Hyperchromicity

Hyperchromicity is the increase of absorbance (*optical density*) of a material. The most famous example is the hyperchromicity of DNA that occurs when the DNA duplex is denatured.[1] The UV absorption is increased when the two single DNA strands are being separated, either by heat or by addition of denaturant or by increasing the pH level. The opposite, a decrease of absorbance is called **hypochromicity**.

Hyperchromicity in DNA denaturation

Heat denaturation of DNA, also called melting, causes the double helix structure to unwind to form single stranded DNA. When DNA in solution is heated above its melting temperature (usually more than 80 °C), the double-stranded DNA unwinds to form single-stranded DNA. The bases become unstacked and can thus absorb more light. In their native state, the bases of DNA absorb light in the 260-nm wavelength region. When the bases become unstacked, the wavelength of maximum absorbance does not change, but the amount absorbed increases by 37%. A double stranded DNA strand dissociating to two single strands produces a sharp cooperative transition.

Hyperchromicity can be used to track the condition of DNA as temperature changes. The transition/melting temperature (T_m) is the temperature where the absorbance of UV light is 50% between the maximum and minimum, i.e. where 50% of the DNA is denatured. A ten fold increase of monovalent cation concentration increases the temperature by 16.6 °C.

The **hyperchromic effect** is the striking increase in absorbance of DNA upon denaturation. The two strands of DNA are bound together mainly by the stacking interactions, hydrogen bonds and hydrophobic effect between the complementary bases. The hydrogen bond limits the resonance of the aromatic ring so the absorbance of the sample is limited as well. When the DNA double helix is treated with denatured agents, the interaction force holding the double helical structure is disrupted. The double helix then separates into two single strands which are in the random coiled conformation. At this time, the base-base interaction will be reduced, increasing the UV absorbance of DNA solution because many bases are in free form and do not form hydrogen bonds with complementary bases. As a result, the absorbance for single-stranded DNA will be 37% higher than that for double stranded DNA at the same concentration.

Hypochromic Effect

Hypochromicity describes a material's decreasing ability to absorb light.

The Hypochromic Effect describes the decrease in the absorbance of ultraviolet light in a double stranded DNA compared to its single stranded counterpart. Compared to a single stranded DNA, a double stranded DNA consists of stacked bases that contribute to the stability and the hypochromicity of the DNA.

When a double stranded DNA is denatured, the stacked bases break apart and thus becomes less stable. It also absorbs more ultraviolet light since the bases no longer forms hydrogens bonds and therefore are free to absorb light. Ways to denature DNA include high temperature, addition of denaturant, and increasing the pH level.

Importance of Hypochromic Effect

The measurement of absorption of light is important in monitoring the melting and annealing of DNA. At the melting temperature (Tm), the DNA is half denatured and half double stranded. By lowering the temperature below the Tm, the denatured DNA strands would anneal back into a double stranded DNA. When temperature is above the Tm, the DNA is denatured.

Because melting occurs almost instantly at a certain temperature, monitoring the absorbance of the DNA at various temperature would indicate the melting temperature. By being able to find the temperature at which DNA melted and annealed, scientists are able to separate DNA strands and anneal them with other DNA strands. This is important in creating hybrid DNAs, which consists of two DNA strands from different sources. Since DNA strands can only anneal if they are similar, the creation of hybrid DNAs can indicate similarities between genomes of different organisms.