THE FUNDAMENTAL BIOCHEMISTRY OF DRY STATE DNA: HYDROLYTIC REACTIONS

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It is currently a matter of routine for a forensic scientist to obtain a genetic profile, consisting of the 13 ‘core’ autosomal STR loci, of an individual who has deposited a biological stain at a crime scene. However sometimes DNA isolated from biological stains recovered from the crime scene is too damaged for current technological analysis. Potential remedies for such damaged DNA are most likely going to depend on the precise chemical nature of the damage. Though extensive studies have been done on the damage incurred by DNA in vivo, we only possess rudimentary knowledge of the nature of the molecular damage incurred in dried biological stains. Knowledge of the types of chemical changes and the kinetics of their formation will facilitate development of protocols to lessen the impact of this damage or even to repair it in vitro.

It is hypothesized that similar hydrolytic and oxidative reactions occur in the dry state DNA molecule as it does in the hydrated molecule. It cannot be assumed, however that these types of activities occur to the same extent and at the same rates. In this work we study in detail the chemistry and kinetics of the hydrolytic process as it pertains to DNA and its components in the dried state. In general the generic process of hydrolysis encompasses two important reactions, that of deamination and of base loss from the 2’-deoxyribose backbone. Base loss is believed to ultimately lead to chain scission.

Here techniques of ion-pairing chromatography and denaturing HPLC (DHPLC) were employed to determine damage to dry state nucleotides, poly (dN)10, and a forty base pair DNA duplex. It was found that to a large extent the same degradation reactions (base hydrolysis, deamination, and strand breaks) do occur in the dry state as in the hydrated state when heat alone is used as an energy source. Reaction rates indicate that base hydrolysis and deamination occur much more slowly, yet have the same energies of activation in both states. The duplex configuration of dry state DNA offers a level of protection for the molecule that is not found in single strand and nucleotide species. Single strand breaks of dry state duplex DNA occur with a half life of 24 ± 2 days and appear to occur in a mechanistic manner. In addition, base loss alone does not correlate with the extent of single strand breaks detected. This is much slower than what was seen with hydrated duplex DNA. Thermodynamic data can lead to the conclusion that DNA degradation in both dry and hydrated states is not a spontaneous process.