A Potentiometric and Spectroscopic Study on the Interaction Between Human Immunoglobulin G and Sodium Perfluorooctanoate in Aqueous Solution

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Summary: The interaction between human immunoglobulin G (IgG) and sodium perfluorooctanate (SPFO) has been characterized by a combination of UV-vis and UV-CD spectroscopies and ion selective electrodes. The study determined that there were true specific unions between SPFO and IgG. The conformational changes at the bulk solution, induced by SPFO, were well characterized by UV-vis and UV-CD spectroscopies. Chemical and thermal unfolding were analysed and the thermodynamic parameters were determined.

Keywords: human immunoglobulin G; monolayers; sodium perfluorooctanoate; unfolding

Introduction

Compounds with perfluorinated carbons chain are chemically and thermally more stable and have lower intermolecular interactions, hence display lower surface tension and higher vapour pressure than any of their hydrocarbon counterparts. The combination of their extreme hydrophobicity and pronounced lipophobic character is unique, inciting paradoxically perfluoroalkylated amphipiles to segregate into organized nano- and micro-size assemblies.[1] The limited information available on membrane protein extraction using fluorosurfactants indicates lesser effectiveness at all, probably due to the lipophobic character of perfluorinated chains. In contrast to fluorocarbons, the pharmacology of fluorinated amphiphiles is still in its infancy. Adsorption, distribution, metabolism and excretion studies are badly needed. The results of these studies will largely determine the extent to which fluorosurfactants may be used in pharmaceuticals.[2] As a promising for the construction of novel pharmaceutical systems, it is important to study the interaction between perfluorinated surfactants and blood proteins.

In this sense and as a continuation of our systematic studies on the interaction of blood proteins with perfluorinated systems,[3–5] we present here an analysis of the interaction between immunoglobulin G (IgG) and sodium perfluorooctanoate (SPFO).

Material and Methods

Materials

Sodium perfluorooctanoate was from Lancaster MTM Research Chemicals Ltd.

IgG Purification

Human immunoglobulin G was purified from human serum collected from healthy donors agree a protocol elaborated in the Department of Biochemistry at the Faculty of Biology (University of Santiago de Compostela).[6]

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Absorbance Measurements

Difference spectra were measured with a Beckman spectrophotometer (model DU 640), with six microcuvettes, which operates in UV-vis region, from 190 to 1100 nm, of the electromagnetic spectrum wavelength.

Potentiometry, Ion Selective Electrode

Potentiometric determinations were made with a millivoltmeter and a CRISON pH meter. The millivoltmeter was used with a perfluorooctanoate-ion-selective electrode, against a saturated calomel electrode. The perfluorooctanoate electrode was made by gluing at one extreme of a poly(vinyl chloride) (PVC) tube a membrane made with dissolved tetrahydrofuran (THF) and dibutylphtalate (plasticiser) and barium perfluorooctanoate.

Circular Dischroism (CD) Measurements

Far-UV circular dichroism (CD) spectra were obtained using a JASCO–715 automatic recording spectropolarimeter (Japan) with a JASCO PTC-343 Peltier type thermostated cell holder. Quartz cuvettes with 0.2 cm pathlength were used. Data are reported as molar ellipticity and was determined as:

$$[\theta]_\lambda = \frac{\theta_\lambda M_r}{ncl} \quad (1)$$

where c is the protein concentration, l is the path length of the cell, $[\theta]_\lambda$ is the measured ellipticity at a wavelength λ, M_r is the molecular mass of the protein, and n is the number of residues.

Results and Discussion

The binding isotherm for interaction of IgG and SPFO at 25°C is shown in Figure 1. The binding data were measured using the SPFO-selective membrane electrode. According this Figure, SPFO manifests an high affinity for IgG molecules.

To obtain the binding Gibbs energy per surfactant molecule bound per monomeric IgG (ΔG_v), the binding isotherm was filled to a polynomial of the form:

$$v = a + b(\log[S]) + c(\log[S])^2 + \ldots \quad (2)$$

where v is the average number of surfactant molecules bound per monomeric IgG molecule and $[S]$ the surfactant concentration. The order of the polynomial was chosen to give the highest correlation coefficient and lowest error over the data

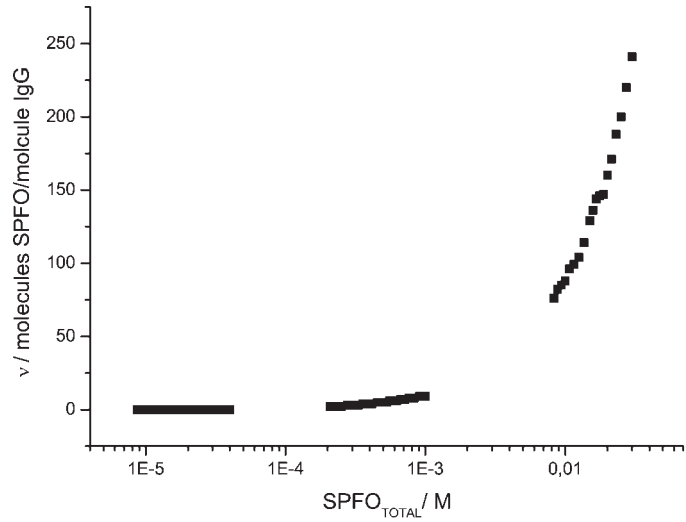

Figure 1.
Binding isotherm for interaction of SPFO with IgG at 25 °C. The IgG concentration was 0.015 mg/ml (0.1 μM).

point range. The polynomial was used to calculated the Wyman binding potential (π) as a function of ν from the following equations:[7]

$$\pi = 2.303RT \int_{(\log[S])\nu(=0)}^{(\log[S])_\nu} \nu \, d(\log[S]) \quad (3)$$

$$\pi = 2.303.RT\{a(\log[S]) + b(\log[S])^2 + c(\log[S])^3 + \ldots\} \quad (4)$$

The equilibrium constant (K) as a function of ν was calculated from the equation:

$$\pi = RT \ln(1 + K[S]^\nu) \quad (5)$$

and hence ΔG_ν as a function of ν from

$$\Delta G_\nu = -\frac{RT \ln K}{\nu} \quad (6)$$

This procedure leads to smooth curve of ΔG_ν *vs.* ν (Figure 2). $\Delta G \to 0$ at $\nu \to 0$ so that the plot shows a minimum corresponding to the most tightly bound ligands at low values of ν, then the curve tend to a limiting value of -7.41 kJ mol^{-1}.

The effect of SPFO on the IgG unfolded process is shown in Figure 3, where difference spectra for the 280 nm band native IgG versus SPFO is plotted. It can be seen one transition region (0.03–6 mM) over which absorbance changes steeply with surfactant concentration. These results suggest that in the interaction with SPFO the protein undergoes a significant change in its conformation.

Thermodynamically, as a first approximation the conformational changes process can be considered as an interaction between native IgG (N) and surfactant (S) expressed by the following equilibrium:

$$N + \nu S \Leftrightarrow DS_\nu \quad (7)$$

where ν is the average number of surfactant molecules bound to the complex (DS_ν). The reaction equilibrium constant (K), can thus be written:

$$K = \frac{[DS_\nu]}{[N][S]^\nu} = \frac{K_s}{[S]^\nu} \quad (8)$$

where K_S is the ratio of surfactant-protein complex and native molecules respectively and [*S*] is the equilibrium concentration of free surfactant. Because in the experiments IgG molarity was very low (0.1μM) it is assumed that [*S*] is negligibly different from the total surfactant concentration in the system. Values of K_S as a function of [*S*] for the first and second transition regions were calculated from the absorbance curve (A_{280}) in Figure 6 from the extent of denaturation

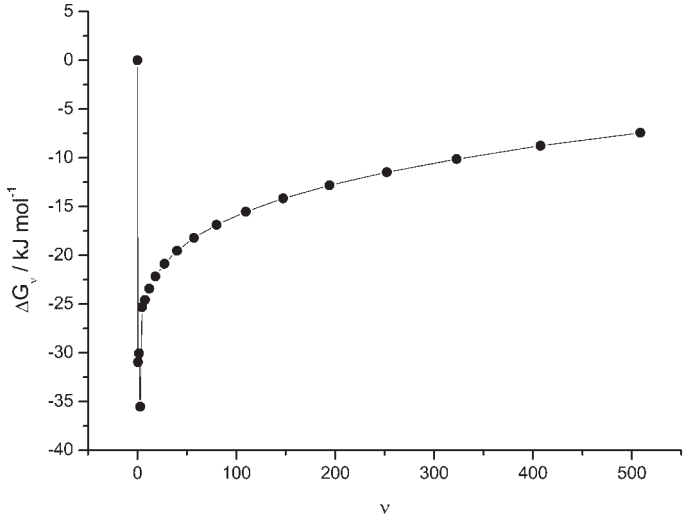

Figure 2.
Gibbs energy of binding (ΔG_ν) of SPFO to IgG as a function of surfactant ions bound (ν) at 25 °C.

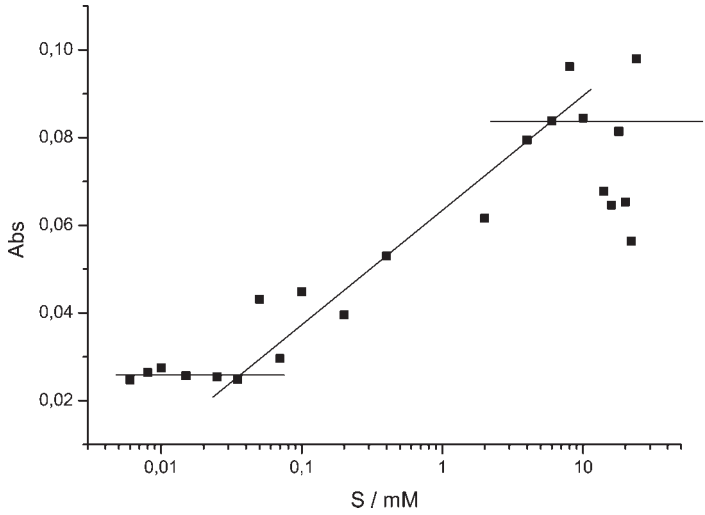

Figure 3.
Absorbance variation of IgG at 280 nm as a function of SPFO concentration. The IgG concentration was 0.015 mg/ml (0.1 μM). Estimated uncertainties are less than 2%.

(α) taken as:

$$\alpha = \frac{A_{280} - A_{280}^{N}}{A_{280}^{D} - A_{280}^{N}} \tag{9}$$

where A_{280}^{N} and A_{280}^{D} are the absorbance for the native and denaturated states respectively, and $K_S = \alpha/(1-\alpha)$. A_{280}^{N} and A_{280}^{D} were taken from the start and finish of the transition region. The pre y post transition curves were fitted by least-squares linear plots and the transition region was fitted by a polynomial; α and K where then calculated by the method described by Pace.[8]

The difference in the standard Gibbs energy between the folded and unfolded conformations, (ΔG^0), with the following relations:

$$\Delta G^0 = \Delta G_W^0 - m[S] \tag{10}$$

$$\ln K_S = \ln K_W - \frac{m}{RT}[S] \tag{11}$$

where ΔG_W^0 is the value of ΔG^0 for the transition in the absence of surfactant and m is a measure of the dependence of ΔG^0 on surfactant concentration. The standard Gibbs energy for unfolding in water ($\Delta GW0$) can be calculated from $\ln K_W$ from the intercept of $\ln K_S$ vs. $[S]$ at $[S] = 0$. From Equation (8) it follows that:

$$\ln K = \ln K_S - v \ln[S] \tag{12}$$

The plot of $\ln K_S$ vs. $\ln[S]$ is not linear as predicted equation (12) but is better represented by the equation:

$$\ln K_s = \ln K + v \ln[S] + b(\ln[S])^2 \tag{13}$$

(the reason for the curvature may be related to the neglect of activity coefficients in Equation (8)).

At a surfactant concentration of 1 mol dm^{-3}, $\ln K = \ln K_S$ and the equilibrium constant might then be considered to correspond to the transition in a surfactant-saturates complex approximating to that in a very hydrophobic environments with a corresponding Gibbs energy change, ΔG_{hc}. The found values of ΔG_{hc} and ΔG_W can be related by the following expression

$$\Delta G_{hc}^0 - \Delta G_W^0 = \Delta t_r G^0(D) - \Delta t_r G^0(N) \tag{14}$$

where $\Delta t_r G^0(D)$ and $\Delta t_r G^0(N)$ are the standard Gibbs energies of transfer of denatured and native IgG water to hydrophobic environments.

Table 1 lists the parameters $[S]_{1/2}$, m, ΔG_W^0, v, ΔG_{hc}^0 and $\Delta(\Delta t_r G^0)$ calculated from

Table 1.
Thermodynamic parameters characterizing the SPFO-induced conformational changes in IgG in aqueous solution at 25 °C.

$[S]_{1/2}$	m	ΔG^0_W	v	ΔG^0_{hc}	$\Delta(\Delta t_r G^0)$
mmol dm^{-3}	kJ mol^{-1}	kJ mol^{-1}		kJ mol^{-1}	kJ mol^{-1}
0.34 ± 0.03	2335.3 ± 274.1	3.1 ± 0.5	4 ± 2	-43.7 ± 20.1	-46.8 ± 20.6

equations 11, 13 and 23 for IgG-SPFO interactions, which are similar to found for other protein-surfactant interactions.[9–10] This suggests that the protein suffer a conformational change due to the chemical interaction with SPFO. We can see that conformational changes on IgG structure begin at very low surfactant concentration. The method did not provide us information to distinguish between electrostatic and hydrophobic interactions. The higher parameters values found, indicates that the interaction is highly coopetarive. This fact was previously inferred from potential data. So, the initial binding of SPFO molecules to the active site could act as a nucleus for the further binding and subsequent unfolding.

Figure 4 shows the CD spectra for native and SPFO-IgG mixture solutions. There is a clear decrease in the ellipticity content; the bands become more positive, as SPFO concentration augmented. A significant fraction of the IgG is denatured. This fact may be due to the binding of surfactant hydrophobic fluorinated tails, which will penetrate into the hydrophobic domains of the globular IgG in order to reduce their contacts with water. Due to such penetration the IgG molecule may deform as we can observe from the CD-UV spectra. The surfactant effect is appreciable even at very low surfactant concentration (0.013 mM), the changes are incessant at intermediates SPFO concentration (0.026–0.5 mM) and remains practically constant at high amphiphile concentration (2–6 mM).There is a clear decrease in the ellipcity content.

Figure 5 shows the thermal ellipticity variation of the mixture IgG-SPFO solutions. It can be seen that the presence of fluorinated surfactant affect the thermal–ellipticity profile. In the presence of the surfactant at the selected concentrations of 0.013; 0.026; 2; the transitions occurs at higher temperature and the second transition overlaps with the firts one. CD data

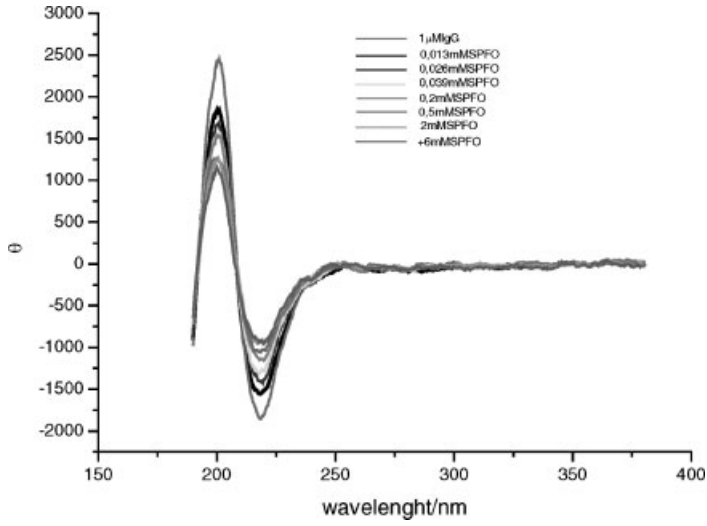

Figure 4.
UV-CD spectrum of pure IgG and SPFO-IgG mixture solution. Protein concentration was 1 μM.

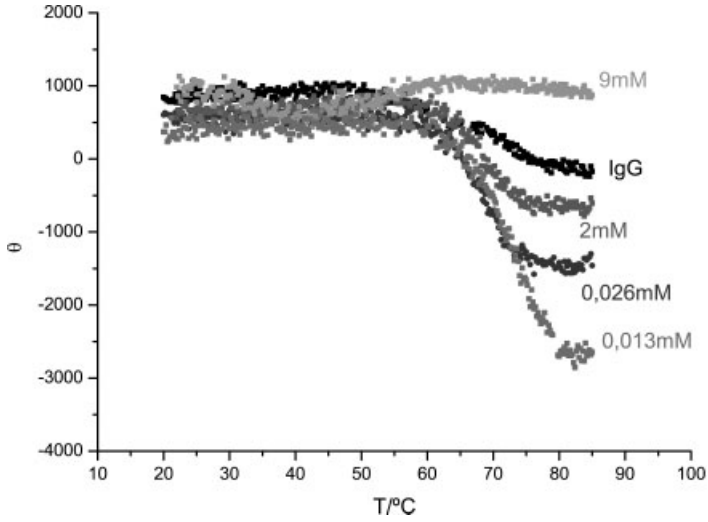

Figure 5.
Thermal elipticity variation of the mixture IgG-SPFO solutions at a heating rate of 30°C. Protein concentration was 1 μM.

demonstrate that SPFO has a notable effect on the thermal stability of IgG.

In the absence of SPFO, the fraction of α-helix, β-sheet, β-turn and random coil conformation of native IgG were 0, 66, 22, and 12% respectively.[11] So much for the native IgG as for the surfactant-protein mixtures (0.013–2 mM SPFO concentration), at higher temperatures the fraction of α-helix and random coil structures increase and those of β-sheet and β-turn decrease. For dissolved, native proteins it is more favorable for the peptide units at the aqueous periphery to form hydrogen bonds with water molecules than among each others. At low surfactant concentration (0.013 mM), part of the hydrophobic interior of the IgG will be exposed to the solution, which, in turn, causes the formation of aggregates. Now at the interfaces between the building blocks, hydrogen bonds between peptide units in the polypeptide chain may be formed, inducing the formation of α-helixes. Timmins et al.[12] and Zulauf[13] previously showed that the adsorbed amount of surfactant increases with increasing temperature.

As surfactant concentration augmented, hydrophobic perfluorinated tails penetrating the hydrophobic IgG domains, it causes a smaller contribution from intramolecular hydrophobic bonding to the stabilization of globular IgG structure in aqueous solution, so the increment in the percentage of α-helix decreased. The presence of a high surfactant concentration induces the transference of a significant fraction of the β-sheets into a random coil. The SPFO destabilizing effect can be easily seen from Figure 5, at high surfactant concentration (9 mM) IgG is completely unfolded.

The thermal unfolded curves are analysed in exactly the same way as in the chemical denaturant unfolding curves, but yields ΔG as a function of temperature rather can be used to determine the melting temperature (T_m), the entropy change at T_m (ΔS_m), the enthalpy change at T_m (ΔH_m) and the difference in heat capacity (ΔC_p) between the folded and unfolded conformations.

The thermal melting curve of IgG and IgG-SPFO was examined using a modified form of the Gibbs-Helmholtz equation:[8,14]

$$\Delta G(T) = \Delta H\left(1 - \frac{T}{T_m}\right) - \Delta C_p\left[T_m - T + T\ln\left(\frac{T}{T_m}\right)\right] \quad (15)$$

where $\Delta G(T)$ is ΔG at a temperature T. Equation (15) was used to fit thermal unfolding parameters by using a non-linear least-squares fit and successive iterations using the Marquardt-Levenberg routine. A minimum of 30 iterations or more was performed until the fractional change in the χ^2 value was within the tolerance limit, which was set to 0.005.

The ΔG thermal variation of native IgG and SPFO (0.013 mM)–IgG mixture respectively, are shown in Figure 6. Similar plots were fit from the analysis of the rest SPFO-IgG mixture solutions obtained data. The values of thermal unfolding parameters are listed in Table 2.

As we can see from the melting curves (Figure 5), the SPFO presence provokes that the transitions occurs at higher temperature and the second transition overlaps with the first one. The obtained melting thermodynamic parameters are in completely accord with those facts.

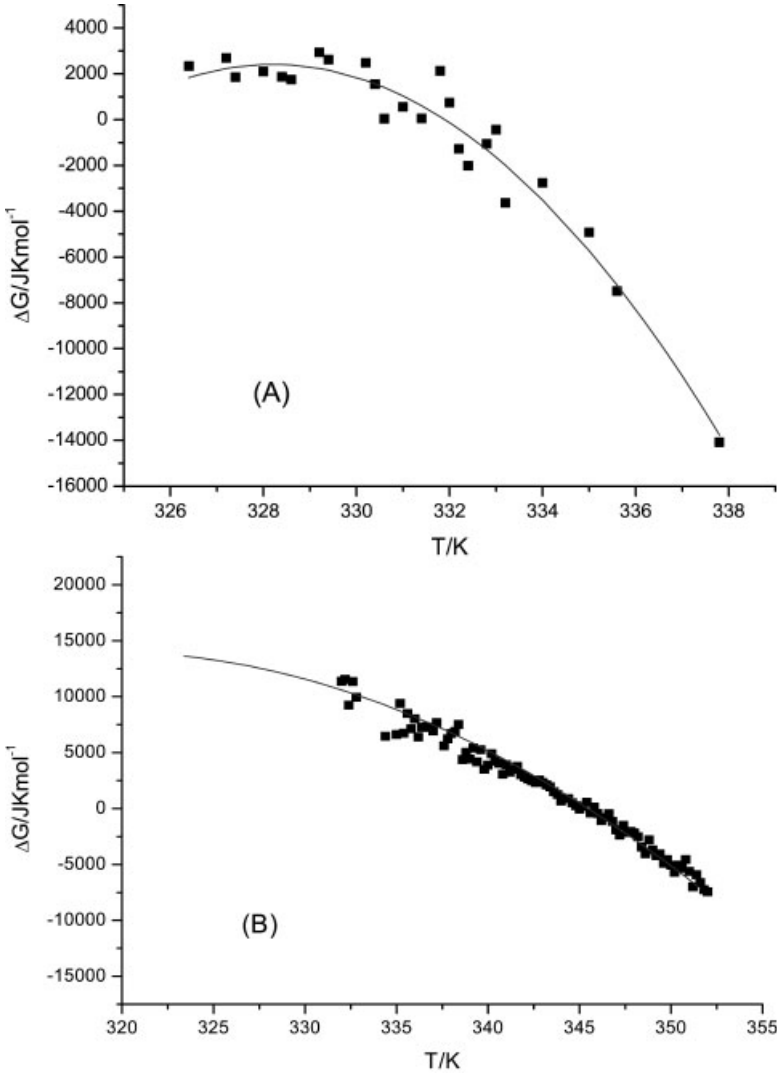

Figure 6.
Gibbs energy of thermal unfolding (ΔG) variation. (A) Pure IgG (1μM). (B) SPFO (0.013mM)-IgG(1μM) mixture. The solid line represents the best fit of equation (14) used to determine the thermodynamic parameters of unfolding.

Table 2.
Thermodynamic parameters characterizing the thermal-induced conformational changes in IgG aqueous surfactant solutions.

	TRANSITION I				TRANSITION II			
[SPFO]	ΔH_m	T_m	C_{pm}	ΔS_m	ΔH_m	T_m	C_{pm}	ΔS_m
mM	kJ mol^{-1}	K	kJ mol^{-1} K^{-1}	kJ mol^{-1} K^{-1}	kJ mol^{-1}	K	kJ mol^{-1} K^{-1}	kJ mol^{-1} K^{-1}
0	432.8 ± 35.1	331.9 ± 0.2	116.5 ± 13.0	1.3±0.1	328.2 ± 12.1	345.3 ± 0.2	2.9 ± 0.5	0.95 ± 0.15
0.013	361.6 ± 15.7	345.5 ± 0.2	13.2 ± 2.2	1.0 ± 0.1				
0.026	366.7 ± 9.5	340.7 ± 0.2	19.9 ± 3.3	1.1 ± 0.1				
2	337.8 ± 10.3	340.8 ± 0.2	18.6 ± 3.0	1.0 ± 0.1				

Conclusions

The interaction between IgG and SPFO has been studied by the employ of UV-vis, UV-CD spectrophotometric methods and potentiometric measurements. From the potential measurements obtained by the application of SPFO selective electrode, it was determined that there were true specific unions between surfactants molecules and IgG structure and SPFO manifests and high affinity for IgG molecules. The binding isotherm were filled to the polynomial form and used to calculated the Wyman binding potential (π) and the binding energy calculated per surfactant molecule bound per monomeric IgG (ΔG_v). This procedure leads to smooth curve of ΔG_v v*s*. v. This plot shows a minima corresponding to the most tightly bound ligands at low values of v, then the curve tend to a limiting value, -7.41 kJ mol^{-1}.

Spectroscopic measurements indicated that IgG allows a conformational transition induced by the binding of SPFO molecules on the protein structure. The conformational changes on IgG structure begin at very low surfactant concentration. The higher parameters values found, indicates that the interaction is highly cooperative. From the CD-UV spectra, we can observe that due the surfactant penetration the IgG molecule may deform. There is a clear decrease in the ellipticity content; the bands become more positive, as SPFO concentration augmented. A significant fraction of the IgG is denatured, which is reflected by a diminution of the β-sheet content. This fact may be due to the binding of surfactant hydrophobic fluorinated tails, which will penetrate into the hydrophobic domains of the globular IgG in order to reduce their contacts with water.

The thermal unfolded curves, analysed in exactly the same way as in the chemical denaturant unfolding curves, yields ΔG as a function of temperature rather can be used to determine the melting temperature (T_m), the entropy change at T_m (ΔS_m), the enthalpy change at T_m (ΔH_m) and the difference in heat capacity (ΔC_p) between the folded and unfolded conformations.

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