COMPLEXITY OF BIOLOGICAL MACROMOLECULES: LIPID TRANSFER AND UNCONSTANT RATE CONSTANT

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Accumulation of cholesterol in different intracellular membranes or lipoproteins could result in diseases such as Niemann-pick type C, hypercholesterolemia and Wolman syndrome, therefore the cholesterol content of membranes has to be highly regulated. Experiments have been designed to show that Bovine Niemann-Pick type C2 (bNPC2) mediates the transport of free cholesterol from one vesicle (donor) to another (acceptor). This mimics how cholesterol is transported from the endoplasmic reticulum where it is synthesized to the plasma membrane in a fast process. Cholesterol transfer has been shown to be dependent on time, temperature, concentration of the protein, hydrogen ion concentration, the lipid composition of the vesicles, ionic strength and the ratio of the donor vesicles to the acceptor vesicles.

The amount of cholesterol transferred increased with time until a steady state is attained. The transfer could only take place at acidic pH, pH values below 5.5. After this pH the transfer was not significant. The rate of cholesterol transfer at temperatures higher than 25^{o}C was very high, reaching steady state in less than 10 minutes of the transfer experiment. For instance, the best time window to monitor transfer experiment assay at 37^{o}C is 2 minutes. Increased concentration of bNPC2 increased the amount of cholesterol transferred, giving a hyperbolic profile resembling a one-site saturation ligand-binding curve.

In chemical reactions, rate constants are expected to be constant but this is not so in some biological macromolecules as illustrated in hemoglobin molecule. The rate constant changed with change in concentration of hemoglobin in its reaction with 5,5'-dithio-(2-nitrobenzoate) (DTNB). We took advantage of this variation to determine the $pK_{4,2}$ of hemoglobin and to determine both the acid and alkaline Bohr Effect of the sample. This turned out to be a simple method for determining the tetramer-dimer dissociation constant amenable to the conditions in many developing countries.