Calorimetric determination of thermodynamic parameters of 2′-dUMP binding to *Leishmania major* dUTPase

Irene Domínguez-Pérez a, Ramiro Téllez-Sanz a, Isabel Leal b, Luis M. Ruíz-Pérez b, Dolores González-Pacanowska b, Luis García-Fuentes a, *

a Dpto. de Química Física, Bioquímica y Q. Inorgánica, Facultad de Ciencias Experimentales, Universidad de Almería, La Cañada de San Urbano, 04120 Almería, Spain

b Instituto de Parasitología y Biomedicina “López-Neyra”, Consejo Superior de Investigaciones Científicas, Avda. del Conocimiento s/n. Parque Tecnológico de Ciencias de la Salud, 18100 Granada, Spain

Received 11 February 2004; received in revised form 29 June 2004; accepted 22 July 2004

Available online 9 August 2004

Abstract

We have investigated the binding of 2′-deoxyuridine 5′-monophosphate (2′-dUMP) to *Leishmania major* deoxyuridine 5′-triphosphate nucleotide hydrolase (dUTPase) by isothermal titration microcalorimetry under different experimental conditions. Binding to dimeric *L. major* dUTPase is a non-cooperative process, with a stoichiometry of 1 molecule of 2′-dUMP per subunit. The utilization of buffers with different ionization enthalpies has allowed us to conclude that the formation of the 2′-dUMP–dUTPase complex, at pH 7.5 and 30 °C, is accompanied by the uptake of 0.33 ± 0.05 protons per dUTPase subunit from the buffer media. Moreover, 2′-dUMP shows a moderate affinity for the enzyme, and binding is enthalpically driven across the temperature range studied. Besides, whereas ΔG° remains practically invariant as a function of temperature, both ΔH and ΔS° decrease with increasing temperature. The temperature dependence of the enthalpy change yields a heat capacity change of ΔC_p = −618.1 ± 126.4 cal·mol⁻¹·K⁻¹, a value low enough to discard major conformational changes, in agreement with the fitting model. An interpretation of this value in terms of solvent-accessible surface areas is provided.

**Keywords:** dUTPase; *Leishmania major*; *Trypanosoma cruzi*; 2′-dUMP; Binding; Microcalorimetry

1. Introduction

DNA repair is indispensable for the faithful conservation of genetic information. The ubiquitous enzyme deoxyuridine 5′-triphosphate nucleotide hydrolase (dUTPase, E.C. 3.6.1.23), catalyzing the hydrolysis of dUTP to pyrophosphate (PPi) and 2′-deoxyuridine 5′-monophosphate (2′-dUMP), plays a preventive role in maintaining dUTP levels low enough to suppress ruinous incorporation of uracil into DNA [1]. Highly uracil-substituted DNA is subjected to excessive excision repair leading to double strand breaks and, eventually, cell death. Thus, control of intracellular dUTP levels by dUTPase is indispensable for cellular organisms. The importance of minimizing dUTP pools is also underscored by the fact that dUTPase is essential in both *E. coli* [2] and the yeast *Saccharomyces cerevisiae* [3], which suggests that its activity might be crucial for DNA replication and cell division. Furthermore, there is indirect evidence implicating increases of intracellular dUTP in the action of cancer chemotherapeutic agents, including thymidylate synthase inhibitors and folate agonists. dUTPase inhibition has also been proposed as an anti-viral strategy.

Abbreviations: dUTPase, deoxyuridine 5′-triphosphate nucleotide hydrolase; *L. major*, *Leishmania major*; *T. cruzi*, *Trypanosoma cruzi*; ITC, isothermal titration calorimetry; 2′dUMP, 2′deoxyuridine 5′monophosphate; 2′dUDP, 2′deoxyuridine 5′diphosphate

* Corresponding author. Tel.: +34 950 015618; fax: +34 950 015008.
E-mail address: lgarcia@ual.es (L. García-Fuentes).

1570-9639/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
Most dUTPsases are homotrimers with three active sites located at the interfaces between the subunits [4]. Each active site consists of five conserved sequence motifs [5] from different subunits. The three-dimensional structure has been determined for the enzymes from E. coli [6,7], feline immunodeficiency (FIV) [8], equine infectious anaemia virus (EIAV) [9] and human [4]. However, it is also known that there are some monomeric dUTPase structures [5].

The enzymes from the protozoan parasites Leishmania major [10–12] and Trypanosoma cruzi [13] are dimeric and show a high degree sequence similarity. However, these dUTPsases do not show sequence similarity with trimeric dUTPsases and lack the five structural motifs.

Another difference between dimeric and trimeric dUTPsases is that the former are able to hydrolyze efficiently 2'-deoxyuridine 5'-diphosphate (2'-dUDP), whereas the latter can bind 2'-dUDP but cannot hydrolyze it. In other words, 2'-dUDP is a competitive inhibitor for trimeric dUTPsases. To date, the crystal structure of L. major dUTPase has not been solved. Recently, however, the structure of T. cruzi dUTPase in the complex with 2'-dUDP has been solved [14]. Two domains, termed ‘rigid’ and ‘mobile’, and angled at about 60° to each other, compose each subunit (Fig. 1). An exhaustive kinetic study has been performed with L. major dUTPase [12]. The enzyme is a dUTPase–dUDPase, which exhibits product inhibition and the K_i for 2'-dUMP is 13.05 μM. However, so far, no thermodynamic data have been reported for this system. In the present paper, we report the first thermodynamic study using isothermal titration calorimetry (ITC) of the binding of 2'-dUMP to L. major dUTPase. The information provided contributes to understand the behaviour of this dimeric enzyme, which constitutes a potential target for pharmaceutical inhibitors.

2. Materials and methods

2.1. Chemicals

2'-dUMP and 2'-dUTP were ultrapure grade from Sigma and Amersham Biosciences, respectively. 2-Mercaptoethanol and EDTA were from Merck. 2-Glycerophosphate, PIPES, BICINE and HEPES buffers were purchased from Merck and Sigma. Centriprep 30 concentrators were from Amicon. DEAE-cellulose DE52 and hydroxyapatite Bio-Gel® HTP gels were purchased from Whatman and Bio-Rad, respectively. All other chemicals were of analytical grade of the highest purity available. All solutions were made with distilled and deionized (Milli Q) water. All solutions were degassed and clarified through a 0.45-μm Millipore filter before use. The concentration of 2'-dUMP was determined from absorbance measurements at 262 nm using a molar extinction coefficient of 10^4 M^-1 cm^-1 [15].

2.2. Expression and purification

Recombinant L. major dUTPase was overexpressed in the E. coli strain BL21 and purified as previously described [11]. Basically, the purification involves two consecutive chromatographic steps, using hydroxyapatite and DEAE-cellulose. The enzyme showed a single-band pattern in SDS polyacrylamide gel electrophoresis. Purified protein was stored at −80 °C in 20 mM potassium phosphate, 2 mM 2-mercaptoethanol and 100 mM MgCl2 at pH 7.5. Protein concentrations were determined spectrophotometrically from the absorbance at 280 nm using an extinction coefficient of 1.065 10^5 M^-1 cm^-1. This coefficient was calculated on the basis of the amino acid sequence as reported by Gill and von Hippel [16], and confirmed by bicinchoninic acid method (Pierce). A molecular mass of 30.3 kDa per dUTPase subunit was used in the calculations. Absorbance measurements were carried out using a Beckman DU-7400 spectrophotometer with cells maintained at 25 °C.

2.3. Enzyme assay

The hydrolysis of dUTP by L. major dUTPase to 2'-dUMP and PP_i was recorded spectrophotometrically by monitoring the decrease in absorbance at 573 nm using cresol red as an indicator [12]. The reaction was initiated by the addition of 35 nM enzyme in 25 μM dUTP, 25 mM MgCl2, 2.5 mM BICINE and 50 μM cresol red (Merck) buffer, at pH 8 and 25 °C [12].

2.4. Isothermal titration calorimetry

ITC experiments were performed using a MCS isothermal titration microcalorimeter from Microcal, Inc. (Northampton, MA, USA). A complete description of its predecessor instrument, OMEGA-ITC, experimental strat-
egies, and data analyses are given by Wiseman et al. [17] and Schwarz et al. [18]. Prior to the titration experiments, both the enzyme and the nucleotide were degassed for 10 min under vacuum. The sample cell was filled either with 1.8 ml (effective volume: 1.38 ml) of buffer (for the control experiment) or an appropriately diluted enzyme. During the titration, the reaction mixture was continuously stirred at 400 rpm. The experiments were carried out by titrating dUTPase solutions with 2'-dUMP.

The background titration profiles, under identical experimental conditions, were obtained by injecting 2'-dUMP into appropriate buffer solutions. The observed heat effects were concentration-independent and were also identical to the heat signals detected after complete saturation of the protein. The raw experimental data are presented as the amount of heat evolved per second following each injection of ligand into the enzyme solution (after correction for the ligand heat of dilution) as a function of time. The amount of heat produced per injection was calculated integrating the area under individual peaks using the Origin software provided with the instrument.

The heat released for every injection, $\Delta Q_i$, was then fitted via a nonlinear least-squares minimization method to the total ligand and total protein concentrations inside the cell, $X_i$ and $M_i$, respectively. $\Delta Q_i$ is calculated from

$$\Delta Q_i = Q_i^{\text{tot}} - Q_i^{\text{pre}}, + \frac{dV}{2V_o} \left( Q_i^{\text{tot}} + Q_i^{\text{pre}} \right)$$

(1)

where $V_o$ is the cell volume and $dV_i$, the volume of titrant added in the solution. $Q_i^{\text{tot}}$ is the total heat generated up to the $i$th injection, according to

$$Q_i^{\text{tot}} = 2M_i \Delta H_b \rho \left[ 1 + X_i / 2M_i + 1 / 2K_{M_i} \right]$$

$$- \left[ \left( 1 + X_i / 2M_i + 2K_{M_i} \right)^2 - 4X_i / 2M_i \right]^{1/2} / 2$$

(2)

which corresponds to a model of two equal and independent sites.

Values of $\Delta H_{\text{obs}}$ determined by ITC may have contributions from the heat of ionization of the buffer if there is any net protonation or deprotonation of the interacting species upon formation of protein–2'-dUMP complex [19–21]. Values of $\Delta H_b$ (binding enthalpy) have been corrected for the contribution due to the heat of ionization of the buffer using Eq. (3)

$$\Delta H_{\text{obs}} = \Delta H_b + n_{\text{H}} \Delta H_{\text{ion}}$$

(3)

where $n_{\text{H}}$ is the number of protons taken up (positive value) or released (negative value) by the dUTPase–2'-dUMP complex upon binding. We note, however, that even after this correction, $\Delta H_b$ still includes contributions from the heat of protonation of the ionizable group (or groups) on the protein or 2'-dUMP.

By means of an iterative fitting analysis (combining Eqs. (1), (2) and (3)) of the experimental data to the above model, using SCIENTIST (MicroMath Scientific Software, Salt Lake City, UT), we can obtain the enthalpy change ($\Delta H_b$), the association constant ($K$) and the number of protons exchanged ($n_{\text{H}}$) upon complex formation.

To calculate the heat contributions due to coupled protonation events upon binding, the buffers used and their ionization enthalpies (in kcal mol$^{-1}$ at 25 °C) were as follows: 2-glycerophosphate (~0.17), PIPES (2.74) and HEPES (5.03) [22]. To calculate the ionization enthalpy at each experimental temperature, we have used the heat capacity change data from Fukada and Takahashi [22]. The pH of the buffer solutions was adjusted at the desired temperature.

A complete thermodynamic description is finally provided by $\Delta G = -RT \ln K = \Delta H_b - T \Delta S$, which allows the calculation of the free energy binding ($\Delta G$) and the change in entropy ($\Delta S$). The standard state is that of 1 mol l$^{-1}$, and these calculations imply the usual approximation of setting standard enthalpies equal to the observed ones.

### 3. Results and discussion

#### 3.1. Isothermal calorimetry experiments

Fig. 2 shows a typical titration of 36.59 μM dUTPase from *L. major* with 29 aliquots (5 μl each) and one preinjection of 1 μl of 2'-dUMP (stock concentration of 5.31 mM) in 50 mM Pipes buffer at pH 7.5 and 20 °C. The top panel in Fig. 2 shows the raw calorimetric data, denoting the amount of heat produced (negative exothermic peaks) following each injection of 2'-dUMP. The area under each peak represents the amount of heat produced upon the binding of 2'-dUMP to dUTPase. As the titration progresses, the area under the peaks progressively becomes smaller due to an increased occupancy of the enzyme by 2'-dUMP. The bottom panel of Fig. 2 shows the plot of the amount of heat generated per injection as a function of the molar ratio of ligand to enzyme.

A model of two equal and independent sites fits adequately the calorimetric data (see materials and methods) under the solution conditions used in this study. Therefore, in the complex 2'-dUMP–dUTPase, one molecule of 2'-dUMP occludes the binding site of each subunit. Overall, these results suggest that the binding of 2'-dUMP to *L. major* dUTPase is a non-cooperative process across the temperature range 15–35 °C under the solution conditions used. The result is similar to that found for trimeric *E. coli* dUTPase bound to inhibitor 2'-dUDP [6,7]. These authors reveal that the crystal structure of dUTPase–2'-dUDP complex is a symmetric trimer with three molecules of 2'-dUDP bound at the interfaces between monomers (suggesting the existence of three active sites per trimer). A comparison of the structure of dUTPase–2'-dUDP complex with the structure of the enzyme without 2'-dUDP reveals no conformational differences, i.e., the binding of 2'-dUDP to trimeric *E. coli* dUTPase will be a non-cooperative process.
corresponds to the theoretical curve, as described in Section 2.

A

mercaptoethanol. (A) Incremental heat released upon titration of 36.59 peaks plotted against the molar ratio of 2 dUMP (stock concentration of 5.31 mM). (B) Integrated areas for the above analysis [14]. However, since in the crystal structure for which the substrate 2 rearrangement (induced by the ‘mobile’ domain) that buries the binding of this nucleotide promotes a structural change [14]. However, in the crystal structure for which only one molecule of 2dUDP is bound, the unoccupied subunit retains the native conformation existent in the unbound state (Fig. 1) [14], the binding of this nucleotide would be non-cooperative. So, the non-cooperative process for the binding of 2dUMP to the dimeric L. major dUTPase could be similar to that observed for the binding of 2dUMP to trimeric dUTPases, for which 2dUDP is an inhibitor. From the T. cruzi dUTPase crystal structure, it is reasonable to reckon that the second phosphate group of 2dUDP, which shows many interactions with the residues in the ‘mobile’ domain, is responsible for the structural change following the binding of the nucleotide.

On the other hand, the absence of cooperativity upon binding of 2dUMP to L. major dUTPase is consistent with results from kinetic studies, which show a typical Michaelis–Menten behaviour [12]. Identical experiments to those described at 20 °C were carried out at 15, 25, 30 and 35 °C. As the temperature rises, the binding enthalpies become more exothermic. In order to measure ionization changes upon binding, the calorimetric titration at each temperature was performed in three buffers with different ionization enthalpies, viz. 50 mM 2-glycerophosphate, 50 mM HEPES and 50 mM Pipes containing 1 mM EDTA and 2 mM 2-mercaptoethanol at pH 7.5. The number of protons exchanged by the enzyme during 2dUMP binding at pH 7.5 was −0.19±0.04, −0.22±0.07, 0.30±0.05, 0.33±0.02 and 0.41±0.12 at 15, 20, 25, 30 and 35 °C, respectively (Fig. 3). Thus, protons are released at temperatures below 21 °C, whereas there is an uptake at physiological temperatures. The protonation changes for 2dUMP–dUTPase binding probably arise from a shift in the pK_a of one or more groups during complex formation. Alterations in the protonation state of certain residues in the vicinity of the binding site may explain a variation in the number of protons when 2dUMP binds to enzyme at pH 7.5.

As we have described before, the number of protons exchanged varies with temperature and, consequently, the enthalpy also changes with pH at a fixed temperature [23], thus suggesting that binding of 2dUMP to L. major dUTPase is a pH-dependent process. In agreement with this observation, Hidalgo-Zarco et al. [12] have shown that the rate of 2dUTP hydrolysis depends on pH. Several amino acids may contribute to the proton exchange. Thus, residues susceptible to changing their pK_a values at this pH as a consequence of binding are His, Asp and Glu. It is also known that the pK_a value in water of lysine (≈10.5) undergoes a significant reduction to approximately 6.5 in a hydrophobic environment or if another Lys residue exists in the vicinity [24]. Besides, the phosphate group of the nucleotide may also be considered. An examination of conserved motifs in dimeric dUTPases of the Trypanosomatidae and the dUTPases–dCTPases of T. phages reveals the existence of several highly conserved acidic residues which are possible candidates to undergo ionization upon

Fig. 2. Representative isothermal titration calorimetric profile of 2dUMP/dUTPase from L. major association. The experiment was performed at pH 7.5 and 20.1 °C in 50 mM Pipes, 1 mM EDTA and 2 mM 2-mercaptoethanol. (A) Incremental heat released upon titration of 36.59 μM dUTPase with 29 aliquots (5 μl each) and one preinjection of 1 μl of 2dUMP (stock concentration of 5.31 mM). (B) Integrated areas for the above peaks plotted against the molar ratio of 2dUMP to dUTPase. The solid line corresponds to fit to the theoretical curve, as described in Section 2.

Fig. 3. The experimentally observed enthalpy change, ΔH_expt, is plotted versus the ionization enthalpy of the buffer, ΔHion, at different temperatures to evaluate the protonation effect. ITC experiments were performed at pH 7.5, in 2-glycerophosphate, PIPES, and HEPES buffers. Symbols ■, ○, □, ●, △, ◆ correspond to the titrations at 15, 20, 25, 30, and 35 °C, respectively. Continuous lines are the linear least squares fits according to Eq. (3).
binding. Three of these residues, Glu51, Glu76 and Asp79, have been shown to be essential for activity [25]. In addition, a motif containing two conserved lysines (Lys 59 and Lys 62), surrounded by tryptophan residues, could also be considered.

The variation in the number of protons with temperature might spring up from two different sources, not mutually exclusive: (1) temperature-dependent variations in the pK values of any involved groups; (2) the occurrence of slight temperature-dependent structural changes in the protein as a consequence of ligand binding. In this latter manner, the folded state may be slightly altered by temperature, modifying the way active-site residues interact with the ligand. A similar folding alteration pattern to that appearing when 2‘-dUDP binds T. cruzi dUTPase might support this possibility.

On the other hand, as shown in Fig. 4, $\Delta H_b$ and $T\Delta S^o$ strongly depend on temperature, while $\Delta G^o$ is almost independent. In Fig. 4, a linear correlation between $\Delta H_b$ and temperature is shown. Likewise, a negative change in heat capacity ($\Delta C_p = -0.62 \pm 0.13 \text{ kcal} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$) was calculated from the slope value. The plot of $\Delta H_b$ vs. $T\Delta S^o$ at different temperatures gives a slope close to 1.0 (Fig. 5), which is common in a wide variety of molecular associations [23,26–28], and has been described as enthalpy/entropy compensation. Therefore, $\Delta G^o$ itself is almost unaffected across the temperature range investigated, which is also true for the affinity constant, the value of which has the same order of magnitude as the inhibition constant obtained by kinetic studies [12]. Table 1 also shows that $\Delta S^o$ is positive at 15 and 20 °C, changing to negative at approximately 24 °C. Clearly, at the intersection point, $\Delta G^o = \Delta H_b$ (23.4 °C and $-6.08 \text{ kcal/mol}$), implying that the entropic contributions to 2‘-dUMP–dUTPase binding are equal to zero. Hence, $\Delta G^o$ for 2‘-dUMP–dUTPase binding is exclusively contributed by $\Delta H_b$ at 23.4 °C. However, at temperatures below 14 °C, the free favourable energy change is entirely entropic, indicating a strong hydrophobic contribution.

The experimental values for the enthalpy, entropy (Table 1), and heat capacity changes are the result of contributions of opposite signs. Van der Waals interactions and hydrogen bonding are generally considered to be the major potential sources for negative $\Delta H$ values [26]. Besides, the net formation of hydrogen bonds should be accompanied by a negative $\Delta S^o$. However, one should consider that the formation of hydrogen bonds between 2‘-dUMP and the protein results in the release of water molecules. These molecules, before binding, were hydrogen bonded to protein and/or ligand; therefore, unfavourable enthalpic and favourable entropic components are expected due to the dissociation of water from protein, ligand or both [30]. Although the three-dimensional structure of 2‘-dUMP bonded to L. major dUTPase is not yet available, the structures of T. cruzi dUTPase [14] and other dUTPases, mainly trimers [7,31–33], are known. In the crystal structure of the E. coli dUTPase complexed with 2‘-dUDP, several H-bonds have been described [7]. Likewise, many H-bonds stabilize the binding of 2‘-dUDP to dimeric T. cruzi dUTPase [14]. In every known structure, the uracil ring and the deoxyribose moiety are bound to the enzyme through hydrogen bonds, and the interactions between the phosphate residues are also basically hydrogen bonds. Similar interactions may also exist in the L. major dUTPase–2‘-dUMP complex, which could justify the thermodynamic parameters obtained.

In the 2‘-dUDP–T. cruzi dUTPase complex structure, $\beta$-phosphate from 2‘-dUDP establishes numerous H-bonds with active-site residues, mainly in the ‘mobile’ domain [14]. It seems that at least two phosphate groups were necessary for the folding of the ‘mobile’ domain, which could explain the lack of a conformational change upon 2‘-dUMP binding, in agreement with our results. Never-
theless, although this structural rearrangement existed in the binding of nucleotide 2′-dUMP, its contribution would be included in the thermodynamic parameters obtained in this study.

Moreover, the sign of the entropic change upon binding to dUTPase provides some clues about the kind of physical processes involved. Above 23.4 °C, the entropy change is negative (Fig. 2; Table 1). Processes with a negative entropy change may be ascribed to hydrogen bond formation, a decrease in the number of isosmertic conformations, and a decrease in soft internal vibrational modes [26]. Processes with a positive entropy change may arise from the burial of electrostatic charges or hydrophobic groups from water [26].

### 3.2. Molecular interpretation of heat capacity change

The binding of 2′-dUMP to dUTPase generates a large negative change in heat capacity, which is frequent in binding studies [26,28,29], and is a distinctive feature of site-specific binding [29,34–36]. The size and sign of $\Delta C_p$ are consistent with those of a binding interaction that involves a net increase in buried apolar surface area. Possible reasons for a change in heat capacity upon binding are (1) conformational changes in protein or ligand, (2) changes in ionization, (3) changes in the water network in the binding site, and (4) release of water from hydrophobic surface upon binding. However, the main contributors to a negative value of $\Delta C_p$ could be an increase in hydrophobic interaction, the burial of nonpolar surface area from water, and the presence of water molecules buried in the interface and/or local folding due to binding [37–40]. Large changes in heat capacity could also arise due to changes in the hydrogen bonding network [37]. Variations in apolar ($\Delta ASA_{ap}$) and polar ($\Delta ASA_p$) solvent-accessible surface areas upon complexation are also estimated by the following equations,

$$\Delta C_p = \Delta C_{p,ap} + \Delta C_{p,p} = 0.45 \Delta \text{ASA}_{ap} - 0.26 \Delta \text{ASA}_p, \quad (4)$$

$$\Delta H(60) = -8.44 \Delta \text{ASA}_{ap} + 31.4 \Delta \text{ASA}_p, \quad (5)$$

where $\Delta H(60)$ is the change in enthalpy at 60 °C. This temperature is taken as a reference because it is approximately the average denaturation temperature of the model proteins used in the analysis [41]. In both equations, it has been assumed that the contributions to $\Delta C_p$ and $\Delta H(60)$ (expressed in cal K$^{-1}$ mol$^{-1}$ and cal mol$^{-1}$, respectively) due to the protonated residues are relatively small, within the experimental error in those parameters.

For our experiments, the calculations of the $\Delta \text{ASA}_{ap}$ and $\Delta \text{ASA}_p$ from Eqs. (4) and (5) result in $-2360\ \text{Å}^2$ (58% apolar surface) and $-1705 \text{Å}^2$ (42% polar surface). On the basis of the X-ray crystallographic data of several proteins, the changes in the water accessible surface areas of both apolar ($\Delta A_{ap}$) and polar ($\Delta A_p$), residues on protein folding, have been calculated. Such calculations reveal that the ratio $\Delta A_{ap}/\Delta A_p$ varies between 1.2 and 1.7 [42]. Using the $\Delta \text{ASA}$ values calculated for the interaction 2′-dUMP–dUTPase from $L.\ major$, the ratio $\Delta A_{ap}/\Delta A_p$ is $\approx 1.4$, which is comprised in the range mentioned.

On the other hand, as shown by Eq. (4), it is possible to estimate $\Delta C_p$ once the solvent accessible surface areas (polar and apolar) upon complex formation with the ligand are known. This determination requires to know the three-dimensional structure of the complex with 2′dUMP. As it has been cited before, this structure is not known yet. However, we could use the structural data of the $T. cruzi$ dUTPase–2′dUDP complex [14] as a first approximation to study our binding process. The surface area calculations indicate a $\Delta \text{ASA}_{ap} = 1630\ \text{Å}^2$ and $\Delta \text{ASA}_p = 1170\ \text{Å}^2$. These values were computed using the NACCESS program [43], using a probe radius of 1.4 Å and a slice width of 0.1 Å, with the Protein Data Bank access codes 1OGL and 1OGK. Eq. (4) gives, then, $\Delta C_p = -430\ \text{cal K}^{-1}\ \text{mol}^{-1}$. This theoretical value correlates well with the experimental value ($-618\ \text{cal K}^{-1}\ \text{mol}^{-1}$), and allows us to discard large conformational changes in the binding. Therefore, the following forces may be mainly implicated in the binding of 2′-dUMP to $L.\ major$ dUTPase: hydrogen bonds, van der Waals interactions, and a strong hydrophobic contribution. The latter arises from the burial of nonpolar surface areas from water. These significant contributions might be related to the high specific molecular recognition between 2′-dUMP and the protein. Antibody–antigen associations, which likewise exhibit a high degree of specificity, are dominated by van der Waals interactions, hydrogen bonds, and the loss of mobility upon association [44]. The hydrogen bonding is also an important determinant of specificity, since the strength of the hydrogen bonding depends on the bond angle between proton and base.
Acknowledgements

We really appreciate the helpful discussion of Dra. C. Barón about calorimetric issues. These studies were supported by research grants from the Spanish (SAF2001-2067, BCM2001-1723, MCYT), Regional Andalusian (PAI groups CVI-199 and CVI-290) Governments and by EC project contract no. QLK3-CT-2001-00305.

References


