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ENERGETICS OF STRUCTURAL TRANSITIONS IN BIOPOLYMERS

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ABSTRACT

Of all structural transitions in biopolymers the helix coil transition of DNA as prerequisition of replication and transcription is considered. The theoretical concept of weak bonds and the experimental procedure of measurements of cooperative processes is outlined. As result we present the complete thermodynamic state functions ΔH, ΔG and ΔS for any DNA sequence.

INTRODUCTORY REMARKS

It is actually quite appropriate and surprising at the same time to look into the history of biocalorimetry to find out that we are now exactly at the bicentennial anniversary of the first application of calorimetry to biology. In 1784 Lavoisier and de Laplace described to the Academie des Sciences in Paris (1) a simple ice calorimeter and its application in showing that the heat evolved to form carbon dioxide by animal respiration is the same as that evolved in a combustion process. This clever experiment did not initiate a firework of similar experiments and it took more than a century until the next major achievement in the field was acquired, the interaction of oxygen with hemoglobin by Barcroft and Hill in 1910 (2). So much of the early history. Let us now turn to a special conceptual problem in the application of physical methods to biology! In his famous book "What is life?" (3) E. Schrödinger stated that living objects "feed on negative entropy". This concept of the surprising order of biological structures at all levels of organization from macromolecules to organisms or the antientropic tendency of life is now a common trend. Indeed, the observation of special and temporal order is a surprising fact. Surprise, however is an emotional category and the only exact measure of the order of a biological system is the thermodynamic state function, primerely the entropy (4). An estimation of entropy changes associated with the formation of biological organization will show that there is no antientropic tendency in the formation of biological systems but biological order has a special meaning already on the level of macromolecules.

It is beyond the scope of this review to cover all structural transitions in biopolymers. Every metabolic step in a living cell is associated with at least one conformational change to switch the affinity of the enzyme involved

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from the substrate to the product, and there are around five thousend enzymes. There is in fact little systematic research in the field of protein biophysics and thus no general conclusions can be drawn from the fragmentary data on the behavior of proteins during conformational changes. So I will leave it with that statement. I will also exclude the lipids from my consideration, not because we are as ignorant about lipids as we are about proteins, but this field is covered by A. Blume extensively. This contribution will focus on our present knowledge on the energetics of structural transitions in nucleic acids and on the description of biological systems with the help of experimental thermodynamic data.

THEORETICAL ASPECTS

Structural transitions as we will consider now in more detail will leave all covalent bonds in the individual molecules intact. Besides the covalent bonds in macromolecules there is an important second class of noncovalent bonds, which form between different parts of the same molecule or between different molecules. They determine both the three-dimensional structure of the main chain and the interaction of these secondary structures via surface interactions. The usual classification of weak bonds considers three types : ionic bonds, hydrogen bonds and van der Waals attractions. There is a fourth kind of noncovalent bond, caused by the tendency of solvent water to minimize the disruptiv effect of solute molecules on the water structure, commonly called a hydrophobic bond. In an aqueous environment, typical for biological systems, weak bonds are about 100 times weaker than covalent bonds. On the other hand the strongest weak bond is in the order of ten stronger than the average collision energy of water molecules at room temperature (6 Kcal/mol). This leads to the situation, where individual weak bonds are constantly made and broken at physiological temperatures. The consequence is that a large number of simultaneous noncovalent bonds are needed to hold two molecular surfaces together. The average lifetime of single secondary bonds is only a fraction of a second. There is no need for a cell to speed up the rate at which noncovalent bonds are made and broken (5). Consequently no enzymes are required to participate in this process. A conformational change of a biopolymer requires always a cooperative interaction of a large number of secondary bonds. An analysis of the energy change of the system accompanying the conformational transition requires either a complete model of the different states of the macromolecule or a modelfree method to determine the energy change experimentally.

METHODS

The method of choice is microcalorimetry. The suitable instrument is a

differential adiabatic scanning calorimeter of the highest quality, because of the high costs and the limited supply of precious biological material. Unfortunately there are not many commercial instruments available, which can work with small amounts of dilute aqueous solutions. The most successful design is the DASM-1 M and its forthcoming successor DASM-4 from the special workshop of biological instruments of the Soviet Academy of Sciences (6). During the scan the additional energy input to match the temperatures of the sample cell and the reference cell perfectly is recorded as a function of time. Since a fixed heating rate is applied the time scale corresponds to a temperature scale. Any conformational change due to thermal denaturation of the macromolecule corresponds to an apparent heat capacity change. The integration of the heat capacity change over the temperature interval of the transition gives the enthalpy change due to the structural transition. Adiabatic conditions take care of heat leakage to the surrounding. The differential principle cancels out any contribution of temperature dependent heat capacity changes of the solvent. This is of great importance since the solvent water accounts for 99.9 % of the total heat capacity. To envision the precision required for a measurement to obtain the transition enthalpy of viroids (7) for example I will briefly sketch the experimental boundery conditions. We are provided with 200 μg of the polynucleotide. For the filling of the sample cell we require 1.3 ml of degased solution, corresponding to 10^{-8} mol base pairs. The experimentally obtained enthalpy change is 0.8 mcal or a temperature difference of 8×10^{-4} deg compared to the sample cell. To determine the molar transition enthalpy within a margin of experimental error of 5 percent the deviation of the exact enthalpy change due to the transition must be less than 50 μcal.

Since heat is by definition an unspecific quantity the correspondence of the thermic effect obtained by the calorimeter to a molecular process has to come from other sources of information. To follow the order/disorder reaction of a DNA double helix registration of the absorbance change of UV light with a wave length of 260 nm as function of temperature has become a standard procedure, the so called melting curve (8). The sharp rise of the extinction in a narrow temperature interval is due to the disruption of stacking interactions of the planar base pairs along the helical axis. From the slope of the optical melting curve at the midpoint of the transition the van't Hoff enthalpy can be calculated presuming that the denaturation of DNA is a reversible process. Besides the hyperchromic effect a variety of other spectroscopic methods like IR-, Raman- or NMR-spectroscopy is capable to follow the structural transitions of biopolymers. The disadvantage

of all these methods is the requirement of large amounts of material.

RESULTS

Let us now go to the real thing, the molecular systems we want to explain. The model of the DNA double helix, first proposed by Watson and Crick in 1953, has been unchallenged for almost 25 years (9). Advances in polynucleotide synthesis and more recently in oligonucleotide chemistry have given access to a large variety of sequences with particular structural features. Besides the traditional B-DNA structure we have to deal with single strands, non-Watson-Crick double strands, triple strands and even quadruple strands. They are all stabilized by the same type of weak bonds, namely H-bonds within the plane of the base pairs and stacking interactions along the axis of the helix rods. For those not so familiar with the structural features of the Watson-Crick double helix structure the main elements are briefly summarized. DNA exhibits in general a double stranded conformation of two antiparallel shugar-phosphate strands. The pucker of the shugar conformation dominates the secondary structure. Each shugar ring is covalently linked via a N-glycosidic bond to a heterocyclic residue, either of a purine or a pyrimidine analog. These bases, one of each chain, are paired in a strict way, that is, adenine exclusively pairs with thymine and guanine does so with cytosine, the first pair contains two internal H-bonds, the second contains three. The helix is assumed to be righthanded. Whether this is always the case is subject of intense experimental research.

From the strict selectivity of base pairing it was intuitively presumed that H-bonds are the dominant stabilizing elements of the double helix. The validity of this assumption can be tested experimentally. To this purpose we have investigated systematically a large number of different DNAs from eucariots, procariots and viral origin (10). The results can be summarized as follows. The enthalpy change per base pair, the transition temperature, the axial phosphate distance along the helix axis and the free enthalpy change are at a given set of solvent conditions can be utilized to identify a certain DNA according to its GC content, since all of these physical properties are linearly dependent of the composition of the base sequence. The linear change of the transition enthalpy reflects the dominance of nearest neighbour interactions in a sequence and the absence of special sequence related short range stabilizing effects. The relative fraction of H-bonds present determines the transition enthalpy. Consequently from the slope of ΔH vs. GC content the contribution of H-bonds and stacking fources to the helix stability can be separated. It can be grossly stated that half of the stabilizing forces stem from H-bonds. In the following the three thermodynamic state functions ΔH,

ΔG and ΔS and the transition temperature T_m are given as function of sequence composition and of ionic strength.

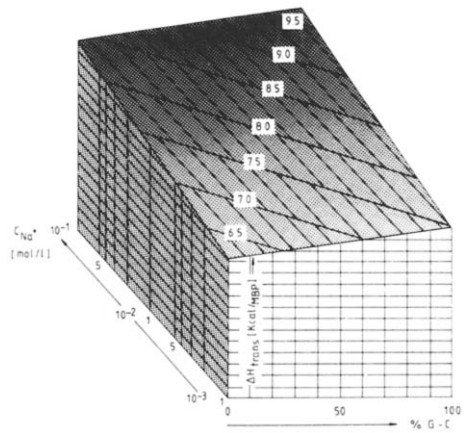

Fig. 1. ΔH-FUNCTION

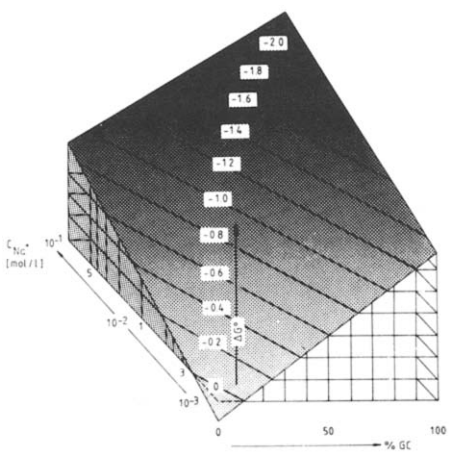

Fig. 2. ΔG-FUNCTION

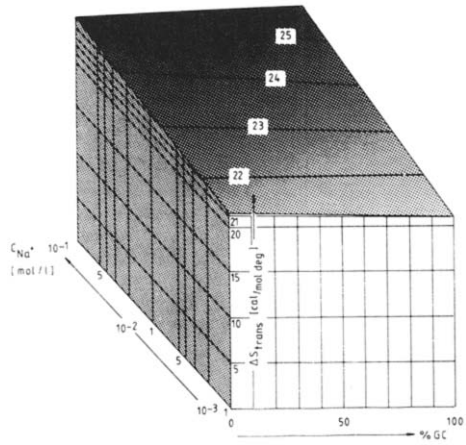

Fig. 3. ΔS-FUNCTION

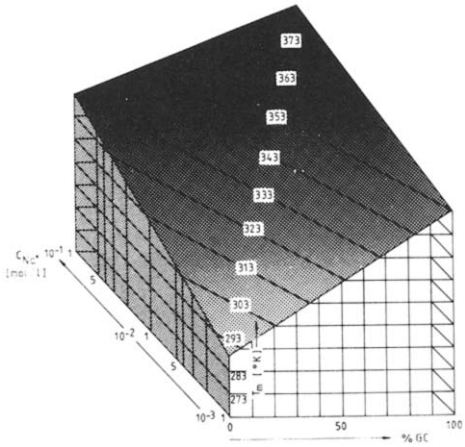

Fig. 4. T_m-FUNCTION

CONCLUSION

At our disposal is the complete thermodynamic data set to predict helix stabilities of any base composition, either using ΔH or ΔG as basis for comparison. This is valid since ΔS is unaffected by any sequence variation, purely reflecting changes in conformational freedom of backbone structures. This achievment will be very valuable in evaluation and comparison of structural features of gene regulatory sequences like promoters or restriction sites.

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