Protein water binding ability correlates with cellular osmolarity

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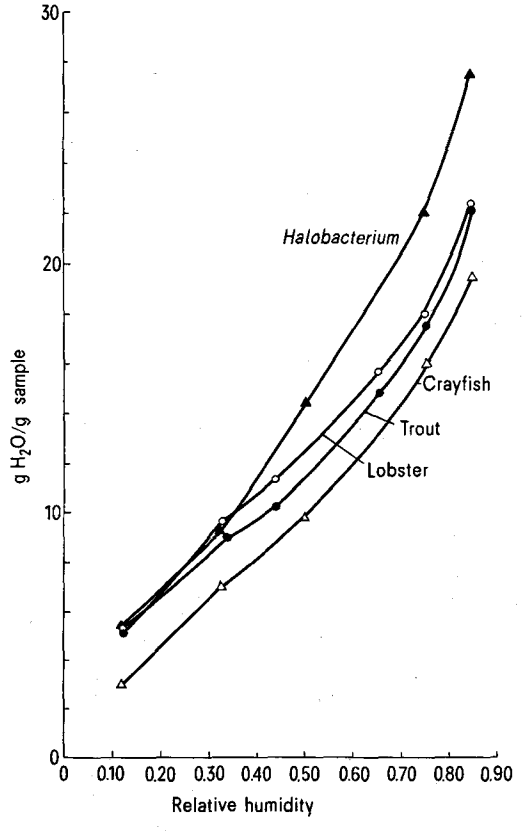
Summary. Water adsorption isotherms were determined for soluble proteins of organisms differing widely in intracellular osmolarity. A positive correlation was observed between protein-water affinity and total cellular osmotic concentration.

The packaging of several 100 types of enzymic reactions into a small volume of intracellular water has created what Atkinson[3] terms a 'solvent capacity problem'. Reducing the number of solute species in competition for this limited water supply in the cell has been achieved by several modifications in both enzymes and substrates, including high enzyme-substrate affinities, high-energy (activated) metabolic intermediates, and the organization of many enzymes into membranes[3].

1 aspect of the solvent capacity problem which has not been explored concerns the intracellular solubilities of proteins which must remain soluble to function. This problem seems especially interesting when species with widely differing intracellular osmolarities are considered, e.g., halophilic bacteria with up to 8000 mosmoles/l salt in their cells[4] and freshwater invertebrates, with total osmotic concentrations of approximately 130 mosmoles/l[5]. These marked differences in intracellular osmotic concentrations have led us to ask whether soluble proteins from cells with high salt concentrations have increased their affinities for water in order to compete more favorably for the limited solvent supply of the cell. Our data let us suggest that this is the case.

Materials and methods. The water binding studies utilized soluble macromolecular fractions obtained either from whole cells (bacteria) or from skeletal muscle (trout, lobster, crayfish). The choice of skeletal muscle was based upon the following reasons: a) muscle tissue is present in large quantities, thus allowing us to obtain adequate amounts of material for the adsorption studies; b) intracellular salt contents of muscle, liver, brain etc., are virtually the same, and thus any water binding 'problems' existing in 1 tissue can be expected to exist in other tissues; and c) the use of liver or hepatopancreas (crayfish and lobster) could pose problems due to the activities of the proteolytic enzymes within these tissues. We felt that muscle tissue would be much less susceptible to proteolytic degradation than, for example, hepatopancreas tissue. Bovine serum albumin and egg albumin were purchased from Sigma Chemical Co., St. Louis, Mo. Bacterial cells were sonicated and animal tissues were homogenized in distilled water. The sonicates/homogenates were centrifuged at 14,000 × g for 1 h. The supernatants were exhaustively dialyzed against distilled water to remove all components having mol. wt less than approximately 12,000 daltons. The dialyzed fractions



Water adsorption isotherms for dialyzed macromolecular fractions from organisms differing in cellular osmotic concentration.

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Organism	Cellular osmolarity (mosmoles/l)	Water adsorption at 50% relative humidity (gH_2O/100 g sample)[f]
Halobacterium halobium	4000–8000[a]	14.3
Marine Mn^{++}-oxidizing bacterium	4000[b]	14.2
Lobster (*Homarus americanus*)	1080[c]	12.1
Escherichia coli	335/410[e]	11.9
Brine shrimp (*Artemia salina*)	200–500[d]	10.7
Bovine serum albumin	310[e]	10.8
Egg albumin	310[e]	10.3
Rainbow trout (*Salmo gairdneri*)	285[c]	11.0
Crayfish (*Cambarus vulgaris*)	130[c]	9.7

[a] Value will vary with concentration of culture medium[4]; [b] unknown species from Dr Kenneth Nealson, Scripps Institution of Oceanography; cultures grown in medium containing 2.0 M NaCl; [c] concentrations given in Prosser[5]; [d] concentrations given by Croghan[11]; values vary with concentration of culture medium; [e] values given in Altman and Dittmer[12]; [f] values were obtained from the curvilinear plots of water adsorption versus relative humidity (e.g. see figure); when sufficient protein was available the adsorption isotherms were repeated and these replicates yielded identical results.

were then lyophilized and the resulting powders used in the adsorption isotherm experiments.

Water binding abilities were measured as described by Bull[6]. A 0.1 to 0.5 g sample of lyophilized material was placed into a vacuum dessicator above approximately 100 ml of a saturated salt solution which gave a known relative humidity at the equilibration temperature (20 °C). The salts (humidities) were: LiCl (12.5%), $MgCl_2$ (33.0%), K_2CO_3 (44.0%), Ca $(NO_3)_2$ (50.5%), $NaNO_2$ (65.5%), $NaNO_3$ (76.0%), KCl (85.0%) and Na_2CO_3 (92.0%)[7]. The dessicators were evacuated and held at 20 °C. After 3 days the vacuum was released and the samples quickly weighed. The samples were then dried at 105 °C, dry weights were measured, and the extent of hydration (g H_2O/100 g sample) was obtained by substracting the dry from the wet weight.

Results. The adsorption isotherms for several samples are shown in the figure. The shapes of all curves were similar and resembled closely the isotherms published by other workers on purified proteins[6,8]. Water adsorption values for egg albumin, bovine serum albumin and brine shrimp agree very closely with published values[7,9]. According to Kuntz and Kauzmann[8] the region of the adsorption curve below approximately 30% relative humidity is due largely to the penetration of water into the void spaces in the protein crystals, and thus does not reflect accurately protein water affinities. At high humidities (above approximately 80%) small changes in humidity lead to large changes in water adsorption, making accurate comparisons in this range difficult. For these reasons the relative water binding abilities of the samples are better compared using data at intermediate relative humidities, near 50% (table), where the adsorption isotherms are parallel (figure). These data reveal that the water binding abilities of the samples correspond positively with the osmolarities of the fluids in which the proteins occur. By far the strongest water binding ability is found in the case of samples from the 2 salt-tolerant bacteria; lowest water binding ability is by the sample from the freshwater crayfish.

Discussion. These results are consistent with the hypothesis that differences in protein-water affinity are correlated with differences in cellular osmolarity. Thus selection may lead to modification of protein surfaces such that their water binding abilities are always adequate to permit the proteins to remain adequately hydrated within the cell. In view of the very high osmotic concentrations of halophilic bacteria this requirement is perhaps not surprising. It is surprising, however, that apparent differences exist among animal species. Even the approximately 8fold differences in total osmotic concentration between tissues of marine and freshwater crustaceans[5] appear to be adequate to favor modification in protein water binding abilities.

While these data are consistent with solubility-related adaptations of soluble proteins, they are clearly but a tentative first step in the analysis of this question. Differential contributions of nucleic acid water adsorption and protein denaturation among the samples may have affected our results. Studies of purified, well-defined soluble proteins are an obvious next step in resolving this question. Ultimate resolution of the question may entail accurate amino acid composition analyses of soluble proteins, since the water binding abilities of amino acids differ markedly[10]. In this regard it is interesting that halophilic bacterial proteins contain extremely high amounts of aspartate and glutamate[4], the 2 amino acids having the highest water binding abilities[10].

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Effect of vasectomy on hepatic drug metabolism[1]

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Summary. 2 months after bilateral vasectomy the metabolism of aniline but not aminopyrine was increased in rat liver homogenates, whereas vasectomy did not affect the metabolism of either compound in guinea-pig liver homogenates.

Vasectomy is a widely used procedure for fertility control in man. There is, however, a paucity of information concerning possible metabolic consequences of vasectomy. In one recent study, increased drug metabolism was observed in liver homogenates prepared from vasectomized compared to control rats[2]. In view of the importance of an effect of vasectomy on hepatic drug metabolism, the present study was conducted in order to independently examine this phenomenon.

Materials and methods. Adult rats (approximately 350 g) obtained from SASCO, Omaha (NE) and adult English Short Hair guinea-pigs (approximately 600 g) obtained from CAMM Research Institute, Wayne (NJ) were housed individually and maintained on commercial laboratory diets ad libitum except that the animals were fasted 24 h prior to surgery. Under pentobarbital (Nembutal) anesthesia rats were bilaterally vasectomized by ligation and removal of a portion of each vas deferens essentially as previously described[2]. Guinea-pigs were similarly vasectomized except that the vasa deferentia were exposed via a lower abdominal incision. Control animals of both species were treated the same as the vasectomized animals except that the vasa deferentia were neither ligated nor cut. 2 months after vasectomy the animals were weighed, immediately sacrificed by decapitation and the livers removed for organ weight determination and analysis of in vitro drug metabolism. Aminopyrine and aniline were used as sub-

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