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Editorial

Reflections on the origins of microcalorimetry of biopolymers☆

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One of the most exciting achievements of 20th century science was the discovery that proteins and nucleic acids under environmental conditions close to physiological have highly ordered three-dimensional structures but on deviating from these conditions their structures break down cooperatively. Thus, on heating the DNA double helix, it melts and the two strands separate, while proteins denature and lose their unique structure and functions. Similar effects are achieved by increasing the presence of various denaturing agents, or by changing pH. The most surprising observation was, however, that on restoring the physiological conditions these polymers refold into their unique native conformation if their chemical structure has not been damaged and they had not been aggregated. Thus the process of unfolding and refolding of biopolymers appeared to be reversible in principle, i.e. folding into the native conformation and association with partners is a thermodynamically driven process. The thermodynamics of these processes attracted attention because it is the key for understanding the forces involved in folding and maintenance of the native structure of biopolymers. Its development required determination of the heat effects associated with the formation of the unique structure of biological macromolecules and of association/dissociation of their specific complexes with their partners. Realization of such experiments was not easy because most of biopolymers were available in very limited amounts and moreover needed to be studied in highly dilute solutions to avoid side effects. One of the pioneers who brought this about was Julian Sturtevant, Professor of Chemistry at Yale University.

The heat of unfolding and dissociation of the DNA double helix was first measured by Sturtevant in 1958 by titrating it with acid in an specially constructed isothermal reaction calorimeter [1,2]. Sturtevant later built a twin adiabatic calorimeter for measuring temperature-induced changes in biopolymer structure [3], which permitted to measure the enthalpy of melting of the two-stranded complex of polyA–polyU [4]. Using a vacuum adiabatic heat capacity calorimeter, we also attempted to determine the enthalpy of the heat denaturation of egg white albumin [5]. These first calorimetric experiments showed that the conformational transition of biopolymers, proteins and DNA, is associated with significant heat effects, i.e. proceeds with a positive enthalpy and entropy. But they also made evident that the detailed studies of the intra and inter macromolecular reactions required more sensitive both isothermal reaction and heat capacity calorimeters. The first super-sensitive instrument for measuring temperature induced changes of heat capacity of solutes appeared in 1964 [6]. The qualitative increase in sensitivity was achieved by essentially decreasing the operational volume of the twine calorimetric cells, shifting from the step-wise to the continuous heating and from measurements of the absolute heat capacity to the difference between the energies required to heat together the twin cells with solution and solvent. Such instruments are now called differential scanning microcalorimeters, or just DSC.

In 1968 I received the letter from Professor Sturtevant informing me that he was planning to be in Moscow on his way to the IUPAC International Conference on Calorimetry and Thermodynamics in Warsaw and asking if he could meet me and visit my laboratory. I was certainly delighted to greet the famous American Thermo Chemist, the more so that at that time the Soviet Union was isolated from the western world. This circumstance, however, created some difficulties in taking Professor Sturtevant from Moscow to my lab at the Institute of Protein Research located 70 miles away in the Academic Center for Biological Research in Puschino: at that time, foreigners were not allowed to leave Moscow. Moreover, visiting the Scientific Institute required special permission from the highest authorities and this was not easy to obtain. Nevertheless, I got this permission and at the time when Professor Sturtevant should arrive in Moscow from Leningrad (now St. Petersburg) I went to the railway station. The express train came on schedule and the flow of passengers passed by me. I was looking for the great American Scientist, but in vain: I could not identify him among the hundreds of passing passengers and was greatly

☆ Dedicated to the memory of Julian Sturtevant.

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disappointed. My search for Mr. and Mrs. Sturtevant in the Moscow hotels was also unsuccessful. One week later, however, I was due to go to the IUPAC meeting in Warsaw and there I found Professor Sturtevant with his wife Elizabeth. To my surprise, he was not tall and arrogant as I had imagined Americans, having seeing them only in Hollywood westerns. We immediately became good friends and traveled together in a tour through Poland. Walking around historical Krakow I discussed with Julian not only science but also politics, being sure that nobody was recording our conversations. Later Julian invited me to the National Calorimetric Conference in the USA, which was to be held in 1972 in Park City, Utah. However, in contrast to Poland, America was not a socialist country and for me it was a considerable problem to get there. Only after the President of the National Academy of Sciences of America wrote a letter to the President of the Russian Academy of Sciences was I allowed to go to the conference in America. On my way to Utah I visited Yale University in New Haven and stopped at the Sturtevant house that had a fascinating garden at the very end of the Indian Neck peninsular overlooking Branford Harbor. Later I became a frequent guest of this hospitable home.

At the Calorimetric Conference in Park City I discussed new designs of differential scanning calorimeters that might be practical for manufacturing and easy working. Later, when the Russian Academy started to manufacture this instrument we presented Julian with one of the first examples of this model, DASM-1 (for the evolution of scanning microcalorimetric instruments see [7]). This was very important since Julian's lab at Yale University was a Mecca for the world's calorimetrists and they could see the possibilities of this new technique and learn under the guidance of Professor Sturtevant how to work with it and develop the thermodynamics of biological macromolecules.

In 1985 Julian and Elizabeth visited me in Moscow and together we traveled to my homeland Georgia for the International Conference on Conformational Changes of Biopolymers. As a professional mountaineer, Julian was particularly interested in the Caucasus, so we had a brief excursion to the high peaks.

Julian needed DSC particularly for studying the melting of lipids, a problem which much attracted his attention at that time. DSC was indeed the most powerful method not only for observing the sharp melting of lipids bilayers but also for direct measurements of the thermodynamic characteristics of this process [8]. Later Julian became interested in heat denaturation of proteins and I provoked him to study the cold denaturation of staphylococcal nuclease and we published our only joint paper on this matter [9]. In the nineties Julian concentrated on protein–DNA interactions, i.e. the recognition problem, which became one of the most hectic topics at that time. The newly invented isothermal titration microcalorimeter had opened exciting prospects for investigating the physical bases of such interactions.

The situation with isothermal calorimetry of biological reactions was even more complicated than with heat capacity calorimetry of biological molecules. Although this method is free from all complications caused by the temperature changes occurring in scanning calorimetry, isothermal measurement of the heat of reaction between the liquid reagents has its own difficulties: it requires efficient mixing of the reagents and this induced significant thermal noise. Moreover, the stirrer used in the conventional reaction calorimeters for liquids required too large a volume and correspondingly large amounts of sample. All attempts to get rid off the stirrer were unsuccessful: proposed continuous flow and batch calorimeters needed too much sample and were not accurate enough to measure the heats of protein association with partners in dilute solution. This technical problem in reaction calorimetry was solved only in 1984 by Stanley Gill who constructed a micro modification of the isothermal titration calorimeter with a reactor of only 0.2 ml volume [10]. This instrument required nanomolar quantities of the reagents and thus opened real possibilities for study the heats of biochemical reactions where the amount of sample is highly limited. Several companies started to manufacture various modifications of Gill's isothermal titration calorimeter (ITC). One among these companies was MicroCal headed by John Brandts. John provided their Omega ITC instrument to Julian Sturtevant and this again was very important for the implementation of this new technique to the practice of biochemical experiments: many molecular biologists learned how to use this technique in Julian's lab.

The pioneering works of Sturtevant with biological macromolecules and his authority in thermochemistry were essential to overcome the widespread skepticism concerning thermodynamic studies of biological molecules, i.e. to break the illusion that structural studies alone are sufficient to understand these macro molecular objects. With time, it became obvious that understanding proteins and their complexes indeed required not only knowledge of their structure but also of their energetic base.

I met Julian for the last time in 1999 at the Calorimetric Conference in Tallahassee (Fig. 1). During this conference, a special Symposium was organized to honor Julian, at which the presence of his numerous students, post-docs and collaborators

Fig. 1. My last meeting with Julian at the Calorimetric Conference in Tallahassee, August 1999.

from all over the world witnessed to the enormous influence he had over the whole science of biocalorimetry. This was the first meeting at which Julian was without Elizabeth.

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