

# کالری و آب در طراحی دارو

جان - ای - لدبری \*

## چکیده:

روشن شدن اینکه چگونه در یک واکنش دوملکولی پارامترهای ترمودینامیکی تحت تأثیر ساختار ملکولی قرار می‌گیرند، بصورت یک نیروی محرکه اساسی در طراحی منطقی دارو درآمده است. اگر ما بتوانیم ساختار یک داروی بالقوه و هدف آن را تعیین کنیم قادر خواهیم بود که ثابت‌های تعادل کنش واکنش بین آنها را بر اساس یک سری روابط ساده پیش‌بینی کنیم. با این اطلاعات قادر خواهیم بود که بطور اساسی مرحله زمان و هزینه بر سنتز آنالوگهای داروی راهبر و تعیین پارامترهای تمایل اتصال دارو به گیرنده را کاهش دهیم.

به نظر می‌رسد که معادلات مرسوم راجع به رابطه تغییرات ظرفیت حرارتی ( $\Delta C_p$ ) و سطح ملکولی دور از حلال که بر اساس مطالعات تاخوردگی پروتئین‌ها و حرارت انتقال در یک حلال آلی تدوین شده‌اند در بسیاری از کنش و واکنش‌های بین ملکول دارو و پروتئین گیرنده آن صدق نمی‌کنند. برای مثال وقتی که ملکولهای آب در سطح تماس وجود داشته‌باشد اختلافات قابل ملاحظه‌ای بروز خواهد نمود.

واکنش بین پروتئین رپرسور trp با یک الیگونکلئوتید براساس توالی اپراتور trp بخاطر در دسترس بودن اطلاعات ساختاری دقیقی راجع به هریک از اجزاء و کمپلکس نهائی، یک مدل ایده‌آل را برای مطالعه روابط ذکر شده فراهم می‌نماید. ارزش  $C_p$  بدست آمده از مطالعات تیتراسیون کالریمتری ایزوترمال تقریباً دوبرابر مقادیر است که از محاسبات سطح تماس براساس روابط مذکور بدست می‌آید. کمپلکس نهائی حاوی تعداد زیادی ملکول آب است که در سطح تماس بین پروتئین و DNA قرار دارند. این ملکولهای آب به نسبت تغییر ملکولهای حلال بمیزان بیشتری مهار شده‌اند و این محدودیت آب در سطح تماس است که در روابط فوق منظور نشده و به نظر ما مسئول اختلاف ذکر شده  $C_p$  می‌باشد.

با استفاده از یک سری سیستم‌هایی که اطلاعات ساختاری دقیقی از آنها در دسترس است اثرات ترمودینامیکی ملکولهای آب در سطح تماس مورد بررسی قرار گرفته‌است و براساس این مطالعات ما اکنون قادریم نتیجه بگیریم که چگونه ملکولهای آب ممکن است در طراحی دارو اهمیت داشته باشند.

۲- پارامترهای ترمودینامیک

۴- رپرسور

کلید واژه‌ها: ۱- طراحی دارو

۳- ملکولهای آب

\* مرکز علوم ملکولی، دانشگاه اکسفورد، انگلستان

# CALORIES AND DESIGNER WATER

J.E. Ladbury\*

## ABSTRACT

*Elucidation of how the thermodynamic parameters are determined by the molecular structures in a bimolecular interactions is becoming a fundamental driving force in the rational design of drugs. If we can determine the structure of the target and the potential drug we should be able to predict the equilibrium constant for their interaction based on simple relationships. With this information the temporally and financially expensive process of synthesis and affinity determination of lead compounds can be dramatically reduced.*

*Using a series systems for which highly refined structural information is available the thermodynamic effect of interfacial water has been investigated. Based on this work we are able to draw conclusions on how water may be important in drug design.*

**Key Words:** 1) Drug Design      3) Thermodynamic Parameters  
2) Water Molecules      4) Repressor Protein

## INTRODUCTION

The Role of water molecules in biomolecular interactions has been an issue of some controversy and great debate over the last few decades. The hydrophobic effect, the concept that interactions are energetically driven by the removal of entropically unfavourable water molecules from exposed hydrophobic surface as this surface is buried in the binding site, is predominant in how most think of

thermodynamic interactions. The appearance of a water molecule in an interface is typically regarded opposing high affinity interactions. In the work some evidence is presented that suggests that water molecules can, under certain conditions, actually contribute to binding. Inclusion of water molecules in an interface can result in a favourable thermodynamic outcome.

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\* Oxford Centre for Molecular Science, University of Oxford, New Chemistry Laboratory, South Parks Road, Oxford, OX13QJ, UK

## RESULTS & DISCUSSION

The relationship between thermodynamic and structural data is of central importance in understanding how biomolecular interactions occur and indeed how they might go astray leading to disease states. In addition, if this relationship is determined the prediction of thermodynamic parameters for an interaction should be possible. This has obvious consequences in the pharmaceutical industry, where, for example, if the structure of a particular target and a series of potential drug compounds are known then the affinities of these compounds can be predicted and hence the temporally and financially expensive processes of synthesis and testing can be circumvented. Study in this area requires the direct measurement of thermodynamic parameters associated with going from one state to another and high resolution structural data. With the recent advances in calorimetric instrumentation and the increasing availability of structural information progress is being made.

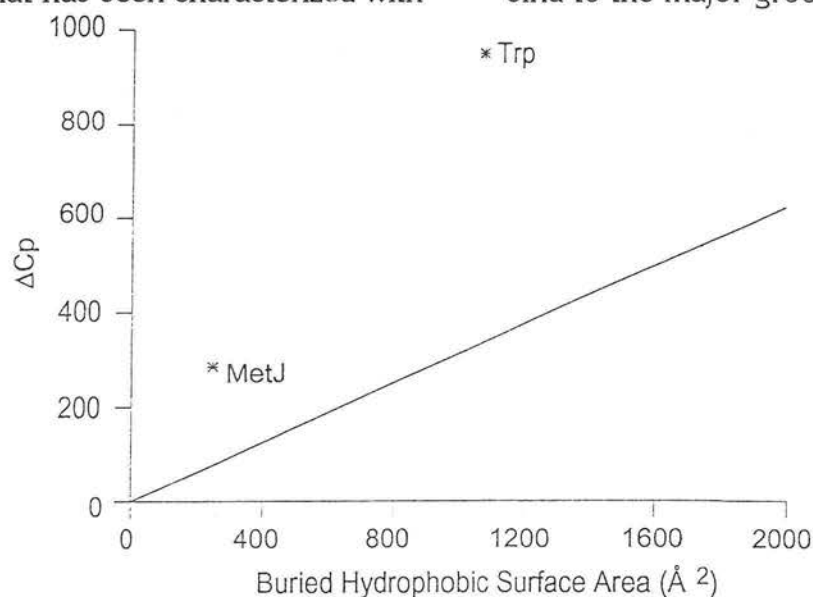
The most successful link between thermodynamic and structural detail has been observed in the relationship between the change in heat capacity,  $\Delta C_p$ , and the burial of surface area. This was based, initially, on studies of heats of transfer of hydrocarbons into aqueous solvents and protein folding/unfolding equilibria<sup>(3,4,11-13)</sup>, however, theoretically the relationship was

believed to be sufficient for all interactions<sup>(21)</sup>. In the cases where discrepancies appeared to exist when burying binding surface area, additional surface area burial could be inferred on conformational changes that putatively occurred on binding. This is an attractive model since it leads to the idea of induced fit<sup>(6)</sup> which adds another level of specificity to the interaction.

We decided to investigate this relationship using the interaction of *trp* repressor protein and a twenty base pair oligonucleotide including the putative *trp* operator connate site. This system was chosen because it is one of the best structurally characterized interactions available. The X-ray crystallographic structures of the free protein<sup>(9)</sup> and oligonucleotide<sup>(19)</sup> as well as the complex<sup>(16)</sup> have been determined to better than 2.0 Å, and NMR spectroscopic structural determinations of the protein<sup>(1)</sup> and complex<sup>(23)</sup> are also available. Using isothermal titration calorimetry (ITC)<sup>(7)</sup> we obtained a value of the  $\Delta C_p$  for the interaction<sup>(8)</sup>. The experimentally determined  $\Delta C_p$  and that obtained from the empirical relationship described above were very different ( $-950 \text{ cal.mol}^{-1}.\text{K}^{-1}$  and approximately  $-380 \text{ cal.mol}^{-1}.\text{K}^{-1}$  respectively). On close inspection of the high resolution structures it was clear that water mediated the interaction of protein and DNA. Water molecules visualized by X-ray

crystallographic methods are presumed to be constrained in the identified position for residence times in excess of nanoseconds; bulk solvent is much more dynamic<sup>(15)</sup>. This led to the hypothesis that this water contributed to the additional  $\Delta C_p$  measured experimentally that was not accounted for in the empirical relationship.

This hypothesis was supported in the only other water mediated protein - DNA interaction that has been characterized with



**Figure 1.** Comparison of  $\Delta C_p$  versus change in surface area for transfer of hydrocarbons protein folding and repressor-operator interactions. Determination of Surface Area Burial.

Solvent accessible surface areas were calculated using the program GRASP<sup>(14)</sup> using atomic radii as described elsewhere<sup>(10,18)</sup>. Solvent accessible surface areas were defined as hydrophobic if the contact atom in the molecule was a carbon or a sulphur. All remaining solvent accessible surface was defined as being hydrophilic.

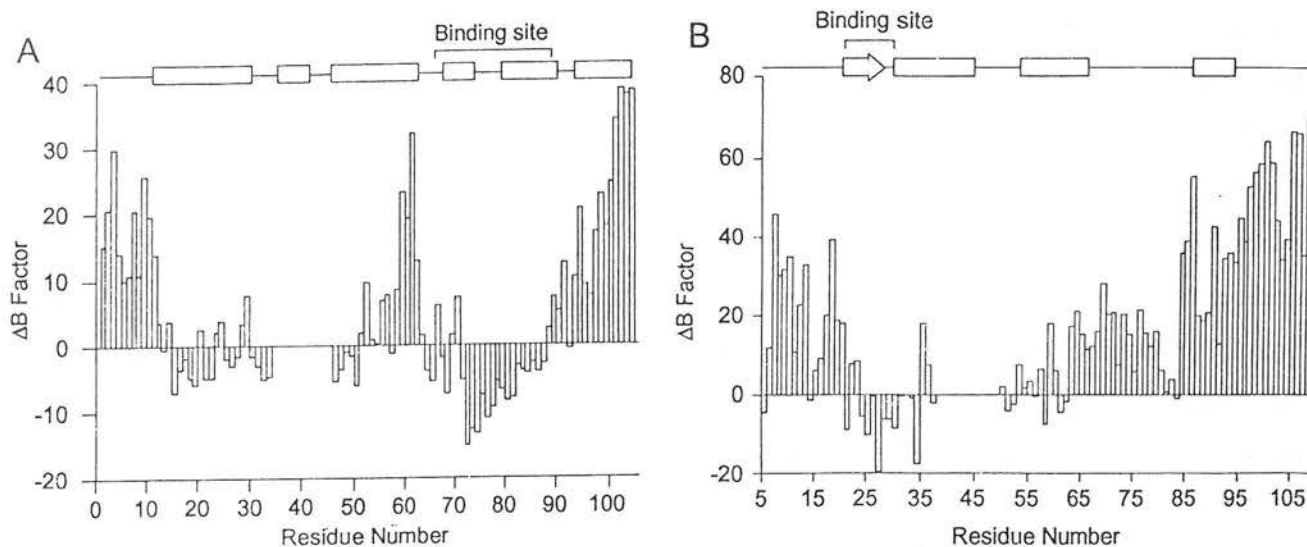
For unbound operators, idealised B-DNA structures were constructed using SYBYL (Tripos Inc. St Louis, USA) and exported as PDB format files for GRASP. Surface areas of unbound repressor complexes were calculated in GRASP by building surfaces on the protein component of the repressor-operator complexes as coordinates for the structure of free repressor were not available for all cases. The crystal structure of the apo -form of the met repressor is used. Strictly the holo -form should be used, however, there is no significant structural difference between the holo- and free apo -repressor<sup>(15)</sup>.

The change in surface area on binding,  $\Delta A$ , is the difference between the sum of the surface areas of the two unbound molecules and the area of the complex.

case of MetJ a ribbon-helix-helix motif is used whereas a helix-turn-helix binds DNA in the *trp* system. Comparison of the experimentally and empirically determined  $\Delta C_p$  showed a large discrepancy (290 cal.mol<sup>-1</sup>.K<sup>-1</sup> and 130 cal.mol<sup>-1</sup>.K<sup>-1</sup>; Figure 1.) The high resolution crystallographically determined crystal structures for the MetJ/metbox interaction show that water plays a mediating role. This adds weight to the above hypothesis.

The question then becomes; how does water increase the  $\Delta C_p$ ? As described by Sturtevant<sup>(22)</sup> there are several other factors that can contribute to the  $\Delta C_p$  in addition to the burial of hydrophobic surface area. The

most important of these arises from the restriction of soft vibrational modes of interacting species. Thus, the restriction of water molecules into a binding interface could have a major contribution to this effect in comparison to their more dynamic state in bulk solvent. One indication of this effect is observed in the crystallographic structures in the form of changes in temperature (B) factors. When the B factor of an atom is reduced on binding with respect to its unbound form this is indicative of a "tightening" of structure. The change in average B factors on going from free to bound state for both *trp* and Met J1 is shown in figure 2. Although caution must be



**Figure 2.** The effect of complex formation on the average B factors. Structure factors were calculated for each coordinate set using XPLOR<sup>(2)</sup> and scaled against each other to allow a relative overall temperature factor for each structure to be determined. B factors for individual residues were then modified to account for the differences in relative temperature factor, allowing a more meaningful comparison of values for residues in the free and complexed structures. The difference in average B factors for each residue of the complex is shown for both *trp* (A) and the MetJ (B) repressors. The position of secondary structural elements in each repressor are represented by the arrows (strands) and rectangles (helices) above the graph with residues that interact directly with the operator indicated.

applied when one looks at these data (since the crystal form and packing will have an effect on these data) it is clear that there are significant net reductions in the B factors of those residues involved in the interaction with the DNA. These interactions involve the residues in the D and E helices of the helix-turn-helix motif for the *trp* repressor (residues 66-85) and the residues in the  $\beta$  strand that forms a single strand of the  $\beta$  ribbon motif in MetJ (residues 21-29).

Thus, we can conclude that water is playing a major role in increasing the  $\Delta C_p$  in mediating these interactions. The unfavourable entropic effect of restricting these water molecules into the binding site has to be compensated for to give a net favourable free energy ( $\Delta G^\circ$ ). It is thus likely that highly advantageous positioning of these water molecules allows hydrogen bonds to form whereby the bond lengths are such that the potential energy barriers to bond formation are at their lowest. These "high strength" hydrogen bonds have a highly favourable enthalpic contribution.

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One additional example where these favourable water interactions may be observed is in stabilizing molten globule formation as intermediates in protein folding pathways.

An intriguing outcome of these observations is that since the presence of water molecules can increase the  $\Delta C_p$ , based on the gibbs-Helmholtz relationship, at a given temperature this will increase the  $\Delta G^\circ$ . Thus, by designing specific sites for positioning of water molecules into drug compounds the efficacy of the proposed interaction could be improved.

The preoccupation of many people with their personal appearance has led to an obsession for watching their weight. This is reflected in rigorous attempts to monitor their calorific intake and eat and drink the right things including water of excessively high purity ("designer water"). It struck me that in this work we have turned up a very different relationship between calories and designer water.

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