Analytical Biochemistry 515 (2016) 61–64

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Technical note

Isothermal titration calorimetry for drug design: Precision of the enthalpy and binding constant measurements and comparison of the instruments

Vaida Linkuvienė [a], Georg Krainer [b, c], Wen-Yih Chen [d], Daumantas Matulis [a, *]

[a] Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio 7, Vilnius, LT, 10257, Lithuania
[b] Department of Molecular Biophysics, University of Kaiserslautern, Kaiserslautern, Germany
[c] B CUBE–Center for Molecular Bioengineering, Technische Universität Dresden, Dresden, Germany
[d] Department of Chemical and Materials Engineering, National Central University, Taiwan

ARTICLE INFO

Article history:
Received 19 September 2016
Accepted 3 October 2016
Available online 4 October 2016

Keywords:
Isothermal titration calorimetry (ITC)
Protein – ligand binding
Enthalpy
Calorimeter
Acetazolamide
Carbonic anhydrase
Nitpic and Sedphat

ABSTRACT

Isothermal titration calorimetry (ITC) is one of the most robust label- and immobilization-free techniques used to measure protein – small molecule interactions in drug design for the simultaneous determination of the binding affinity (ΔG) and the enthalpy (ΔH), both of which are important parameters for structure-thermodynamics correlations. It is important to evaluate the precision of the method and of various ITC instrument models by performing a single well-characterized reaction. The binding between carbonic anhydrase II and acetazolamide was measured by four ITC instruments – PEAQ-ITC, iTC200, VP-ITC, and MCS-ITC and the standard deviation of ΔG and ΔH was determined. Furthermore, the limit of an approach to reduce the protein concentration was studied for a high-affinity reaction ($K_d = 0.3$ nM), too tight to be measured by direct (non-displacement) ITC. Chemical validation of the enthalpy measurements is discussed.

© 2016 Elsevier Inc. All rights reserved.

Isothermal titration calorimetry (ITC) is one of the main technique choices for a researcher to determine an affinity between two molecules interacting in aqueous solution. Most often ITC is used for protein – ligand interactions in drug design but could be used to measure interactions between almost any biochemicals, proteins, nucleic acids, lipids, carbohydrates, and other organic compounds [1–5,28,29] as previously reviewed [6–10]. Despite the fact that most studies use ITC for affinity, an advantage of ITC is that it is the only method that directly determines the enthalpy, which is a useful parameter for structure – thermodynamics correlations in the design of novel drug-like molecules [11–13].

As with every technique, it requires validation experiments, positive and negative controls, and an understanding of the limitations of the method. ITC is a relatively expensive technique that needs relatively large amount of purified protein and is time consuming. Therefore, proper statistical analysis of the data is rarely performed. Furthermore, ITC data is currently obtained by using several different instruments that yield data of slightly different precision. Quite often the claims of precision are unrealistic. Unfortunately, large scatter in the enthalpy determination could lead to an erroneous enthalpy – entropy compensation [14] or incorrect structure – thermodynamics correlations.

Here we compare ITC data obtained by four instruments, PEAQ-ITC, iTC200, VP-ITC, and MCS-ITC, made by Microcal Inc. (part of Malvern group), determine the precision of the data by repeating the same well-characterized reaction (between carbonic anhydrase II (CA II) and acetazolamide, AZM), and the limit of affinity (ΔG, K_d) and ΔH precision that could be achieved by performing direct (non-displacement) ITC experiments. The new PEAQ-ITC calorimeter was found to be able to achieve the most precise direct determination of tight binding affinity.

1. Materials and methods

Human recombinant CA II and CA XII, prepared as described previously [15–17], were used in ITC cell. Acetazolamide (AZM) was purchased from Sigma-Aldrich (Saint Louis, MO, USA). A 10 mM solution was prepared in 100% DMSO and stored up to 2 weeks.

* Corresponding author.
E-mail addresses: matulis@ibt.lt, daumantas.matulis@bti.vu.lt (D. Matulis).

http://dx.doi.org/10.1016/j.ab.2016.10.005
0003-2697/© 2016 Elsevier Inc. All rights reserved.

Table 1
Calorimeter instrument models used in this study.

Instrument model	Year of manufacture	Cell volume (ml)	Syringe volume (μl)	Typical experimental parameters		
				Injection volume (μl)	Number of injections	Reference power
PEAQ-ITC	2015	0.2	40	2	20	5
iTC200	2008	0.2048	40	2	20	5
VP-ITC	2001	1.4315	270	10	25	4
MCS-ITC	1995	1.3446	270	10	25	30%

Compound EA4-2 was synthesized in the laboratory as described previously [18].

ITC experiments were performed nearly simultaneously in four instruments, the cell containing 0.5–20 μM protein and the syringe a 10× greater concentration of the ligand. Both the cell and the syringe in the calorimeter contained 2% DMSO, carefully matched to be as identical as possible in the cell and syringe. Both the cell and the syringe also contained 50 mM sodium phosphate buffer (pH 7.0) and 100 mM NaCl. Experiments were performed at 25 or 37 °C. Both the protein and ligand solutions were centrifuged for 2 min at 2000 g. Centrifugation helped reduce the appearance of large unexpected peaks in the titrations by eliminating bubbles. First injection was small and disregarded for each experiment. Instrument parameters are listed in Table 1.

The data obtained by different instruments were transformed to *.itc files and analyzed by Origin 5.0 software. The new PEAQ ITC software was also used for comparison. Baseline selection was performed by a standard Origin procedure with manual adjustment. Combined Nitpic/Sedphat analysis was performed to improve baseline selection and peak integration to evaluate the effect of automated, user-unbiased analysis on data precision [19,30,31].

2. Results and discussion

A large set of ITC titrations was performed with four calorimeter instruments using the same protein – ligand solutions. The performed reaction was between a well-characterized protein that is easy to produce and the ligand that is commercially available, between recombinant human CA II and acetazolamide that has been measured by numerous techniques, primarily by the stopped-flow CO_2 hydration inhibition assay (SFA) [20], fluorescent thermal shift assay (FTSA) [15], and by ITC [21] and shown that the dissociation constant was within the narrow range where ITC is practically most precise, between 20 and 50 nM.

The binding reaction was performed in a relatively wide concentration range to determine the lowest concentration where the data is still reliable. If the protein concentration is reduced then the heat signal is diminished and eventually disappears in the experimental noise. However, strong, nanomolar binding reactions

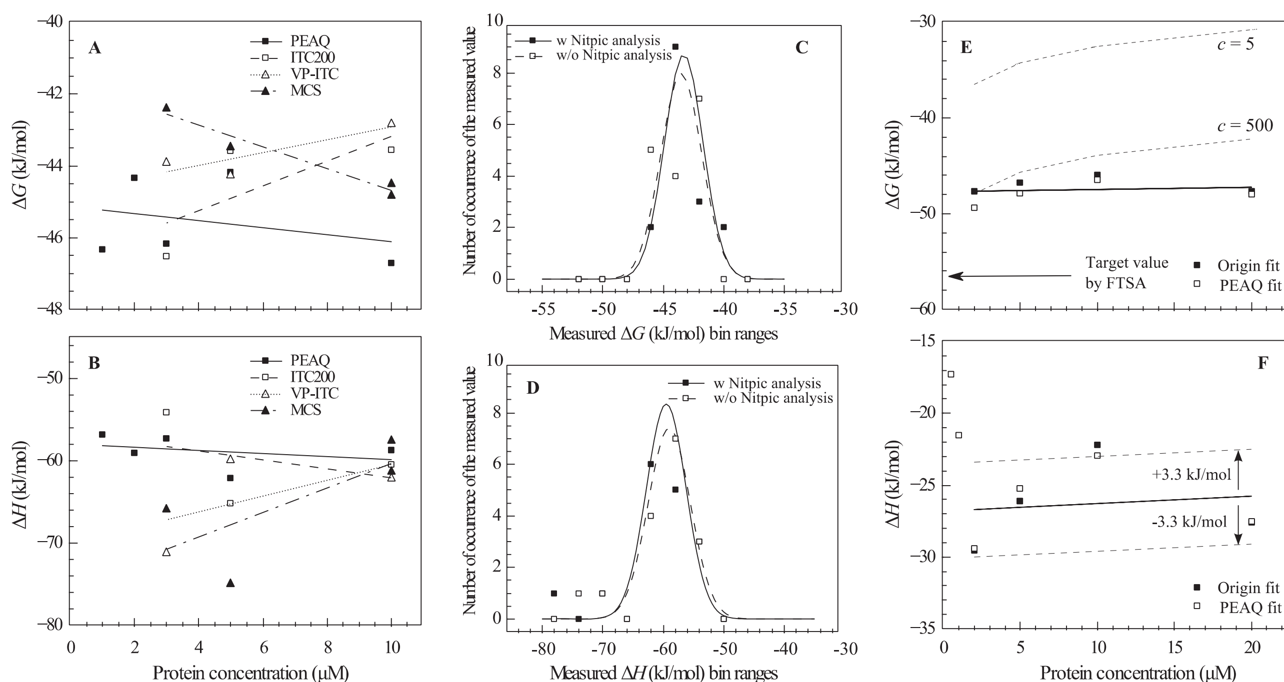

Fig. 1. Panels A and B show the ΔG (**A**) and ΔH (**B**) of CA II titration with AZM performed at different protein concentrations using four instrument models. Lines are linear fits through the datapoints obtained experimentally (37 °C). **Panels C and D** show the Normal Gaussian distributions of the experimental measurements of ΔG (**C**) and ΔH (**D**). Filled symbols represent data analysis after applying Nitpic/Sedphat software while open symbols represent data with a regular Origin analysis. The statistical parameters are listed in Table 2. **Panels E and F** show the analysis of a tight binding reaction between compound EA4-2 and CA XII. This reaction is too strong to be determined by direct ITC and has a K_d = 0.33 nM (ΔG = −56.3 kJ/mol as determined by FTSA). Datapoints show ΔG (**E**) obtained by PEAQ ITC and the solid line is a linear fit of the data. Measurements are limited by the Wiseman factor *c* which should be between 5 and 500 for the ITC curve to have reliable sigmoidal curve, shown as dashed lines. At higher protein concentrations (10 and 20 μM) the reliable data could be obtained even outside this range. The approach of reducing protein concentration did not reach the target value of −56.3 kJ/mol. **Panel F** shows that despite inability of ITC to determine the ΔG, the enthalpies are well determined for such tight-binding reactions. The PEAQ-ITC instrument was able to determine an approximate enthalpy at 0.5 μM protein in the cell. An error of ±1σ is shown as dashed lines.

Download English Version:

https://daneshyari.com/en/article/5131783

Download Persian Version:

https://daneshyari.com/article/5131783

Daneshyari.com