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THEORETICAL APPROACH TO LIFE PROCESSES
Part -VI. Evidence from Anhydrobiosis in Nematodes

Mirza Arshad Ali Beg
136-C, Rafahe Aam Housing Society, Karachi-75210, Pakistan

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The postulates of the theory concernings life processes and aging *vis-a-vis* hydrogen bonding in water and hydrogen bondable substances have been discussed in terms of anhydrobiosis observed among some nematodes. These organisms have the capability to modify their protein structure and yield small molecular weight carbohydrates and glycerol at the onset of desication. These metabolites, hydrogen bondable and lyophilic as they are, replace the structural and bulk water of their body. The replacement of water by these materials, which in turn are non-supporters of life processes, inhibits and slows down the metabolism infinitesimally and hence the organisms go into a state of anhydrobiosis. However, they have the remarkable capability to revive on rehydration. This, according to the theory, becomes possible by the lyophilic character of the glycerol and carbohydrates which get replaced by the surrounding water.

Key words: Life Processes, Nematodes, Dehydration, Oxidative dehydration, Aging.

Introduction

The "Dehydration" theory on aging suggests that for the maintenance of life processes an appropriate degree of hydration must be maintained. Water has several roles in this connection, some of them being as (i) a nutrient, (ii) a carrier of nutrients and metabolites, (iii) a medium for biochemical reactions including enzymic reactions and (iv) a lubricant for the proteins, membranes, cellulosic chains etc. to sustain the hydrogen bonding network among the different strands [1]. Dehydration is the key factor in aging as deduced from evidences from plant processes [2,3]. Experiments on rehydration of dehydrated vegetables have demonstrated that the survival of cellular structure depends upon the amount of cellular water that can be lost without undergoing irreversible damage. For each vegetable there is a critical level of water beyond which lies the point of no return [4].

The essentials of the theory are that water is needed to retard tissue aging; loss of water from membranes by desiccation causes membrane damage and loss of activity resulting in enhanced aging. This paper suggests that small hydrogen-bondable molecules like glycerol substituting for water in membranes can permit reversible dehydration to occur.

Slowing down of biological activity and its final cessation as a result of loss of water from the body beyond a critical limit seems to be an almost universal phenomenon among plants and animals since the life processes in both have to depend on membranes and enzymes. For example, during the study on variation of ionic concentration of blood sera of fasting human volunteers during the month of Ramadan, lyophilic materials were lost along with water and lyophobic materials accumulated in the blood as a result of physical dehydration [5]. Results already on record show that a point of no return is observed for all organisms if the functional and structural integrity of biological membranes and biopolymers is challenged [6-8].

Biological membranes are coordination polymers which have an organic casing studded with specific metal ions to permeate the desirable ions and solutes [9]. Besides water, the casings comprise biopolymeric units whose presence is necessary to control permeability of the membranes by keeping them adequately hydrated. Hydration of membranes constitutes formation of a superstructure having water on the outer sphere or surface. Dehydration of the macro-environment induces reduction in the span of the hydration sphere. If optimised, this could lead to the continuation of life processes, but if a stress situation leading to desiccation is induced, irreversible damage can be done to the membranes and the biopolymers leading initially to disease and finally to death, as observed in the study of impact of air pollution on vegetative growth [10].

Methods

Adaptation of anhydrobiotes to dehydration stresses. Support of the hypothesis, suggesting dehydration as the key factor in aging and hydration as the basis for continuation of life processes, has been sought from studies reported on animals. Indeed diverse groups of animal can undergo nearly complete dehydration through the removal of practically all the intracellular water without losing their lives [11,12]. A majority of these animals are either vulnerable to long periods of drought as well as quick drying or they rely on the level of hydration in the macroenvironment which can undergo rapid changes in the water content [13,14]. Because of these emergent situations, the animals have adapted themselves to dehydration stresses. Nematodes are among the class of anhydrobiotes which have adapted to the diversity of their habitats [12-15]. Studies on their longevity have been on record for long time [16]. Their dehydration as passage into an anhydrobiotic state provides argument in favour of the important roles of water outlined earlier [1].

Studies conducted on the survival of nematodes demonstrate their ability to reduce their rate of evaporative water loss [12,13]. Slow as against fast drying has been found to be more effective in inducing anhydrobiosis [15,21]. However, the diverse species of these organisms have to adapt to different rates of dehydration stress. The nematodes occuring in the upper soil profile can be placed under desiccation conditions for a long time. Their ability to control the evaporative loss of water favours anhydrobiotes to survive [12]. The continuation of life or viability of the anhydrobiotes depends on their ability to maintain biological integrity at desiccation and therefore, it is suggested that complex processes leading to biochemical adaptations adopted at the cellular level become effective [12]. Cellular level integrity must be maintained and slow drying has been found to prepare the anhydrobiotes to face anhydrobiotic conditions metabolically. Thus, nematodes which are fast drying as compared with others are expected not to survive to a great extent since the drying might be too quick to permit metabolic adjustment [15,22]. However, anhydrobiotes may or may not need a preparatory phase for the above adjustment depending on their individual ability to survive rapid as well as prolonged desiccation [12]. This suggests that nematodes such as *plactus* sp. are preadapted towards anhydrobiosis at the cellular level. This is most likely the result of modification of the membrane phospholipids to increase the fluidity of the bilayer and alternation in the micro- environment of the biopolymeric structure [23].

Environmental stresses of the above type are quite pertinent to the present hypothesis because it suggests the presence of a lubricant to preserve the hydrogen bonding network among the different strands of proteins and carbohydrates. Slow drying takes away monolayer after monolayer of water from the hydration sphere while rapid drying does so with 2, 3 or a number of layers at the same time. It appears that the nematodes which undergo anhydrobiosis rapidly have the capability to pass through different aquatic phases to act as hydrated liquid crystals, fluidsol and semi-solid gel by releasing different metabolites so that their skeleton can maintain some fluidity for the continuation of their biological processes particularly locomotion [2,23].

The theory suggests that disturbances in the macro- environment emanating from thermal shocks, radiation damage, stress situation, etc. should be kept to a minimum, otherwise given time, these forces would make macro-environment vulnerable to attack [2]. In this particular case, the degraded macro- environment is the desiccated habitat of the animal and the desiccated conditions through evaporative water loss are the stress situations imposed on the micro-environment i.e. the one which is in the immediate vicinity of the reactant, be it inside or outside. The latter condition first disturbs the hydrogen bonding network by withdrawing the lubricant and reducing the fluidity. Further dehydration would initiate the anhydrase activity e.g.

$$CO_2 + H_2O \xrightleftharpoons{\text{Carbonic anhydrase}} H_2CO_3$$

and would thus prepare for metabolic adjustments at the cellular level where enzymes are located. Metabolites in the form of small proteins or carbohydrates or glycoproteins would be lost to make up for the evaporative water. The protein structure would be modified appropriately to enable the organism to adapt at the cellular level. Alternatively or simultaneously, the membrane phospholipids could be modified thus altering the molecular frame work and increasing the fluidity of the bilayer [23].

Mechanisms for adaptation to anhydrobiosis. Several mechanisms are proposed for the biochemical adaptations to anhydrobiosis. Similar adaptations have been reported for the nematode *Aphelenchus avenae,* a slow drying free living nematode and the encysted embryos of the brine shrimp *Artemia salina,* both species containing a high concentration of glycerol and trehalose [23]. Carbohydrate accumulates at the expense of glycogen and lipid reserves as the nematodes dry [24]. However, there are nematodes e.g. *Ditylenchus myceliophagous,* also slow drying, which utilize lipids and glycogen to accumulate glycerol and ribitol rather than trehalose [23]. Other species of nematodes have been found to deplete lipids with the start of desiccation conditions [23]. Some slow and fast drying nematodes, e.g. *Anguina tritici and Ditylenchus dipsaci,* accumulate varying amounts of trehalose but not glycerol and this is not at the expense of lipids reserves which increase to higher levels [25,26]. The species *Plectus* sp. *and Orrina phylobia* [15,22] accumulate very little carbohydrate but survive rapid drying. Based on the above observations, although great variations exist in the biochemical adaptations to anhydrobiosis, yet the potential role of specific carbohydrates in stabilizing dry biological systems and thus preserving their biological identity has been evidenced.

Most nematodes accumulate low molecular weight carbohydrates, a situation similar to other organisms faced with thermal stress [23,27]. Inspite of the variation in the kind and concentration of carbohydrates, a strong correlation exists between anhydrobiotic survival and the accumulation of trehalose [22]. Therefore, the suggested role assigned to the small molecular weight carbohydrates is the capability to replace bound water, bulk water and inhibition of oxidative damage. One of the postulates of the present hypothesis is that oxidative dehydration reactions leading to hydrophobic substance formation must be kept to a minimum; otherwise lipid reserves will be formed [1]. It is thus interesting to find examples of living systms where these small molecular weight substances are capable of inhibiting oxidative dehydration conditions and of the damage that is likely to occur.

Replacement of water by hydrogen bondable substances. The structure and function of biopolymers and membranes have been shown earlier to be maintained by the presence of a hydration sphere, whose shrinkage and removal damages the functional integrity. It has been demonstrated from the study of some anhydrobiotes, that the damage can be avoided by the capability of the organisms to replace structural or bound water [23]. In the case of *Escherichia coli*, the integrity of its air dried cells can be maintained by the addition of carbohydrates in various proportions, prior to drying [19,28]. The results suggest that the preservation is due to the replacement of bound water by carbohydrates when the former was removed from the DNA molecules [29].

Inositol has also been found to accumulate in *A. tritici* [25] and has pronounced effect on its survival under desiccation conditions. Conversely, glycerol is protective to cells dried slowly at high relative humidities [30]. The observation on *Artomea salina* suggests that the protective ability is a mechanism to stabilize the proteins whose hydrogen bonding network is maintained by glycerol instead of water [6]. The same mechanism also suggests that since trehalose inhibits the reaction between the reducing groups of sugars and free amines of the dried proteins, it would allow the interaction of this carbohydrate and dried protein and hence the partial restoration of the hydrogen bonding network in the absence of water [31]. The reducing disaccharide maltose also preserves the functional identity of phosphofructokinase to the same extent as trehalose [23]. Thus it seems that hydrophilic and hydrogen bondable materials like disaccharides and polyhydric alcohols are able to provide a hydrogen bonding environment in the absence of water, perhaps by doing so, they may avoid bilayer perturbations which occur on shrinkage of the hydration sphere around the phospholipids of the membranes. Trehalose is superior to the other naturally occuring carbohydrates in maintaining structural and functional integrity and membrane fluidity, thereby depressing the phase transitions within the lipid bilayer [23,24]. The postulate accordingly suggests that nematodes alter the phospholipid composition of their membranes such that the hydrogen bonding environment persists at least partially, to increase their fluidity and depress phase transitions at low temperature [26].

Observation on membrane changes and the role of carbohydrates on drying suggest that dehydration is the main cause of membrane damage and subsequent loss of biological activity. The soluble proteins and the intracellular membranes which are separated by bulk water, interact on induction of anhydrobiosis through their side chains, e.g. the SH group, giving rise to disulphide linkages in the initial stages followed by fusion of the membrane bilayers. On rehydration, the structural and the fused bilayers do not separate out with the result that functional integrity and hence the biolgical activity is lost [24]. Fusion of bilayers occurs even in the presence of small concentration of carbohydrates [23]. Protection against fusion of the phospholipid vesicles induced by dehydration is provided by carbohydrates, viz. trehalose followed by maltose and sucrose, provided they are present on both sides of the bilayer and are capable of interacting with the phospholipid, maintaining bilayer fluidity and preserving biological membranes [23,27]. Trihydric alcohol being highly fusogenic, does not behave in the same manner as carbohydrates in protecting the bilayer. However, since it is found in appreciable concentration, probably it not only acts as a replacement of bulk water and serves as a lubricant but also as an antioxidant [24].

Inhibition of free radical attack by hydrogen bondables. Direct molecular oxidation gives rise to formation of superoxide free radicals which are highly destructive for dry biological systems [32]. They are effective in peroxidation of membrane lipids and of proteins, particularly those pertaining to structure and function. Nematodes stored in the dry state lose their viability with the passage of time [33], because the process of production of oxygen free radicals is slow. They need to be activated by enzymes or by photosensitization [24,25]. In both cases they would oxidize and degrade the biological membranes when they have accumulated to build up a potential. In the presence of antioxidants this pathway for damage would be restrained and the process of aging would in general be inhibited [1]. Glycerol is one such antioxidant [24], while the carbohydrates play a secondary role in inhibiting free radical attack. It is also possible that the production of free radicals is controlled by adsorbent and scavenging reactions. The latter argument applies to the observation on the increase in superoxide dismutase (SOD) activity in *A. avenae* on rehydration [23].

The observations on oxidation processes among these organisms therefore also support the Dehydration theory on aging which suggests that antioxidants can slow down the aging process by providing a pool of reducing agents. The latter absorb the active oxidizing capabilities which exceed the normal energy levels and thus the process of quenching of free radical attack is common among living organism.

Conclusion

The adaptation of anhydrobiotes to dehydration proceeds by a mechanism which involves the replacement of structural as well as bulk water by hydrogen bondable metabosiles like glycerol and trehalose. This allows biochemical adaptation at cellular level and hence maintenance of biological integrity. Anhydrobiosis thus supports the dehydration theory which suggests that the presence of hydrogen bondable substances is necessary for the continuation of life processes.

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