Absorption of Water and Nuclear Lens Protein by Nuclear Lens Tissue[1]

ALBERT C. SMITH[2]

ABSTRACT: Intrinsic water and soluble protein were removed from insoluble nuclear lens tissues of bigeye tuna, yellowfin tuna, and squid. These lens tissues were then used to absorb water and protein from extracts of nuclear tissue from these species. The quantity and type of absorption between the tunas were similar; between the tunas and squid, dissimilar. This indicates that nuclear lens tissue can be used to demonstrate both close and distant phylogenetic relationships.

THERE ARE three features of the eye lens nucleus which suggest that this tissue would make a good absorbent of protein in solution: (1) some of the nuclear protein is usually readily solubilized (Smith, 1969) and so can be removed from the tissue for the absorption of other proteins; (2) the nuclear tissue is relatively dry (Amoore, Bartley, and van Heynigen, 1959; Davson, 1963; Paterson, 1970) and so can readily be ground into small particles, thereby increasing the surface area of the tissue to proteins in solution; and (3) soluble lens protein can interact with, and be bound by, lens fiber membranes after disruption of the lens (Maraini and Fasella, 1970).

The present research was conducted to test the possibility that nuclear lens tissue from different species, after removal of intrinsic water and soluble protein and after grinding, might differentially absorb water and protein in a way that would reveal phylogenetic relationships. The species used were the bigeye tuna (*Thunnus obesus*), yellowfin tuna (*T. albacares*), and squid *Nototodarus hawaiiensis*.

MATERIALS AND METHODS

Experiment 1

Lenses were obtained by dissection from 107 bigeye tuna, 22 yellowfin tuna, and 71 squid; nuclei from each species were pooled. The pooled nuclei were then ground into a powder with a mortar and pestle. In order to conduct the experiment in triplicate, the powder was divided into three samples, each of which was extracted with a volume (ml) of 0.018 g% saline solution equal to seven times the wet weight of tissue. This saline solution solubilizes both albumins and globulins (Smith, 1968, 1970*a*). The samples were extracted for 24 hours at 5° to 10° C under constant agitation.

The nuclear tissues were treated to remove any soluble protein remaining after the extraction process. This was done by washing the tissues in large volumes of 0.018 g% saline solution followed by large volumes of distilled water. The saline solutions were changed each hour for 10 consecutive hours, and the distilled water was changed each hour for 5 consecutive hours. All of the washings were maintained at 5° to 10° C under constant stirring. The washed nuclear tissues were placed on a filter paper to air-dry for 48 hours.

Each of the extracts was cleared by centrifugation and then divided into four portions. One portion was stored frozen, and the remainder were individually subjected to absorption by nuclear lens tissue. The absorptions were conducted in a process similar to the serological method described by Cushing (1964), as follows. Extracts were added to test tubes containing the prepared, dry and soluble protein-depleted, nuclear lens tissues, which previously had been gently packed. The final volume of extract added was approximately equal to the volume of extract-saturated tissue. The tubes were allowed to incubate for 1 hour under constant, gentle agitation, but were swirled vigor-

[1] Manuscript received July 31, 1970.

[2] University of Hawaii at Hilo, Hilo College, Division of Natural Sciences, P.O. Box 1357, Hilo, Hawaii 96720.

ously every 10 minutes on a vortex mixer. After the absorption period, the extracts were centrifuged and the cleared solutions were stored frozen.

Protein concentrations of the thawed, unabsorbed, and absorbed extracts were determined with a Bausch & Lomb protein refractometer (catalogue number 33-45-87).

Experiment 2

Lenses were obtained from 22 bigeye tuna, 20 yellowfin tuna, and 42 squid. Unabsorbed and absorbed extracts were prepared as in Experiment 1.

The extracts were processed electrophoretically on cellulose acetate according to a method that produces multiple, simultaneous protein patterns (Smith, in press). This method is like that described in previous reports (e.g., Smith, 1970*a*, *b*), except that three samples rather than one can be processed on each cellulose acetate membrane.

RESULTS

The extracts of nuclear lens tissue from the bigeye and yellowfin tunas and from the squid reveal species-specific differences in variation in protein concentration due to absorption. These differences are indicated in Table 1.

The electrophoretic patterns of the proteins in the extracts correspond in overall staining intensity with the concentrations of proteins —the higher the concentration, the more intense the staining.

DISCUSSION

The variations, i.e., the increases or decreases in protein concentration of the extracts, as revealed by both refractometry and electrophoresis, were probably due to the ratio of water and protein absorbed by the treated nuclear tissue.

The overall staining intensity of the electrophoretic patterns showed good correspondence with the protein concentrations determined in the first experiment. This indicates the validity of the observed variations in protein concentration of the extracts due to absorption.

The nuclear tissue from each species absorbed water or protein differently. The absorptions were similar by nuclear tissues from the two tuna species, and different by the tissues from either tuna species and the squid.

These data indicate that nuclear lens tissue varies least between closely related species, such as the two tunas, and varies most between distantly related species, such as the tuna and the squid. The basis for this variation may be in the relative concentrations of urea-soluble and urea-insoluble proteins, which are known to vary among soluble-protein-depleted nuclei from different species (Zigman, Schulz, and Yulo, 1970). Furthermore, since urea-insoluble protein has been demonstrated to bind considerable amounts of some soluble proteins (Maraini and Fasella, 1970), variation in the concentration of the urea-insoluble protein may be the major cause of the differences in absorption of protein by the nuclear tissue of the different species used in this study. Nuclear tissue can thus be used to demonstrate proximity, as well as distance, in phylogenetic relationships.

TABLE 1

REFRACTOMETRY READINGS OF THE CONCENTRATIONS OF PROTEIN IN UNABSORBED AND ABSORBED EXTRACTS OF NUCLEAR LENS TISSUE FROM BIGEYE AND YELLOWFIN TUNAS AND FROM SQUID

	BIGEYE TUNA	YELLOWFIN TUNA	SQUID
Protein (g%) in Unabsorbed Extract	0.30	1.00	0.20
	0.31	1.00	0.20
	0.30	1.00	0.21
Protein (g%) in Extract after Absorption with Treated Nuclear Lens Tissue from:			
Bigeye Tuna	0.60	0.73	0.12
	0.60	0.73	0.12
	0.60	0.74	0.13
Yellowfin Tuna	0.70	1.02	0.25
	0.70	1.03	0.26
	0.70	1.02	0.25
Squid	1.10	1.22	0.10
	1.10	1.23	0.10
	1.10	1.22	0.11

NOTE: The readings were performed on extracts of three samples from each species.

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