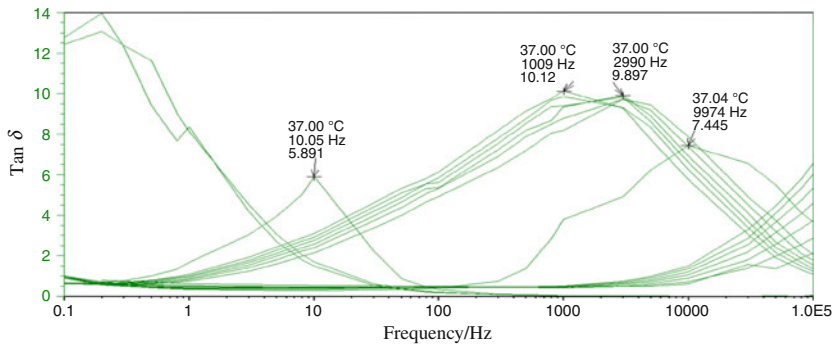
Debye plots of tan δ vs. log frequency and temperature dependent conductivity

Tan δ curves (Debye plots) for cytokines and soluble cytokine receptors showed reproducible distribution in critical frequency ranges. Dielectric loss spectra at approximately 37 °C, with sharp peaks were obtained for all proteins (see Fig. 1a–f). Charge mobility for IL-2Rα was very high at the frequency range examined (Fig. 1b). Higher values of tan δ indicate more mobility of the charged amino acid residues on the proteins examined, while low tan δ values indicate reduced mobility of the charges on the proteins. Moderate and high tan δ values were obtained for the different proteins. Electrical response varied by time and frequency. Three different concentrations of proteins were examined. However, values for 20 μL at a concentration range of 0.02–2 μg are shown. Cytokines and soluble cytokine receptors showed 2–3 peaks at a frequency range of 50–300 Hz and at 10,000 Hz. Tan δ peak heights ranged from 7.6 to 25, with values of 111.2 Hz obtained for sIL-2R (Fig. 1).

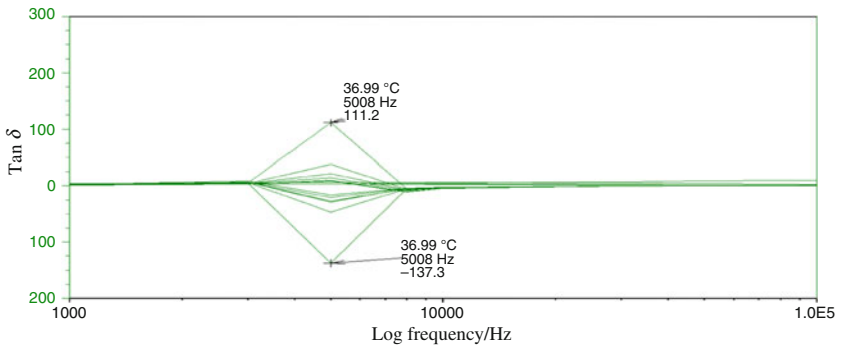
DETA spectra and polarization times for cytokines and soluble cytokine receptors

A frequency dependent increase in ionic conductivity (Eq. 1) was observed for all proteins analyzed. Clear dispersions with conductivity peaks between 10,000 and 100,000 Hz were observed (Supplementary Figure 1). At higher frequencies, a plateau was observed, potentially a peak at the end of our test range. The conductivity values collected for the cytokines and soluble cytokine receptors are summarized in Table 1. The proteins were characterized by the conductivity peak spectra (pS/cm), tan δ (loss factor/permittivity) vs. Frequency/Hz and the conversion of the latter peak frequencies to polarization times. The conductivity is an earmark of the charge conductivity and is probably related to the collective hydrophilic and hydrophobic amino acid residue content of the proteins. The polarization time (or response time) is derived from the peak frequency in the tan δ vs. frequency plot. The polarization time is equal to $10^6/2\pi \times$ critical peak frequency in μs (see Eq. 3). It appears that the matching of DETA data is most appropriate for the polarization times and the tan δ peak heights. The latter are related to the "amount" of mobility of the charge transfer species. It is our hypothesis that other charging values for amino acids, e.g., the hydroponics index will also be related to these tan δ and conductivity values. It is these DETA properties that we will use to characterize the unique dielectric signatures of the various cytokines and their soluble receptors.

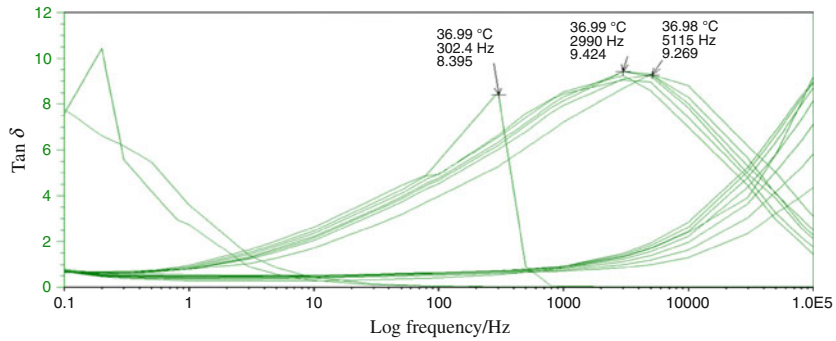
A frequency of 10^4 Hz was selected from plots of log ionic conductivity vs. log frequency to show the differentiation and the resulting tan δ values obtained in the spectra. Two peaks at 10^6 pS/cm were examined (Table 1). Cytokines and soluble cytokine receptors were conductive with cytokines generally less conductive than receptors. Receptors were more conducting with sTNFR2 more conducting than sTNFR1. The tan δ reflecting mobility strength for sTNFR1 is stronger than that of sTNFR2 and shows a faster response time. Tan δ values for sTNFR1 are similar to TNFα, with a similar strength of signal. Similar (moderately fast charging) polarization times at 16 μs, were obtained for TNFα, IFNγ, IL-1Ra, and sTNFR2. Soluble IL-2R had a high polarization time of 32 μs and sTNFR1 had polarization time of 3.2 μs, indicating a very fast charging species. The rate of occurrence of charging species within the proteins or the speed of response to the electric field is dependent on the amino acid composition of the proteins and association of the proteins with water. The tan δ and polarization time data supports our hypothesis. Additional cytokines and cytokine receptors will be investigated to confirm the dielectric profile observed in the current study. Intrinsic dielectric properties of the cytokines and soluble cytokine receptors combined with the patterns of adsorbed water provide a means to rank characteristics of the proteins. The Polaron theory or the "hopping model for conduction" is used to describe the transfer of charge between various cations in the cytokines and their receptors [19, 20]. According to this theory the transfer of electronic charge is accompanied by a corresponding phonon field involving polar modes. This composite of the electron and the associated phonon field are treated as a quasiparticle, i.e., the polaron. A slow moving electron in a dielectric induced crystal interacting with the



(a) IL-Ra, Tan Delta vs Log Frequency, 20 μl, 37 °C



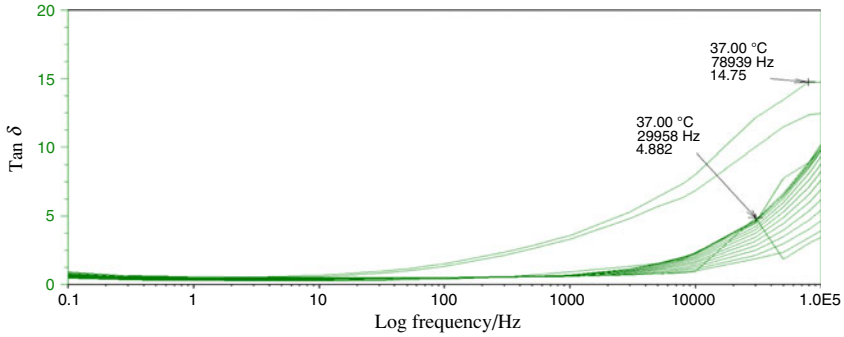
(b) sIL-2Rα receptor, Tan Delta vs. Log Frequency, 20 μl, 37 °C



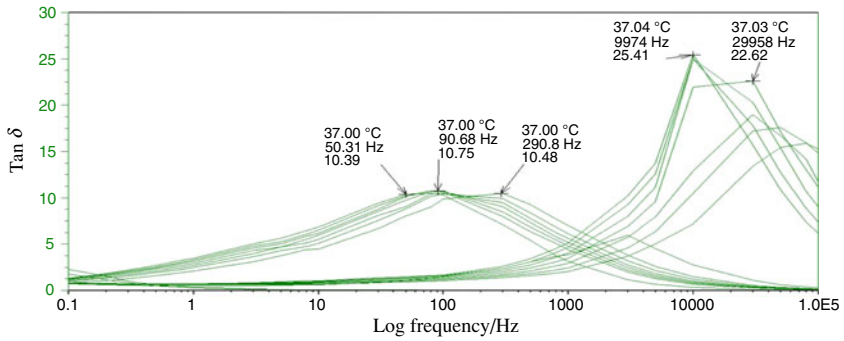
(c) IFN-Y, Tan Delta vs. Log Frequency, 20 μl, 37 °C

Fig. 1 Debye plot of tan δ vs. log frequency, 20 μL, 37 °C. A comparison of charge mobilities for cytokines and soluble cytokine receptors is shown in the spectra. Following DETA analysis, the ratio of the dielectric loss factor (ε'') to the permittivity (ε') was plotted against log frequency. The critical frequency ranges of charge mobilities of the proteins are compared using the Debye plot. Peak frequencies and tan δ values are shown in the plots. **a** IL-1Ra; **b** sIL-2Rα; **c** IFNγ; **d** sTNFR2; **e** TNFα; **f** sTNFR1

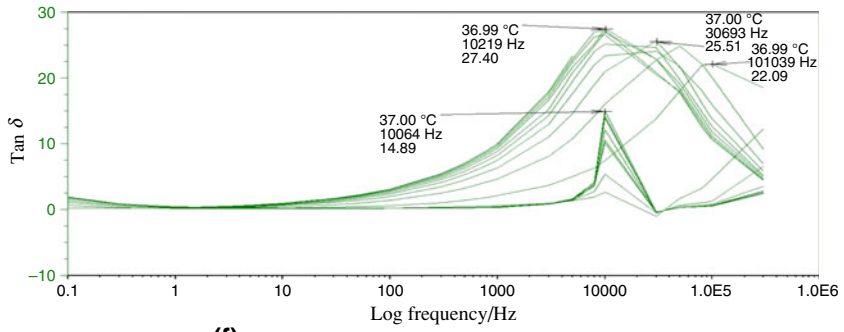
Fig. 1 continued



(d) sTNFR2,Tan Delta vs. Log Frequency, 20 µl, 37 °C



(e) TNF alpha,Tan Delta vs. Log Frequency, 20 µl, 37 °C



(f) sTNFR1, Tan Delta vs. Log Frequency, 20 µl, 37 °C.

Table 1 DETA spectra of cytokines and soluble cytokine receptors: conductivity, tan δ, and polarization time

	Cytokine	Receptor	Conductivity/pS/cm × 10^6		Tan δ intensity	Tan δ frequency (f_c)/Hz × 10^4	Polarization time/µs
			Peak 1	Peak 2			
A	TNF Alpha		1.3	0.26	25	1.00	16
B	IFN Gamma		17	0.028	8.7	1.00	16
C	IL-1-Ra		91	0.0092	7.9	1.00	16
D		sTNFR1	0.46	34	25	5.00	3.2
E		sTNFR2	110	38	7.6	0.85	19
F		sIL-2Rα	69	11	11	0.50	32

Key: Polarization time (µs) = 1,000,000/6.28 × f_c critical peak frequency in Hz

lattice ions through long range forces will be surrounded by a field of lattice polarization caused by the moving electron. The charge in this system jumps or hops discontinuously from site to site. The electron transfer requires the pre-stressing or the polarization of the dielectric surrounding the cations. The latter are probably from the residual amino acids associated with the proteins of interest. The activation energy for pre-stressing in an electric

field or polarization of the lattice or phase can be related to the carrier sites [19, 20]. Studies by Suherman et al. [21] detected low frequency dispersions (LFD), in the study of hydrated ovalbumin. Permittivity increases at low frequency were observed [21]. This increase was attributed to low-mobility charge carriers and associated with a quasi-dc process. In the current study, LFDs were not observed in the conductivity of the cytokines and soluble cytokine receptors. Dispersions were frequency dependent, increased with higher frequencies and were probably due to high mobility charge carriers (Supplementary Figure 1). Similar to the data in the current study, LFDs occur due to proton hopping in the hydrogen bonded water molecules [21].

Conductivity and thermogravimetry

Water association with cytokines and soluble cytokine receptors was further analyzed by TG (Fig. 2). Due to the changes in ionic conductivity observed between 20 and 60 min in DETA (Supplementary Figure 1), thermogravimetric analysis was performed on soluble cytokine receptors; sTNFR1, sTNRF2, IL-1Ra and sIL-2Rα, and cytokines; IFNγ and TNFα. The changes observed in ionic conductivity were attributable to water loss for the first 30 min followed by conductivity of the dried protein samples. An overlay of DETA and TG is shown in Fig. 2. Changes in frequency peaks in response to time were analyzed at low (0.1 Hz), medium (100 Hz), and high

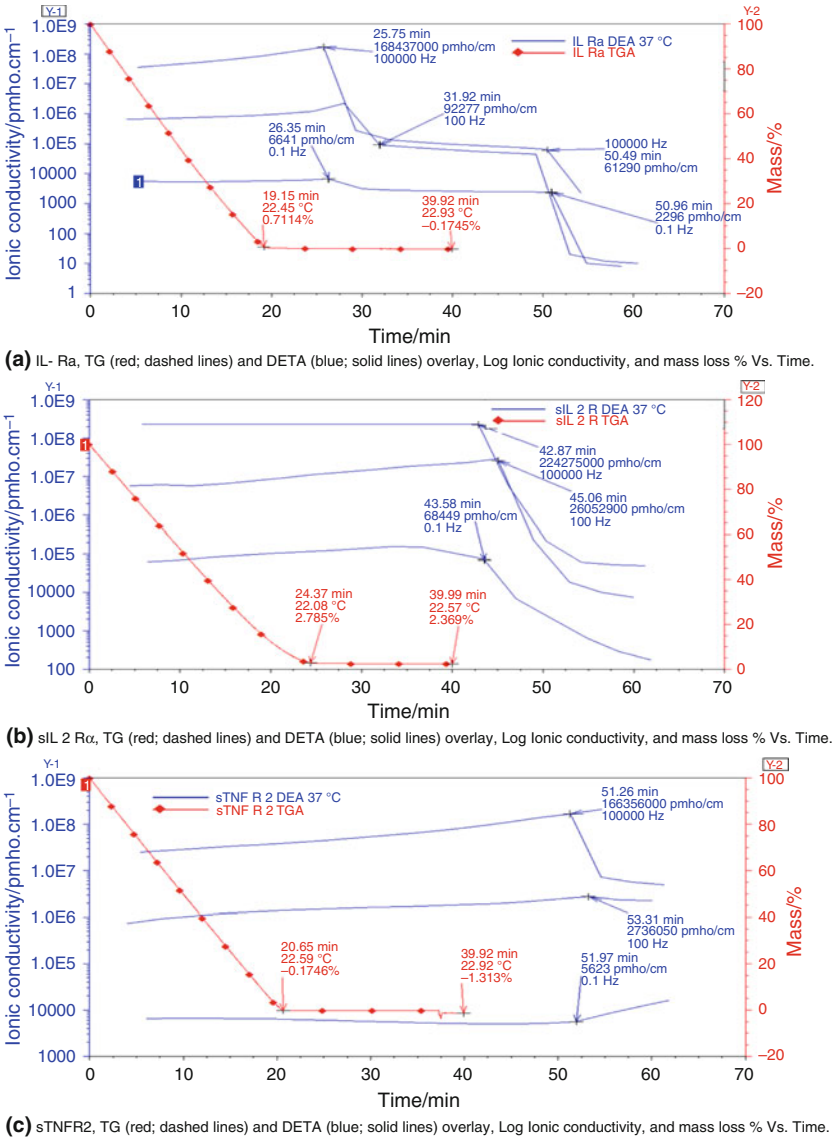
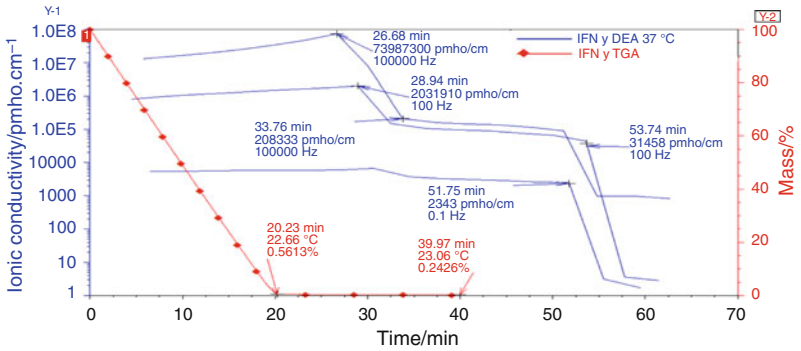
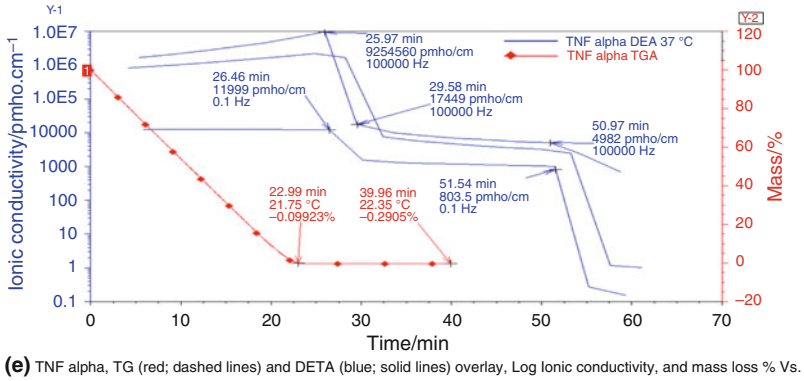


Fig. 2 Overlay of DETA and TG curves for log ionic conductivity and mass loss % vs. time, for cytokines and soluble cytokine receptors. Plots show changes in conductivity due to water loss in protein samples. Changes in peak frequency as a response to time were analyzed at low (0.1 Hz), medium (100 Hz), and high (100,000 Hz) frequencies. Time, temperature and % water loss are shown in the plots. **a** IL-1Ra, TG (*red*; *dashed lines*) and DETA (*blue*; *solid lines*) overlay; **b** sIL-2Rα, TG (*red*; *dashed lines*) and DETA (*blue*; *solid lines*) overlay; **c** sTNFR2, TG (*red*; *dashed lines*) and DETA (*blue*; *solid lines*) overlay; **d** IFNγ, TG (*red*; *dashed lines*) and DETA (*blue*; *solid lines*) overlay; **e** TNFα, TG (*red*; *dashed lines*) and DETA (*blue*; *solid lines*) overlay; **f** sTNFR1, TG (*red*; *dashed lines*) and DETA (*blue*; *solid lines*) overlay

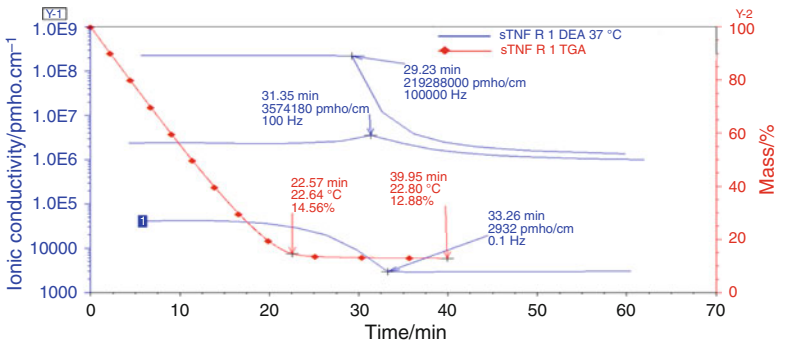
Fig. 2 continued



(d) IFN γ, TG (red; dashed lines) and DETA (blue; solid lines) overlay, Log Ionic conductivity, and mass loss % Vs. Time.



(e) TNF alpha, TG (red; dashed lines) and DETA (blue; solid lines) overlay, Log Ionic conductivity, and mass loss % Vs. Time.



(f) sTNFR1, TG (red; dashed lines) and DETA (blue; solid lines) overlay, Log Ionic conductivity, and mass loss % Vs. Time.

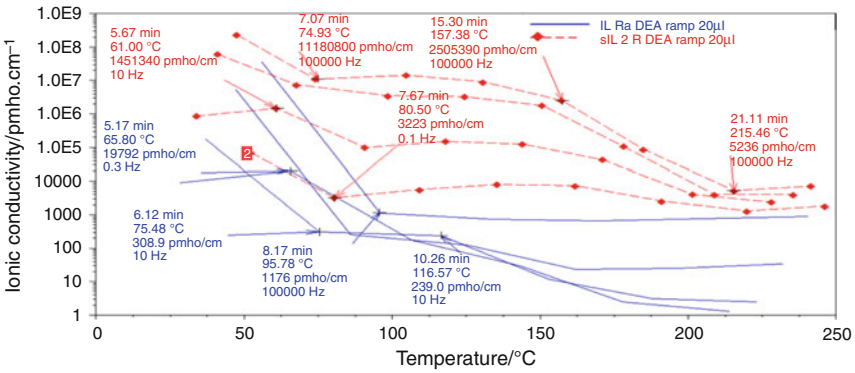
(100,000 Hz) frequencies. As water loss increased, conductivity at mid and high frequencies remained between 10^6 and 10^8 pS/cm. Soluble TNFR1 showed an 86% water loss (14% water retention), followed by sIL-2R with 97% water loss (3% water retention). Soluble TNFR2, TNFα, and IFNγ showed close to 100% water loss in 20–40 min at 23 °C. The two receptors, sTNFR1 and sIL-2Rα appeared to have a higher capacity for interacting with water followed by IL-1Ra (0.6% water retention).

Overall, water retention by the receptors was on average approximately 4% compared to cytokines at approximately 0.4%. The conductivity observed was mainly due to the proteins in their dried state. The fluid environment surrounding the proteins can influence the relaxation of the charged amino acid residues [10]. The different aqueous solutions used to reconstitute lyophilized recombinant proteins were also subjected to DETA analysis in order to determine the level of conductivity of the solutions. It is important for the conductivity of the proteins dissolved in the solutions to be differentiated from the solution. The solvents used such as water, PBS, and Tris maintain native protein structure and do not interact with the proteins. An earlier study examined influence of the "conducting media" on DETA of proteins dissolved in the media [22]. Three frequency regions (a low, middle and high frequency), showing variations in sample responses to the electric field and variations in sample polarization were identified. At high frequencies the proteins under analysis behaved as a "pure dielectric".

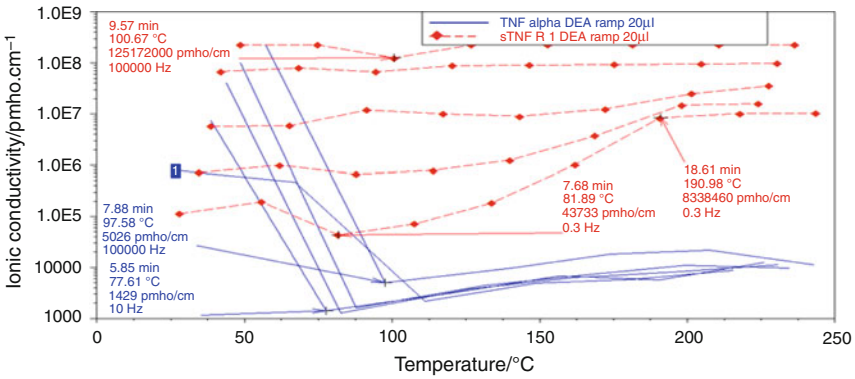
Conductivity versus temperature

Soluble cytokine receptors; sTNFR1, sTNRF2, IL-1Ra and sIL-2Rα, and cytokines; IFNγ and TNFα were analyzed further by ramping the temperature at 10 °C/min up to 250 °C (Fig. 3a–c). Cytokines lost water precipitously resulting in a conductivity drop from 10^8 to 10^1 (pS/cm). It appears that water is more loosely associated with the cytokines and more tightly associated with the soluble cytokine receptors. IFNγ and TNFα showed distinct peak frequencies, with IFNγ being more active than TNFα (Fig. 3b, c). The water loss for TNFα is between the conductivities of 10^8 to 10^3 pS/cm. For soluble receptors water loss was observed between the conductivities of 10^8 and 10^5 pS/cm and 10^8 and 10^3 pS/cm. Water is more tightly absorbed in most of the receptors, in agreement with the results of TG (Fig. 2). At 50–70 °C, a loss of adsorbed water could be observed. The loss may be due to water dissociating from the proteins or from protein denaturation. The water loss is frequency independent and appears to be a first order thermodynamic transition. Hydration appears to be a marker for differentiating the proteins at the frequencies studied.

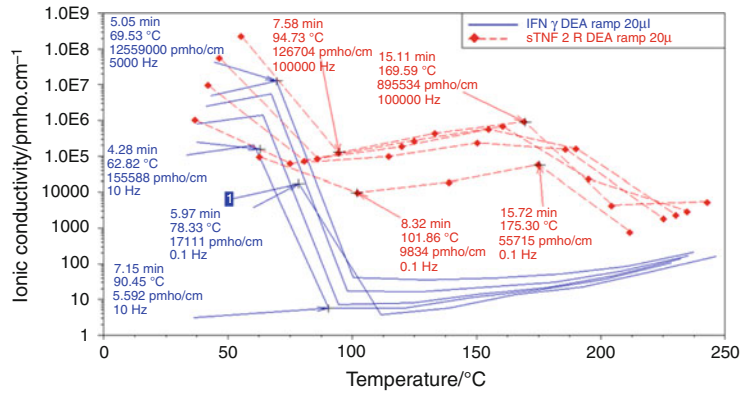
A comparison of sTNFR1 and sTNFR2 shows distinct fingerprints or barcodes with sTNFR2 showing more structural change compared to sTNFR1. Both proteins show two frequency dependent peaks at higher temperatures. Between temperatures of 50–75 °C, both proteins



(a) Cytokines IL-1Ra (blue; solid lines) and sIL-2Rα (red; dashed lines), temperature ramp ups.



(b) Cytokines TNF alpha (blue; solid lines) and sTNR1 (red; dashed lines), temperature ramp ups.



(c) Cytokines IFN γ (blue; solid lines) sTNFR2 (red; dashed lines), temperature ramp ups.

Fig. 3 Overlay of DETA curves for log ionic conductivity vs. temperature for cytokines and soluble cytokine receptors. Plots show frequency independent water loss indicating a first order thermodynamic transition. Ionic conductivity, peak frequency, time, and temperature of transitions are shown in plots. **a** IL-1Ra (*blue*; *solid lines*) and sIL-2Rα (*red*; *dashed lines*); **b** TNFα (*blue*; *solid lines*) and sTNFR1 (*red*; *dashed lines*); **c** IFNγ (*blue*; *solid lines*) and sTNFR2 (*red*; *dashed lines*)

had a similar behavior with desorption observed in both proteins. Loss of water or protein denaturation at these temperatures could be the cause of the observed desorption. The changes observed could also be due to the charges in the amino acid side chains. At 155–170 °C (10^6 conductivity) and at 175–185 °C (10^5 conductivity) two sharp peaks representing conductivity of the proteins can be observed. Conductivity of IL-1Ra shows variation in frequency probably as a result of protein interaction with different types of water (Fig. 3a). Surface adsorbed water and bulk water associated with the interior of the folded proteins are responsible for the observed desorption, with water loss seen at high frequencies. Similarly, conductivity of sIL-2Rα shows desorption at 50 °C with frequencies varying between 10^8 and 10^5 pS/cm (Fig. 3a). Between 110 and 140 °C conductivity due to the protein was observed. Through analysis of pure distilled water by calorimetry and dielectrometry, we have a sensitive tool for detecting the amount of water associated with the protein. The temperature at which peaks occur, the conductivity and the peak frequency determines how tenacious the interaction is between the proteins and water. The knowledge of associated water and the thermodynamic variations during vaporization can be used as a ranking determinant for the interaction of water with the proteins and can therefore define the hydration state of the protein [13, 18]. Differentiation of unique peaks for each cytokine and soluble cytokine receptor is probably due to the different amino acid residues and the side chains. The amino acid composition and hydroponics index for the cytokines and cytokine receptors are shown in Table 2. Global distribution of amino acid side chains determine the hydrophobicity of the cytokines compared to the hydrophilicity of the soluble receptors. Water is intimately bound to each protein but varies depending on the protein.

Table 2 Percent frequency of amino acid composition in cytokine and soluble cytokine receptors (Protein Information Resource)

Residues	IFNγ	TNFα	IL-1Ra	sIL-2R	sTNFR1	sTNFR2	Score[a]
A	5.6	18.3	5.9	5.0	1.2	6.3	1.8
C	–	1.3	2.6	5.0	14.9	12.9	2.5
D	6.9	3.2	5.9	2.7	3.7	4.6	−3.5
E	6.3	6.4	7.2	10.5	6.8	4.6	−3.5
F	6.9	2.5	5.9	2.7	3.1	1.7	2.8
G	3.5	7.0	6.3	5.5	5.6	5.7	−0.4
H	1.4	1.9	1.3	2.7	4.3	1.1	−3.2
I	4.9	5.1	3.9	2.7	2.5	2.3	4.5
K	13.9	3.8	5.9	4.6	6.2	3.4	−3.9
L	6.9	11.5	7.8	2.7	6.8	3.4	3.8
M	3.5	–	3.9	4.6	1.2	1.7	1.9
N	6.9	4.5	5.2	2.3	7.4	2.9	−3.5
P	1.4	6.4	5.2	7.8	3.1	7.3	−1.6
Q	6.9	6.4	5.2	8.2	5.6	5.2	−3.5
R	4.9	5.7	5.2	5.0	4.3	6.3	−4.5
S	7.6	8.3	7.2	7.3	9.3	10.3	−0.8
T	3.5	3.8	5.2	11.4	6.2	10.3	−0.7
V	5.6	9.3	6.5	3.7	4.3	5.7	4.2
W	0.7	1.3	1.3	1.4	0.6	1.7	−0.9
Y	2.8	4.5	2.0	3.2	3.1	2.3	−1.3

Amino acid names: *A* alanine, *C* cysteine, *D* aspartic acid, *E* glutamic acid, *F* phenylalanine, *G* glycine, *H* histidine, *I* isoleucine, *K* lysine, *L* leucine, *M* methionine, *N* asparagine, *P* proline, *Q* glutamine, *R* arginine, *S* serine, *T* threonine, *V* valine, *W* tryptophan, *Y* tyrosine

[a] Kyte–Doolittle amino acid hydropathy scores [26]: hydrophilic residues, −0.4 to −4.5; hydrophobic residues, 1.8–4.5

Conclusions

To our knowledge this study represents the first DETA and TG analysis of cytokines and soluble cytokine receptors for the identification of thermally induced signatures. Cytokines and cytokine receptors possessed differentiating thermally induced dielectric loss spectra with DETA and TG. Cytokines could be differentiated from soluble cytokine receptors based on the hydration pattern of the proteins. The signatures obtained from dielectric spectra can be applied as a means to differentiate the individual cytokines and other inflammatory host mediators induced during malaria infection. Levels of the mediators as well as ratios of the mediators can be obtained and can form the basis for detecting the proteins in human samples such as serum, urine or saliva. In studies where a clear association has been established between host mediators and clinical malaria, DETA and TG can be applied for parasite and host protein detection in human bodily fluids. High levels of ANG-2 and decreased levels of ANG-1 were shown to be prognostic for the development of cerebral malaria [6]. In other studies, malaria symptomatic children had higher ratios of IFNγ:IL-10 and IFNγ:IL-12 compared to asymptomatic controls [3]. Furthermore, high levels of procalcitonin (PCT) have also been associated with severe malaria [23]. An immunochromatographic PCT-Q test used initially for testing systemic bacterial infection was used to evaluate imported malaria cases with moderate success [23].

The use of proteomics approaches to identify human protein profiles in normal and pathogenic states is yielding a catalogue of biomarkers that can be screened in different human samples [24]. A combination of proteomics and two dimensional gel electrophoresis, protein microarrays and matrix-assisted laser desorption/ionization techniques is being used to identify changes in expression patterns of proteins in normal and pathogenic states [24]. DETA and

TG can be applied similarly to discriminate unique dielectric signatures of specific proteins in human samples from malaria patients. Investigations of inflammasomes which are structures containing multi-protein complexes that can activate specific cytokines is another area where thermal analytical techniques will be applicable to protein detection. Experimental studies using the mouse model of cerebral malaria are currently being used to delineate molecules participating in cytokine activation [25].

In this study, the soluble cytokine receptors were generally associated with more water than the cytokines. Water is intimately bound to each protein but varies depending on the protein. In future studies we plan to investigate additional recombinant soluble cytokine receptors and cytokines as well as examine human serum (from malaria infected and uninfected individuals) for detection of cytokines using DETA and TG. We will determine if the levels of water interaction as well as the temperature dependent conductivity of the proteins reveal unique DETA signatures. In addition, the relaxation regions and polarization times influencing hopping polarization of charges in the proteins will be investigated.

Overall, the data show that distinct and reproducible differences in dielectric spectra can be obtained by analyzing microgram concentrations of hydrated recombinant cytokine and soluble cytokine receptors. The data supports our hypothesis showing that charging values for amino acid residues is related to the tan δ, conductivity and polarization values. The use of unique dielectric protein signatures for detection of human and malaria biomarkers will be of great benefit in public health surveillance of malaria cases, assessments of effective drug treatments for malaria and for diagnosis of malaria infection at POC.

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