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ORIGINAL ARTICLE

Potential enzyme toxicity of perfluorooctanoic acid

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Abstract Using equilibrium dialysis, isothermal titration calorimetry (ITC) and circular dichroism (CD), the interactions of perfluorooctanoic acid (PFOA) and lysozyme were investigated under normal human physiological conditions, i.e., at pH 4.40, 6.00 and 7.40 at 37°C in 0.15 M electrolyte. A simple and rapid spectrophotometric method was developed for determining PFOA concentrations. Interactions between PFOA and lysozyme were found to result from non-specific non-covalent bonds—F/N and F/O affinity, ion-pair attraction, hydrogen bond, hydrophobic interaction and van der Waals force—and were affected by chemical adsorption to monolayers. The results indicated that binding of PFOA altered the secondary structure and activity of lysozyme. This work provides a useful experimental strategy for research into the enzyme toxicity of organic chemicals, e.g., food additives and organic contaminants, and it may help to elucidate the molecular toxicology of human health risks.

Keywords Lysozyme · Perfluorooctanoic acid · Non-covalent binding · Enzyme toxicity · Structural change

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Introduction

Knowledge of biomolecular interactions is important for our understanding of cellular processes including signal transduction, gene regulation, and enzyme reactions (Kraut et al. 2003; Almogren et al. 2006; Hunter et al. 2005). Lysozyme (EC 3.2.1.17), also known as muramidase or *N*-acetyl muramide glycanohydralase, is widely distributed in nature, including in tears and saliva and in skin, blood, liver and lymphatic tissues of humans and other animals. Its primary structure was one of the earliest to be solved; it contains a single chain of 129 amino acid residues (Blake et al. 1965). Under physiological conditions, lysozyme is folded into a compact, globular structure with a long cleft in the protein surface (Collins et al. 2005). It has many pharmacological functions, e.g., antiseptic, antioxidant, antiviral, and antineoplastic effects; it acts against Gram positive bacterial cells by hydrolyzing the polysaccharide component of the wall. It can improve human blood circulation and enhance immunity. However, intake of any extraneous chemical is likely to affect the activity of the enzyme in human tissues, either enhancing it (Marolia and D'Souza 1999), with potential medical significance, or inactivating it (Miller et al. 2005) if an organic contaminant or toxin is involved.

It is well known that the Teflon event involving the Dupont Company of USA drew serious international attention to perfluorooctanoic acid (PFOA). PFOA is formed from the raw materials used in the production of Teflon-lined non-stick cooking appliances. Fluoropolymers such as Teflon have very good performances as fire retardants and for oil and fat resistance; their byproducts such as PFOA can be formed by cooking, burning, and environmental degradation. PFOA is still widely used in basic processes in the aviation, automobile, building materials,

chemicals, electronic, semiconductor, and textile industries. It is persistent and non-biodegradable and becomes widely distributed in nature, e.g., water (Wania 2007), biological bodies (Lau et al. 2004), human tissues (Maestri et al. 2006) and foods (Karrman et al. 2007). It can certainly enter the gastrointestinal tract via the intake of foods and water and then it is absorbed and permeates into the blood and various tissues. Sampling studies have revealed the presence of PFOA in the bloods of over 90% of US residents (Calafat et al. 2007). It may have serious effects on the endocrine, immune and nervous systems and it can be delivered to the fetus through the umbilical cord and can accumulate (Abbott et al. 2007). It can also cause cancers of the liver, testis, pancreatic and mammary glands (Martin et al. 2007), and can lead to embryonic deformities and other diseases (Maras et al. 2006). In recent years, PFOA has become a significant issue in many aspects of environmental ecology, toxicology, pathology, and life sciences (Service 2005; Washburn et al. 2005). However, the detailed studies about enzyme toxicity of PFOA are very rare (Mulkiewicz et al. 2007). In this work, we investigated the interaction of PFOA with lysozyme under normal physiological condition by equilibrium dialysis, fluorospectrometry, isothermal titration calorimetry (ITC) and circular dichroism (CD) in order to elucidate the general principle underlying molecular toxicity in human health risk of PFOA.

Materials and methods

Instruments and chemicals

Model S-4100 spectrophotometer (Sinco Co., Korea), which was computer-controlled by Labpro Plus firmware (Version 060105); Model MSC-ITC system (MicroCal Inc., USA) with measurement software (VP-Viewer 2000); Model J-715 CD Spectropolarimeter (Jasco Instrum., Japan) with secondary structure Estimation-Standard Analysis Measurement software (715/#B014460524, JASCO); Model RC 30–5 K semi-permeable membrane (Molecular Weight Cut Off 5 KDa, Shanghai Green Bird STD). 1.00 mM lysozyme (Egg white; Amresco, USA) was prepared and stored at 4°C. The other solutions were prepared: a standard PFOA solution (New Jersey, USA) (5.00 mM); Enrichrome cyanine R (1.00 mM, ECR, A. R., Sigma) solution; 1.00 mM CPC (cetylpyridinium chloride, purity >99%, Shanghai Reagents). Lysozyme activity detection packages (Nanjing Jiancheng Bioengineering Institute, China) containing bacterial powder (5.0 mg), solvent (100.0 ml), and standard lysozyme (20000 U mg^{-1}). A series of Britton–Robinson (BR) buffer, pH 4.40, 6.00, and 7.40, were prepared from an acidic solution that contained 0.04 M each of H_3PO_4, HOAc, and H_3BO_3 by adjusting to appropriate pH using 0.2 M NaOH.

Determination of PFOA

Some approaches for determining PFOA have been developed using sophisticated tools, e.g., SPE-LC-MS (Karrman et al. 2007). In this work, a simple, sensitive and rapid method for determining PFOA was developed because of the long dialysis time. PFOA, as a type of anionic surfactant, reacts with cationic surfactants cetylpyridinium chloride (CPC) by ion-pair binding (Gao et al. 2004). The anionic color ligand eriochrome cyanine R (ECR) was used to bind CPC to form a CPC–ECR complex (supplementary material online Fig. S1 A). After preexperiment, the system pH value (3.81) and absorption wavelength (522 and 626 nm) were determined (supplementary material online Fig. S1 B). The following were mixed thoroughly in a 10.0-ml of volumetric flask: 2.0 ml pH 3.81 acetate buffer, 0.50 ml 1.00 mM ECR, and 0.30 ml 1.00 mM CPC. The dialysis solution was added (1.0 ml diluted to 10.0 ml). After mixing for 15 min, the absorbances A_{522} nm and A_{626} nm of the solution were measured at 522 and 626 nm against water. Then, the PFOA concentration (C_L) in the dialysis solution was calculated from the standard curve (supplementary material online Fig. S1 C). The spectral correction method (Gao et al. 2006) was applied to eliminate the interference of excess reactant. The correction absorbance (A_β) of the equilibrium reaction was calculated according to the relation: $A_\beta = A_{522} - \beta A_{626}$ nm where the symbol β denotes the correction constant and calculated to be 1.44 by $\beta = A_{522}/A_{626}$ nm. The regression equation: $A_\beta = 3.14C_L - 0.017$ was used to calculate C_L of PFOA between 0.01 and 0.05 mM.

Equilibrium dialysis

Unlike the color ligand (Gao et al. 2008), PFOA does not absorb light when it interacts with proteins so the interaction cannot be characterized by light spectrophotometry. Therefore, equilibrium dialysis was used (Housaindokht et al. 2002). We designed and made a new dialysis device as shown in Fig. 1, 12.0 ml solutions containing 3.0 ml of BR buffer (pH 4.40, 6.00, and 7.40), 0.15 M NaCl, 0.32 mM lysozyme (C_{M0}), a series of PFOA concentrations from 0 to 1.75 mM (C_{L0}) and distilled water were mixed and transferred to dialysis bags (1). A solution (36.0 ml) containing 0.15 M NaCl, 9.0 ml of BR buffer and distilled water was added to the dialysis cup (3). The temperature (2) of the water bath (4) was kept constant at 37°C by adjusting the thermostat magnetic stirrer (5). After 6 h, 1.0 ml of the dialysis solution (3) was collected from the

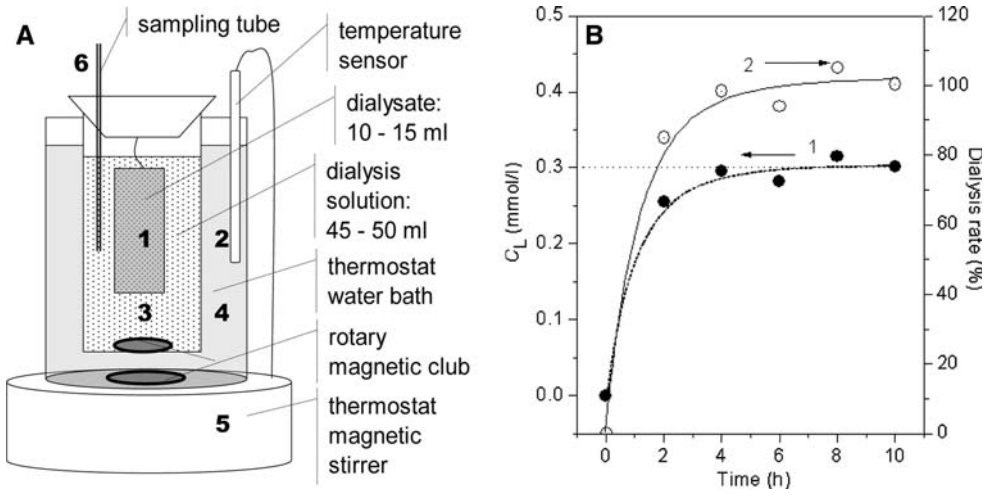

Fig. 1 **a** The new dialyzer was designed and used as illustrated. (1) Semi-permeable membrane with 15.0 ml of dialysate; (2) the temperature sensor for maintaining the reaction at a constant 37°C; *3* dialysis solution less than 50.0 ml; (4) water bath at constant 37°C. The apparatus was placed on a thermostated magnetic stirrer (5) and rotary magnets were used to mix solutions (3) and (4) thoroughly. The PFOA concentration in solution (3) was determined at various times from the sampling tube (6). **b** Equilibrium dialysis of PFOA: change of PFOA concentration (C_L) (1) and the PFOA dialysis rate (2). The dialysis solution initially contains 9 ml of pH 7.40 BR buffer and 27 ml of distilled water and 3.0 ml of pH 7.40 BR buffer, 3.0 ml of 5.00 mmol l^{-1} PFOA, and 4.0 ml of distilled water were mixed in the semi-permeable membrane bag

sampling tube (6) and the PFOA concentration (C_L) was determined by the above spectrophotometric method.

ITC measurement

0.15 mM lysozyme solution contained 0.15 M NaCl (in pH 4.40, 6.00, and 7.40 BR buffer) was placed in the sample-cell of ITC device and the cell was kept at 37°C. A measure of 2.50 mM PFOA solution contained 0.15 M NaCl (in pH 4.40, 6.00, and 7.40 BR buffer) was taken in the injector and it was injected into the isothermal sample-cell for 40 times in 6-μl increments at 3-min intervals. Heats of dilution of PFOA, obtained separately by injecting into the buffer, were used to correct the data. The corrected heats were divided by the number of moles injected and analyzed using the Origin software (version 7.0) supplied by the manufacturer.

CD measurement

BR buffer (1.0 ml, pH 4.40, 6.00 or 7.40) was mixed with 0.15 mM lysozyme in three flasks; 0 or 1.00 mM PFOA was added and the solutions were diluted to 10.0 ml with distilled water. Simultaneously, a reagent blank without PFOA was prepared. Before measurement, all the solutions were diluted from 0.10 to 10.0 ml with 10% BR buffer. Each sample was allowed to equilibrate for 15 min, then injected into a 0.1-cm light path cell, and the mean residue ellipticity (MRE) of lysozyme was measured between 190 and 250 nm. From the MRE curves, the relative contents of secondary structure forms of lysozyme—α-helix (feature λ: 190, 208, and 222 nm), β-pleated sheet (195, 215–217 nm), β-turn (180–190, 200–205, and 225 nm) and random coil (<200, 218 nm)—were calculated in all the solutions with the relation, $\theta_\lambda = f_\alpha\ [\theta]_{\lambda,\alpha} + f_\beta\ [\theta]_{\lambda,\beta} + f_\gamma[\theta]_{\lambda,\gamma}$, where $[\theta]_{\lambda,\alpha}$, $[\theta]_{\lambda,\beta}$, and $[\theta]_{\lambda,\gamma}$ are the MRE and f_α, f_β, and f_γ are the mass percentage of α-helix, β-pleated sheet, and random coil at λ.

Assay of lysozyme activity

The turbidimetric method for determining lysozyme activity was used. Following the instruction manual, a bacterial suspension was prepared by mixing the powder with the solvent and a series of standard lysozyme concentrations in distilled water, and six samples containing lysozyme (0.15 mM) and PFOA (0 or 1.00 mM) at pH 4.40, 6.00 or 7.40 were added to it and incubated at 37°C. The absorbance of each suspension was measured at 530 nm after 5 and 125 s. A reference was prepared and measured with the standard lysozyme provided in the activity detection package. After spectrophotometric measurement, the lysozyme activity of each sample was calculated according to the instruction manual.

Results

Equilibrium dialysis of PFOA

Using the determination method described above, the PFOA concentration in the dialysis solution (Fig. 1) was

measured at 37°C at various dialysis times with no protein in the membrane. The results indicated that the PFOA dialysis rate approaches 90% at 2 h and exceeds 98% after 4 h from curves 1 and 2 (Fig. 1b). Therefore, PFOA passes freely through the semi-permeable membrane until equilibrium reached. A lysozyme solution was put into the dialysis membrane as dialysate instead of PFOA, but they were found in the dialysis solution. This indicates that equilibrium dialysis is a suitable method for investigating the interaction of PFOA with lysozyme. The dialysis solution was sampled and measured spectrophotometrically to determine C_L after more than 6 h of dialysis.

Interaction of PFOA with lysozyme

The interaction of PFOA with lysozyme is summarized as follows:

	NPFOA + lysozyme $\overset{K_b}{\rightleftharpoons}$ lysozyme(PFOA)$_N$		
Initiation	c_{L0}	c_{M0}	0
Equilibrium	$c_L = c_{L0} - Nc_{M0}$	$c_M \dashrightarrow 0$	c_{M0}

where both C_{L0} and C_{M0} are the initial mole concentrations of PFOA and lysozyme, C_L is the equilibrium concentration of PFOA. N is the saturation binding number of PFOA. The effective fraction (f) of PFOA bound to lysozyme and its molar binding ratio (γ) are calculated by the relations: $f = 1 - \eta \frac{C_L}{C_{L_0}}$ and $\gamma = f \frac{C_{L0}}{C_{M0}}$ η is the dilution times of PFOA during the equilibrium dialysis. The γ value will approach N with increasing PFOA in the lysozyme solution.

As it is well known, pH varies widely among normal tissues in the human body: for example, less than pH 3.0 in gastric fluid, pH 6.0–7.0 in saliva, pH 5.6–6.6 in the skin, and around pH 7.4 in blood and intestinal tract. Normal human temperature is 37°C and the electrolyte concentration is between 0.8 and 0.9% (approximately 0.15 M). Therefore, the interaction of PFOA with lysozyme was studied at pH 4.40, 6.00 and 7.40. All the experiments were conducted in 0.15 M NaCl at 37°C. By measuring a series of PFOA solutions containing lysozyme, the f and γ values for each were calculated according to the above equations. The variation in γ with PFOA is shown in Fig. 2. The γ values increase with increasing PFOA concentration and approach the following maxima: 1 when C_{L0}/C_{M0} is more than 3.7 from curve 1 in Fig. 2a; 1 when C_{L0}/C_{M0} is more than 1.5 from curve 2 and 0.9 when C_{L0}/C_{M0} is more than 3.0 from curve 3. These results indicate that PFOA binding to lysozyme is saturable. Thus, the number (N) of PFOA molecules bound per molecule of lysozyme is approximately 1 at pH 4.40, 6.00, and 7.40. Those N values are given in Table 1. There is no correlation between the saturation binding number of PFOA and the basic amino acid contents of lysozyme. Unlike the sulfonic azo ligand (Gao et al. 2008) and metal chelate (Ajloo et al. 2007), the binding of PFOA to proteins does not result from ion-pair electric attraction. Hydrogen bond and hydrophobic

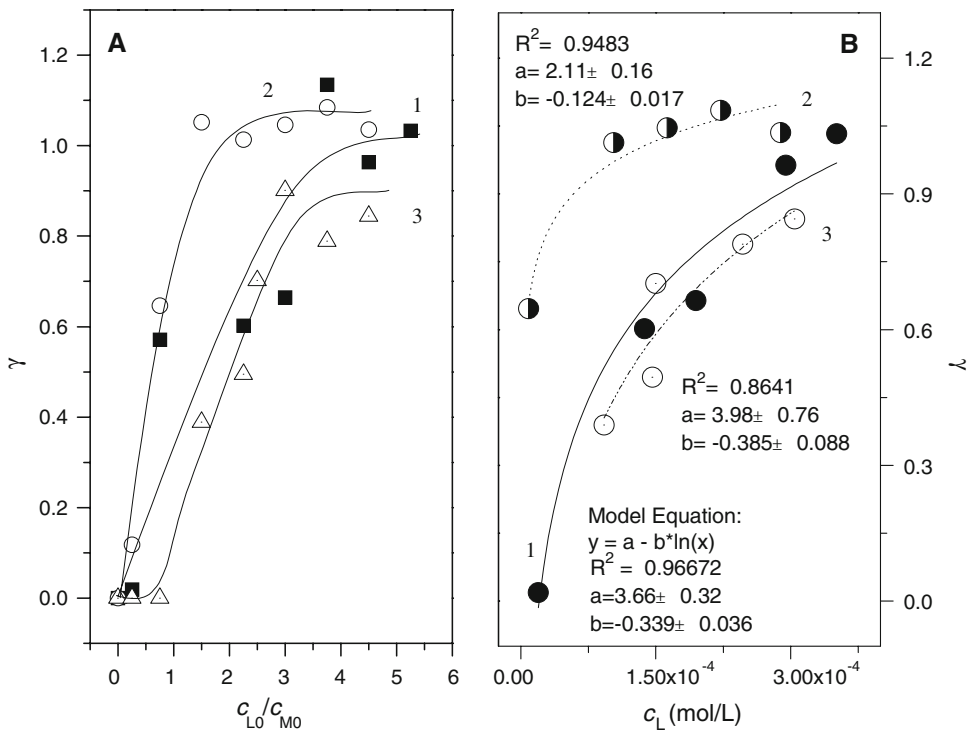

Fig. 2 **a** Effect of PFOA (initial concentration from 0 to 1.75 mM in the dialysate) on binding to lysozyme (initially 0.333 mM). 1-, 2-,and 3-plots of γ versus C_{L0}/C_{M0} at pH 4.40, 6.00, and 7.40. **b** Plots of γ versus C_L. All the solutions were in 0.15 M NaCl at 37°C

Table 1 Determination of the thermodynamic parameters of PFOA–lysozyme binding reaction at pH 4.40, 6.00, and 7.40 at 37°C

pH	ΔQ (kcal mol^{-1})	$K_a \times 10^4$ (M^{-1})	N	K_b (M^{-1})	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})	ΔG (kcal mol^{-1})
4.4	−1.8 ± 0.2	4.9 ± 0.5	1	236 ± 26	−11 ± 1	−14	−6.6
6.0	−5.0 ± 0.7	2450 ± 340	1	148 ± 13	−35 ± 3	−62	−15.7
7.4	−1.6 ± 0.3	3.1 ± 0.6	1	698 ± 47	−3.9 ± 0.2	−14	0.47

ΔQ and K_a are calculated by the Temkin model

K_b, ΔH, and ΔS are calculated from ITC measurements

interaction may be involved. The Temkin isothermal models (Tsai et al. 2006) were used to fit the above experimental data:$\gamma = -\frac{NRT}{\Delta Q}\ln(K_a C_L)$, where K_a is the adsorption constant (M^{-1}) and ΔQ the adsorption energy (J mol^{-1}) of a saturating concentration of PFOA; T is the temperature in Kelvin and R the gas constant, 8.314 J mol^{-1} K^{-1}. Plots of γ versus C_L for the PFOA–lysozyme solutions are shown in Fig. 2b. PFOA binding to lysozyme fitted the Temkin model. Therefore, the interactions of PFOA with lysozyme correspond to chemical monolayer adsorption. From the gradients and intercepts of the regression equations in Fig. 2b, ΔQ and K_a were calculated as shown in Table 1 for the PFOA–lysozyme complexes. Because all ΔQ values are less than 10 kcal mol^{-1}, the binding of PFOA is non-covalent (Yang 1998) and the adsorption is exothermic. This indicates that no strong bond was formed between PFOA and lysozyme. Comparison of the ΔQ and K_a values (Table 1) shows that the binding of PFOA to lysozyme at pH 6.00 is the most stable. A possible reason is that lysozyme is most active at pH 6.00. This is further confirmed in the lysozyme activity experiments.

Thermodynamic characterization of PFOA–lysozyme interaction and secondary structure change of lysozyme

ITC measurements provide information on thermodynamic quantities such as enthalpy and heat capacity changes during the molecular interaction directly from the heat produced by the reaction, and have been used to study, for example, protein interactions (Cooper et al. 1994), DNA triplex formation (Kamiya et al. 1996), and HIV protease activity (Luque et al. 1998). From Fig. 3, treated from the isothermal titration profiles obtained by injecting PFOA into the ITC cell containing lysozyme at 37°C, PFOA binding to lysozyme indicated a single-step model. This was confirmed by the saturation number of PFOA, which was only 1. Values for the equilibrium constant (K_b), enthalpy change (ΔH) and entropy change (ΔS) of the PFOA–lysozyme reaction were obtained from curves and calculated by the Gibbs free energy (ΔG) equation: $\Delta G = -RT\ln K_b = \Delta H - T\Delta S$. The thermodynamic parameters derived from these curves are summarized in

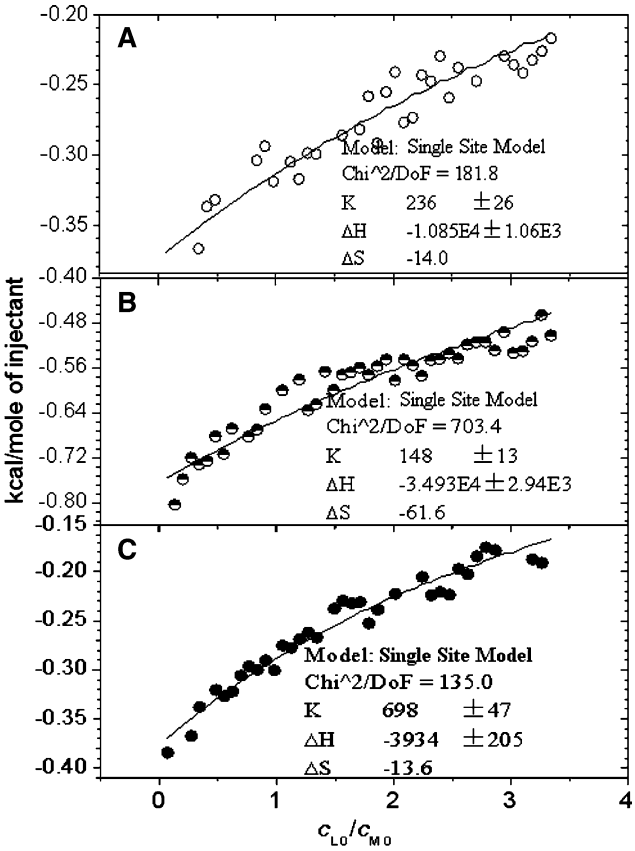

Fig. 3 The area of each peak integrated from the ITC titration profiles of PFOA–lysozyme solutions at pH 4.40 (**a**), 6.00 (**b**) and 7.40 (**c**). They all have been corrected for the heat of dilution, which was estimated in a separate experiment by injecting the PFOA into the BR buffer. The corrected heat was divided by the moles of injectant and the values were plotted as a function of the PFOA/lysozyme molar ratio, C_{L0}/C_{M0}. The titration curve was fitted by a nonlinear least-squares method. The temperature was 37°C and all the solutions contained 0.15 M NaCl and 20% BR buffer. Each pulse corresponds to a 6-μl injection of 2.50 mM PFOA into the ITC cell (1.4685 ml) containing 0.15 mM lysozyme

Table 1. Because all ΔH are much less than 60 kcal mol^{-1} (Yang 1998), the PFOA–lysozyme interactions are non-covalent. As it is well known, PFOA is more lipophilic in aqueous solution and it decreased obviously the aqueous surface intensity. Fifteen electrophilic F-groups in PFOA can attract strongly the lone pair electrons of N- and O-groups on the side chain of protein. Thus, the above

non-covalent bonds involve H-bond, ion-pair attraction, strong dispersion force i.e., F/O and F/N affinity, hydrophobic interaction, and van der Waals force. From Table 1, all the negative ΔH values indicated that the PFOA–lysozyme interactions are exothermic at the various pHs examined. In contrast, the negative ΔS values indicated that the lysozyme structure always approaches a high potential energy state in the presence of PFOA, perhaps becoming more order. Only an unstable conformation resulted in a K_b value less than 1,000 M^{-1} (Table 1). In particular, both the ΔH and ΔS values are most negative at pH 6.00. Thus, most of the heat released was consumed in a conformational transition of the lysozyme molecule. Such an obvious change in structure was confirmed from the CD spectra (Fig. 4b). Unlike K_a, K_b indicates the potential stability of PFOA binding and it was minimal at pH 6.00. This is presumably because the PFOA–lysozyme complex was in the highest-energy structural state at pH 6.00, as indicated by the most negative ΔS value. From curves in Fig. 4, the addition of PFOA at pH 6.00 and 7.40 caused an increase in the β-pleated sheet from 33.4 to 44.5% and from 32.5 to 41.6% (Table 2) and turn content of lysozyme from 5.1 to 11.8% and from 6 to 12.6% and a decrease in α-helix from 26.3 to 18.1% and from 26.4 to 19.2%. These changes confirmed the folding of lysozyme in PFOA solution. From the ΔS and ΔH values (Table 1), PFOA may bridge between two α-helixes via H-bond and F/O and F/N affinity and be inserted into the hydrophobic cavity of lysozyme via hydrophobic interaction. Thus, the helix structure was spread into sheet form, so PFOA may inhibit lysozyme. This was confirmed by further experiments. In contrast, from curves in Fig. 4a, the α-helix content increased from 25 to 28.7% (Table 2) and the β-pleated sheet decreased from 38 to 34.1% at pH 4.40. From the ΔH value (Table 1), PFOA may bridge between two β-pleated sheets mainly via hydrogen bond and F/O and F/N affinity to cause an increase of α-helix; the possible PFOA binding site is located on the exposed surface of lysozyme. The increase in α-helix content will increase the activity of the lysozyme.

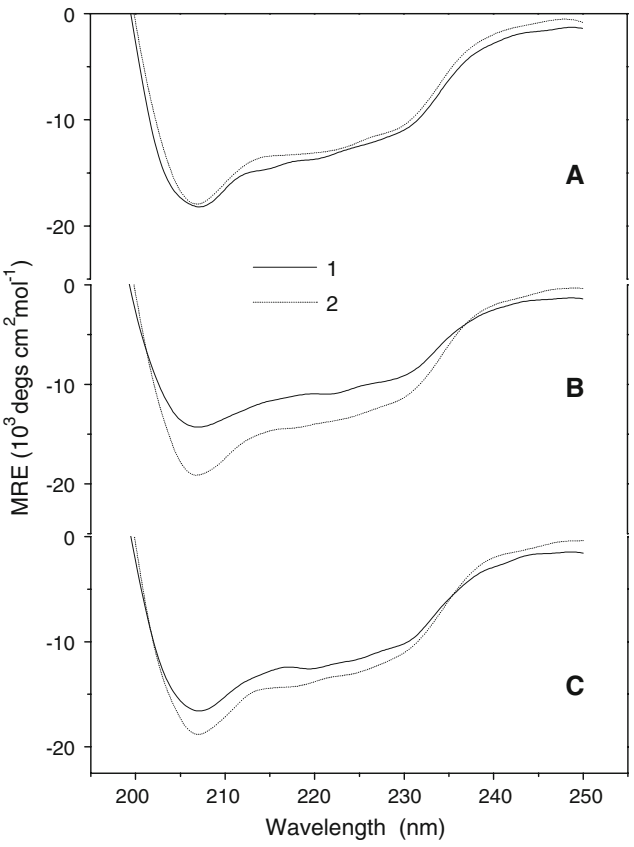

Fig. 4 Molar ellipiticity curves for lysozyme (0.15 mM) solutions at pH 4.40 (**a**), pH 6.00 (**b**), and pH 7.40 (**c**), containing no PFOA (*curve* 1) or 1.00 mM PFOA (*curve* 2)

Table 2 Change of the secondary structure of lysozyme in the presence of PFOA

pH	Factor	PFOA	
		0 mM	1.0 mM
4.40	α-Helix (%)	25	28.7
	β-Sheet (%)	38	34.1
	Turn (%)	3.1	3.5
	Random (%)	34	33.8
6.00	α-Helix (%)	26.3	18.1
	β-Sheet (%)	33.4	44.5
	Turn (%)	5.1	11.8
	Random (%)	35.2	25.6
7.40	α-Helix (%)	26.4	19.2
	β-Sheet (%)	32.5	41.6
	Turn (%)	6	12.6
	Random (%)	35.1	26.6

Change of lysozyme activity

Structural transformations that occur during protein folding and functioning are of great significance in organisms (Hu and Xu 1999). During the formation of a complex, a small organic compound may insert into the inner part of the protein, altering structure and function (Xie et al. 2005; Liu et al. 2000). Non-covalent binding is often weak and non-specific but a union of many non-covalent bonds may alter protein conformation and function (Piekarska et al. 1996). As it is well known, lysozyme attacks a specific component of certain bacterial cell walls, the peptidoglycan composed of *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) cross-linked by peptide bridges, by hydrolyzing the bond between NAG and NAM. This increases the permeability of the wall and causes bacterial lysis (Park et al.

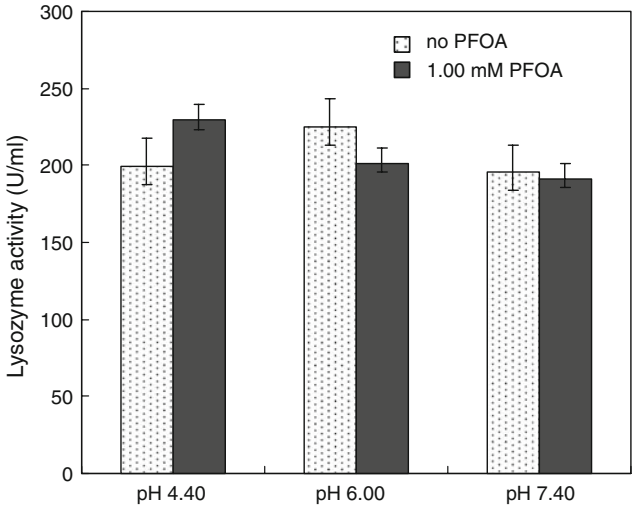

Fig. 5 Change of lysozyme activity in six samples containing 0.15 mM lysozyme with no PFOA (*mesh column*) or 1.00 mM PFOA (*black column*) at pH 4.40, 6.00, and 7.40 in 0.15 M NaCl

2006). Polysaccharides with sulfonate groups (Derham and Harding 2000), sodium dodecyl sulfate (Waehneldt 1975), and native ovalbumin (Cunningham and Lineweaver 1967) were shown years ago to inhibit lysozyme. The effect of PFOA on lysozyme activity was determined and the change in activity is shown in Fig. 5. Lysozyme itself has the highest physiological activity at pH 6.00. When 1.00 mM PFOA was added, the activity increased by 10% at pH 4.40. This resulted from the increase in α-helix content in the presence of PFOA (Fig. 4a). In contrast, the lysozyme activity decreased at pH 6.00 and 7.40 owing to the decrease in α-helix content (Fig. 4b, c). The level of inhibition (over 10%) is more obvious in the presence of 1.0 mM PFOA at pH 6.00 because the K_a value is highest and the ΔH and ΔS values are most negative (Table 1). A possible reason is that insertion of PFOA into the hydrophobic cavity of lysozyme (Maras et al. 2006) prevented the entry of bacterial cell wall peptidoglycan into the cleft, thus inhibiting hydrolysis. Therefore, non-covalent binding of PFOA affected the activity of the enzyme by altering its structure and overlapping or exposing the active site.

Discussion

Ligand binding is often associated with denaturation of macromolecules (folding or unfolding), in particular covalent binding with specific residues, as in identification of enzyme active sites (Brazeau et al. 2004), pharmaceutical development (Petitpas et al. 2003), and heavy metal toxicity (Giaginis et al. 2006). However, non-specific non-covalent interactions—ion-pair attraction, hydrogen bond, dispersion force, hydrophobic interaction, and van der Waals force—are more numerous in cells. Although a non-covalent bond is often weaker than a covalent bond, the union of many non-covalent bonds will produce a stronger association. In fact, the interaction of an organic chemical with a protein is very complicated although the crystal structures of a number of proteins have been analyzed (Durek et al. 2007) and possible binding regions identified (Brazeau et al. 2004). In this present work, the non-covalent interactions of PFOA with lysozyme under normal human physiological conditions were investigated by equilibrium dialysis, ITC, and CD melting techniques. There is only one PFOA binding site in lysozyme. The thermodynamic characterization of PFOA–lysozyme binding reaction indicated the interaction induces a combination of multiple non-covalent bonds. The results found may be general for the toxic effects of PFOA because it bound to the model protein e.g., human serum albumin (Wu et al. 2008). This work provides a useful approach for research into the enzyme toxicity of organic chemicals, e.g., food additives, organic contaminants, and drugs.

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