CHANGES IN METABOLISM OF *SCENEDESMUS OBLIQUUS* AFTER RELIEF OF SALINIZATION STRESS

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SUMMARY: This was an investigation into the changes in some metabolites of salinized algal cells, after being relieved from salinity stress. 7-day salinized. Scenedesmus obliquus cells showed considerable increases in proline, soluble proteins and soluble carbohydrates, but with concomitant decrease in other free amino acids, insoluble-and total proteins and insoluble-and total carbohydrates. Upon rehydration, free proline, soluble proteins and soluble carbohydrates declined rapidly with time reaching, 24 hours later, the level found in the control culture. The levels of other free amino acids, insoluble and total proteins, and insoluble-and total carbohydrates increased progessively from the time of rewatering. During the periods of salinization and rehydration, none of these metabolites was recorded in the extracellular medium.

Key Words: Salinity stress, Agar cells, scenedesmus obliquus.

INTRODUCTION

The retarded growth of some fresh water algae under salinity stress could be due the synthesis of some organic compatible solutes to balance the external salts. These solutes have been found to be accumulated in cytoplasm (Cytosolutes) at high salinity and are less toxic to the metabolic functions than the inorganic ions (6). It was speculated that, with exception of proline, accumulation of these cytosolutes is of adaptive value and not a reflection of impaired metabolism. Proline accumulation has been regarded variously as a consequence of stress induced damage to cells (9), as a consequence of osmatic adjustment and is not due to cell death (16), as an anti-stress organic molecule (25), as an indication of a salt induced water deficit or as a temporary deviation of the metabolic pathway of NH_4 assimilation from glutamic acid to proline (10). In addition, proline has also been thought to be a cytosolute (23), a storage compound (2) or a protective agent to cytoplasmic enzymes and cellular structures.

Upon rehydration, some metabolic changes were expected, as shown by the decline of proline level in rehydrated wilted bean plants and that the fate of the metabolized proline depended on the amount of carbohydrates present (22). Consequently, the fate of proline in relation to other soluble and insoluble metabolites needs also to be investigated after stress relief.

The aim of this investigation was to follow the changes in soluble metabolites of the salinized green cells after being relieved.

Scenedesmus obliquus, which could be easily cultured in the laboratory under controlled conditions, was used in this investigation.

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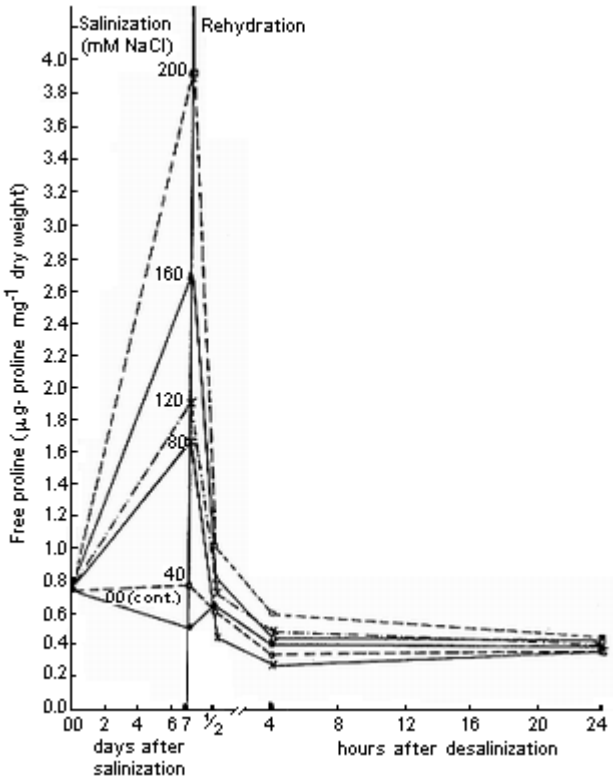

Figure 1: Free proline of Scenedesmus obliquus cultures subjected for 7 days to various levels of NaCl and then for 1/2, 4 and 24 hours to rehydration. The data are presented as absolute values on the bases of dry weight (µg. proline mg^{-1} dry weight).

MATERIALS AND METHODS

The chlorophycean algal species: *Scenedesmus obliquus* (Turp.) Kutz, which was found to be dominant in Nile water was used as a test organism in this investigation. Beijerinc nutritive medium (Stein, 1966) was used for enrichment and growth of this alga. The alga was cultured for 7 days under different salinities (00, 40, 80, 120, 160 and 200 m M NaCl). At the end of this incubation period, aliquots of the variously algal cultures were harvested for assay and analytical procedures. The remaining part of each algal culture after being centrifuged and washed with normal medium to remove the salinizing NaCl, was left to grow in the normal medium for 24 hours (rehydration). During this period, algal samples were harvested after 1/2 -, 4-and 24 hours for assay and analytical procedures.

Free proline was extracted from algal cells by homogenization with 3% suflosalicylic acid and colorimetrically estimated according to the method of Bates *et al.* (3).

Other free amino acids (only traces of proline and hydroxyproline are encountered) were colorimetrically determined as Ug leucine mg^{-1} dry weight using ninhydrin reagent acording to the method of Moore and Stein (15).

Water-soluble proteins were estimated in the water extract by the method of Lowery *et al.* (14). The water-insoluble proteins were estimated in the previous water-insoluble residue remaining after water extraction by homogenization with a certain volume of 1 N NaOH for at least 1/2 hour. One ml. of the homogenate was then colorimetrically determined using the method of Lowery *et al.* (14). The total protein content was calculated by summing the soluble-and insoluble fractions of the same sample.

The total content of carbohydrates was colimetrically estimated as Ug glucose mg^{-1} dry weight according to the anthrone sulphuric acid method recommended by Fales (7). The water soluble carbohydrates were estimated in the water extract by the same method. The water insoluble carbohydrates were calculated as the difference between the total and the water soluble carbohydrates of the same sample.

RESULTS

Free Proline and Other Free Amino Acids

When *Scenedesmus obliquus* cultures were subjected for 7 days to various NaCl levels, free proline was rapidly

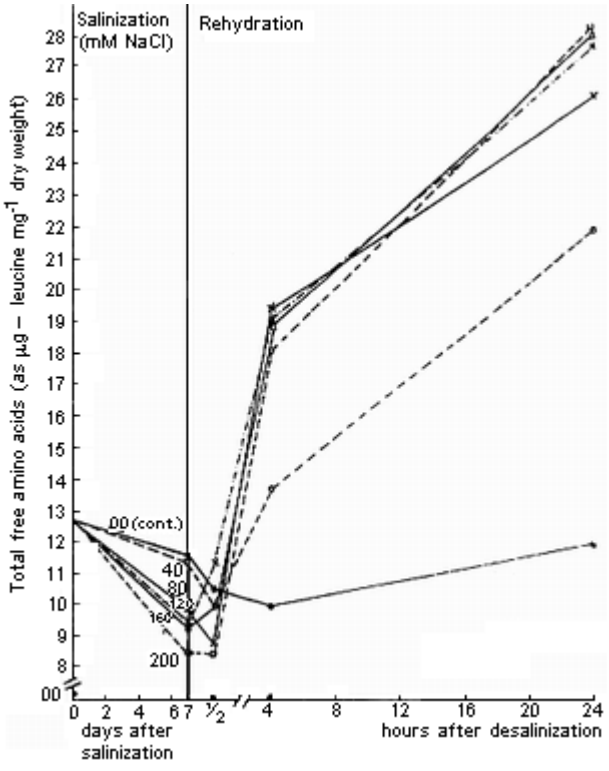

Figure 2: Total free amino acids of *Scenedesmus obliquus* cultures subjected for 7 days to various levels of NaCl and then for 1/2, 4 and 24 hours to rehydration. The data are presented as absolute values on the bases of dry weight (µg. leucine mg^{-1} dry weight).

Figure 3: Protein fractions (soluble, insoluble and total) of *Scenedesmus obliquus* cultures subjected for 7 days to various levels of NaCl and then for 1/2, 4 and 24 hours to rehydration. The data are presented as absolute values on the bases of dry weight (μg. albumin mg^{-1} dry weight).

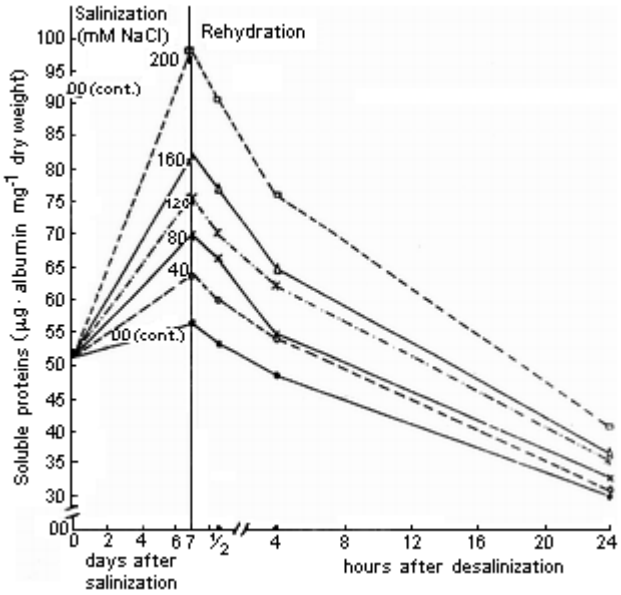

Figure 3a: Soluble proteins.

accmulated with the rise of salinization level, reaching a maximum content (8 fold that of the control) at the highest tolerable level (200 mM NaCl) used (Figure 1). On the other hand, the content of other free amino acids decreased gradually with the rise of salinization level (Figure 2).

Upon rehydration, the content of free proline declined markedly with time reaching, 24 hours later, values which were similar to that of the control culture. The contents of other free amino acids were generally elevated from the time of rehydration. This elevation was more pronounced (2 1/2 fold that of the control) in culture relieved from higher stress. It should be pointed out that during these periods of salinization and rehydration, no proline or amino acids were recorded in the outer medium.

Proteins

The contents of soluble proteins increased markedly with the rise of salinization level. Upon rehydration, a decline in these proteins was recorded from the time of rehydration. However, the contents of previously salinized cultures always remained higher than that of control culture (Figure 3a). Contrary to the soluble proteins, the contents of insoluble-and total proteins (Figures 3b and 3c) in salinized *S. obliquus* cultures decreased considerably with the rise of salinization level. Upon rehydration, and after 4 hours, these contents increased again to reach after 24 hours values which were similar to that of control culture.

Carbohydrates

The contents of soluble carbohydrates did not exhibit any regular trend with the rise of salinization level. Upon stress relief, the soluble carbohydrates decreased considerably with time to always reach their minima after 24 hours of rehydration. However, the minimum values of originally salinized cultures were always lower than that of control culture. After 24 hours of rehydration, the higher the previous salinization treatment the lower the soluble carbohydrate content (Figure 4a).

The levels of the insoluble and total carbohydrates (Figures 4b and 4c) of variously salinized cultures decreased considerably with the rise of salinization until the 4th hour of rehydration, thereafter, these increased again either to reach the level of control (40, 80 and 120 mM NaCl) or to remain below (160 and 200 mM NaCl).

DISCUSSIONS

The accumulation of proline recorded in *Scenedesmus obliquus* as a reponse to salinity stress was also reported in other salinized eukaryotic algae (1,12, 17-20). This accmulation of proline could be at the expense of the

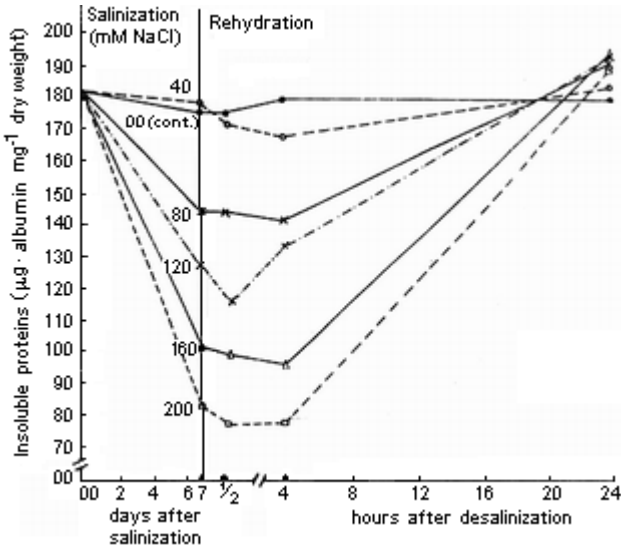

Figure 3b: Insoluble proteins.

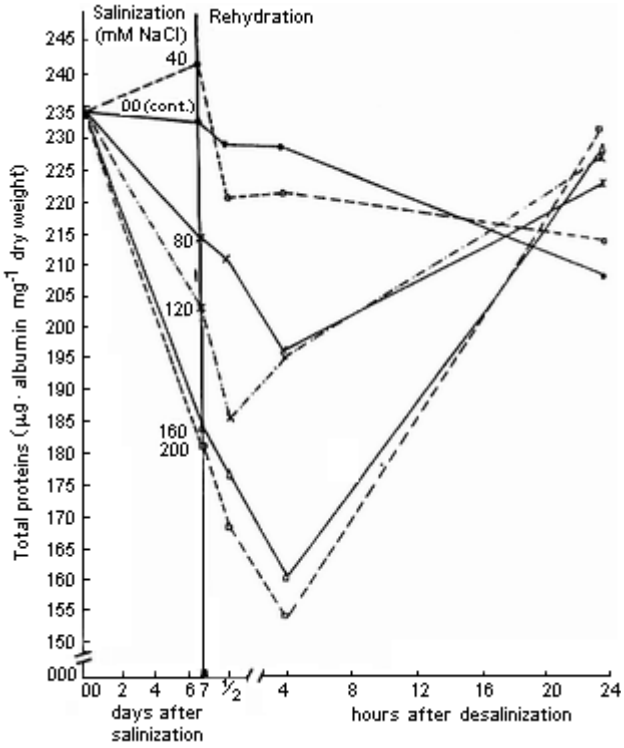

Figure 3c: Total proteins.

other free amino acids, the contents of which were recorded to decrease with these rise of salinization treatments.

Therefore, it is propaple that salinization may retard or inhibit, at least, some of the enzymes necessary for amino acid formation while those responsible for proline synthesis were not drastically affected.

Kaneshiro *et al.* (11) recorded, in addition to proline, an increase in alanine and glycine in *Miamensis avidus* under osmatic stress. Levy (13) recorded a marked increase in proline and amino nitrogen in *Solanum tuberosum* in response to water stress. Fukutoku and Yamada (8) reported that proline and asparagine accumulated extensively in *Glycine max* exposed to water stress.

Upon rehydration of the salinized *S. obliquus*, a rapid decline in free proline levels and an elevation in the content of other free amino acids were reported. This could be due to proline transformations to other free amino acids which in turn could be transformed to proteins. Such results were also reported by some other authors. Schobert (19) reported that, in the diatom *Phaeodactylum tricornutum*, the conversion of ^{14}C-proline into metabolic products is greatly inhibited during water stress conditions, and that a sudden reversal of osmotic stress from 1.9 to 0.77 osmolar in the algal medium leads to a rapid decrease of cellular proline concentration. Stewart (22) found that rehydration of wilted bean leaves resulted in a decline of the proline level Bengthson *et al.* (4) reported that after rewatering of stressed wheat, the levels of proline rapidly declined and reached, 10 hours later the levels found in unstressed seedlings.

That in this investigation no extracellular proline r other free amino acids were recorded in the outermedium during the periods of salinization and rehydration indicates that the proline and the other free amino acids are interconvertable under stress or stress relief. This interconvertability leads to the assumption that proline under stress could partially serve a pool for organic nitrogen, which could be further utilized after rehydration. In accordance with this, the contents of both insoluble-and total

Figure 4: Carbohydrate fractions (soluble, insoluble and total) of *Scenedesmus obliquus* cultures subjected for 7 days to various levels of NaCl and then for 1/2, 4 and 24 hours to rehydration. The data are presented as absolute values on the basis of dry weight (µg. albumin mg^{-1} dry weight).

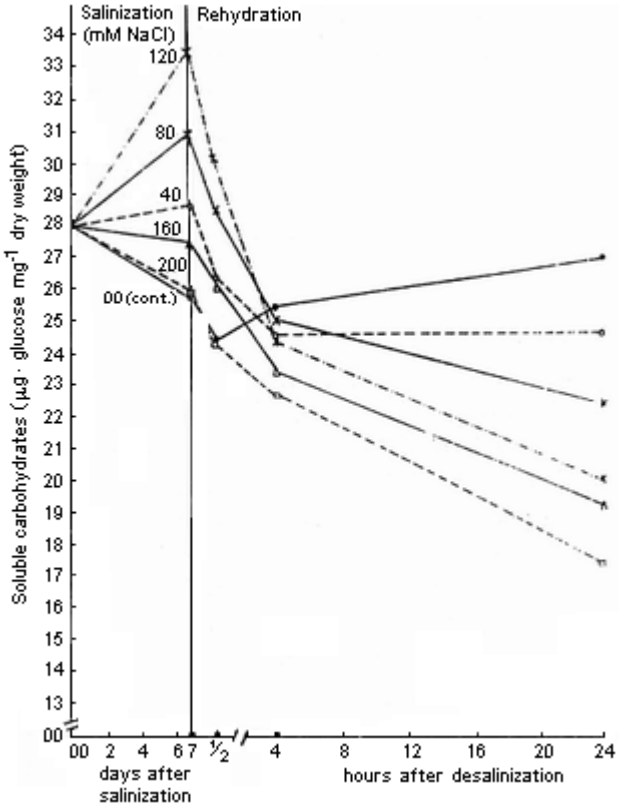

Figure 4a: Soluble carbohydrates.

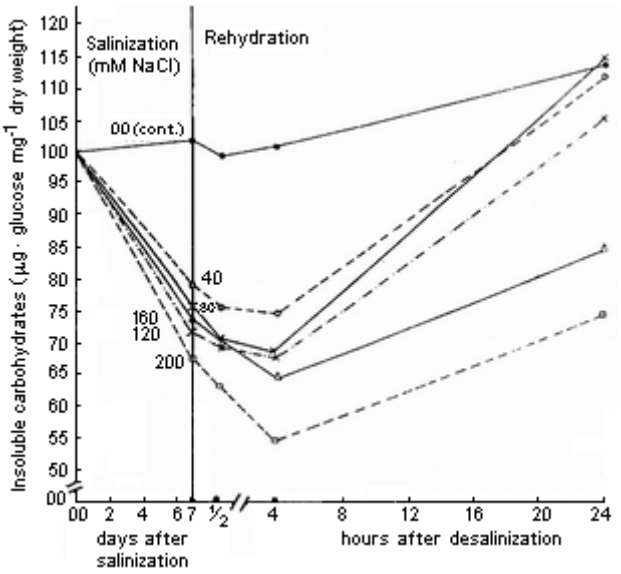

Figure 4b: Insoluble carbohydrates.

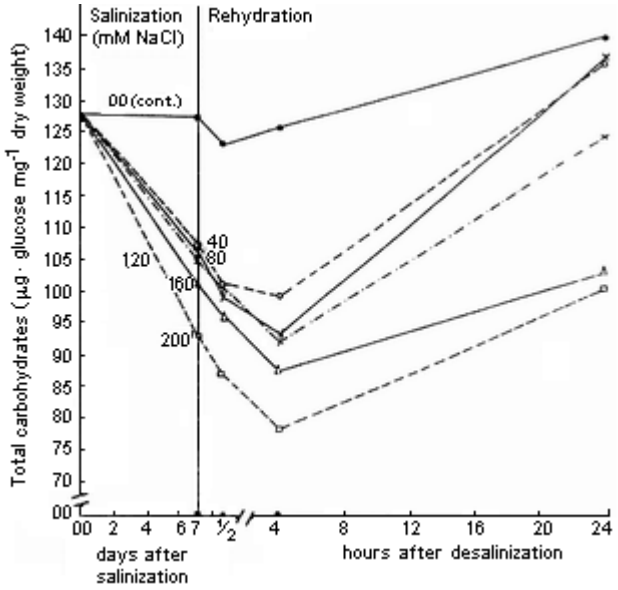

Figure 4c: Total carbohydrates.

proteins increased gradually with time, which means that upon rehydration the cells could retain their ability to synthesize the insoluble building proteins. This was also reported by Bewley *et al.* (5) who found that growing mesocotyl tissues of water stressed maize could recover full protein synthesis upon return to a fully hydrated condition following stress.

Also upon stress relief, the level of soluble carbohydrates decreased after rehydration, while the levels of insoluble carbohydrates as well as of total carbohydrates increased markedly with time, however, the maximum values always remained lower than that of control culture. The decrease in soluble carbohydrates after rehydration could be due to their consumption in metabolic processes responsible for recovery. In this respect, Stewart (22) reported that the rate of decrease and the fate of metabolized proline depend on the amount of carbohydrates present. In accordance with this, Wetherell (24) recorded that the soluble carbohydrates play a role during recovery from osmotic shock.

Generally, it can be said that for the salinization treatments used, *Scenedesmus obliquus* upon rehydration retained its activity, not only to recover, but also to build units to sustain life and to compensate the retardation in growth.

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