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INSULIN FROM EGYPTIAN TILAPIA BROCKMANN BODIES

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ABSTRACT

Certain teleost fish have large anatomically discrete islet organs called Brockmann bodies (BBs) that are much more easily harvested than pancreatic islets. Brockmann bodies (islet organs) of both types were excised from freshly killed Tilapia fish at the Fisheries Center, Faculty of Agriculture of Ismailia and immediately frozen. Approximately 5.1 g of islet tissue was obtained from 81 fish of approximate weight that ranged from 150-350 .These Brockmann bodies (BBs) were subjected to acid/alcohol extraction and gel filtration. Ultraviolet and infrared spectral measurements were performed. The fraction with the highest absorption was studied further and compared to standard insulin. They both showed the presence of alcohol/phenol OH groups and the amide C=O stretch.

KEY WORDS: Brockmann bodies, Nile Tilapia, Insulin

RESUMO

O presente trabalho trata da obtenção de insulina a partir dos corpos de Brockmann (ilhôtas de orgãos) de Tilapia do Nilo. Aproximadamente 5,1 g de tecido de corpos de Brockmann foram obtidos de 81 peixes (Tilapia Egípcia) com peso variando entre 150 e 350 g. O pescado fresco foi obtido do Laboratório de Piscicultura da Faculdaade de Agricultura de Ismailia , Egito. O material foi congelado e subsequentemente extraído com ácido/álcool e filtração com gel. O extrato foi estudado com espectroscopia nas regiões ultravioleta e infravermelha e comparado com padrões de insulina. Ambos indicaram a presença de grupos álcool/fenol e do grupo amida C=O.

PALAVRAS CHAVE: Corpos de Brockmann. Tilapia do Nilo, Insulina

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1. INTRODUCTION

Brockmann body is a discrete organ, occurring in fish, that contains tissue corresponding to that found in the islets of Langerhans of mammals. One or more Brockmann bodies occur in various teleost species. They are free of pancreatic acinar tissue, which makes them particularly suitable for biochemical studies and a good source of fish insulin. The principal islet weighs in the region of 1 — 50 mg; smaller secondary islets may also occur (1).

Diabetes mellitus is a heterogeneous group of diseases characterized by high blood glucose levels due to defects in insulin secretion, insulin action, or both. With the number of cases expected to increase rapidly in the years to come, diabetes is a growing health challenge worldwide. Of the approximately 16 million diabetics in the United States, about 1.5 million suffer from type 1 diabetes. In this catabolic disorder afflicting predominantly young individuals, blood insulin is almost completely absent, leading to hyperglycemia and alterations in lipid metabolism. Type 1diabetes is thought to be induced by a toxic or infectious insult that occurs in genetically predisposed individuals. With recent advances in the understanding of the involved immunology and cellular and molecular mechanisms, researchers strive to battle the disease with new preventive and corrective strategies (2).

Safley*et al.* 2014(3) reported that encapsulated tilapia islets normalized random BG levels for up to 210 days in NOD-SCID mice. In diabetic NOD mice, encapsulated tilapia islets were rejected on day 11 ± 4 with a peritoneal infiltrate of macrophages, eosinophils, B cells, occasional neutrophils, but few T cells. Immunohistochemical staining demonstrated the presence of murine IgG on tilapia islets within capsules of rejecting, non-immunosuppressed mice, as well as murine IgG-positive lymphocytes in the layer of host cells surrounding those capsules. These findings suggested that barium (Ba)-gelled alginate capsules are permeable to IgG and that anti-piscine antibodies may be involved in the rejection of encapsulated tilapia islets in untreated mice. No single

immunosuppressive agent prolonged encapsulated tilapia islet survival in NOD mice, but the combination of CTLA4-Ig plus anti-CD154 mAb extended tilapia islet graft survival until rejection at 119 ± 20 days and inhibited host cell recruitment to the peritoneal cavity. Triple treatment with CTLA4-Ig, anti-CD154 mAb, and anti-CD4 mAb allowed graft survival for 157 ± 35 days with little evidence of a host cellular reaction. IV and oral glucose tolerance tests (GTTs) of recipients with functioning xenografts demonstrated remarkably normal metabolic function. It was concluded that microencapsulated tilapia islets can survive long term with excellent metabolic control in diabetic mice given targeted immunosuppression, suggesting that cross-species physiological incompatibility may not compromise the applicability of that novel approach for future clinical applications. It was predicted that an improved microcapsule that prevents the entrance of IgG will enhance tilapia islet survival in that model, possibly allowing the application of this technique with limited or no immunosuppression.

2. MATERIALS AND METHODS

Brockmann bodies (islet organs) of both types were excised from freshly killed Tilipia fish at the Fisheries Center, Agriculture Faculty of Ismailia and immediately frozen. Approximately 5.063 g islet tissue, obtained from 81 fish with approximate weight ranged from 150- 350g, was stored at - 80°C until extraction was carried out. Following the addition of 18 ml ice-cold acid ethanol (2% concentrated HC1 in 95% ethanol) plus 3 ml water, the islet tissue was homogenised for 2 min at 4°C in an Ultra-Turrax disperser then stirred at 4°C for a further hour. The homogenate was centrifuged at 8000 x g for 15 min and the resulting pellet rehomogenised in 7.5 ml acid ethanol plus 2.5ml water, and centrifuged. The pH of the combined supernatants was adjusted to 8.5 by addition of concentrated NH_40H and, after standing for 20 min at 4"C, the resulting precipitate was removed by centrifugation at 8000 x g for 15 min. Addition of concentrated HCl was made to lower the pH to 5.4 followed by 1 m12 M ammonium acetate and 0.8 ml

20% sodium chloride, then 2.2 vol. ice-cold absolute ethanol and 4 vol. ice-cold anhydrous ether. An immediate precipitate formed but the mixture was allowed to stand overnight at 4 "C before decantation. The precipitate was air-dried and dissolved in 9 ml 3 M acetic acid before being loaded on to a column (89 x 2.5 cm) of Bio-Gel P-30 (100-200 mesh) and eluted in the same solvent at a flow rate of 30 ml/h. The ultra violet and infra- red was measured for the Fractions (5 ml) corresponding to the obvious peak.

3. RESULTS AND DISCUSSION

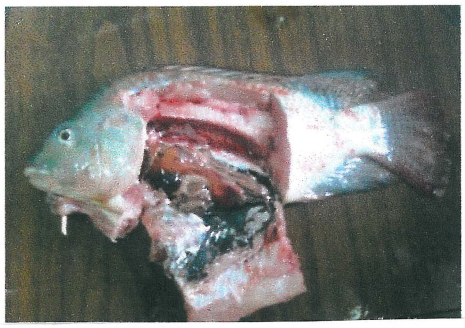

Fig.1 Dissected Tilapia fish.

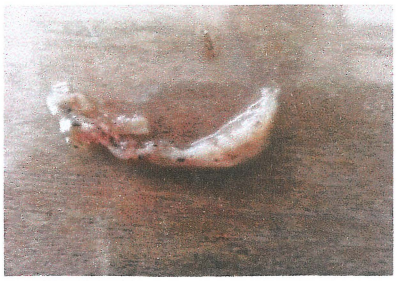

Fig. 2 Brockmann body isolated from Tilapia fish.

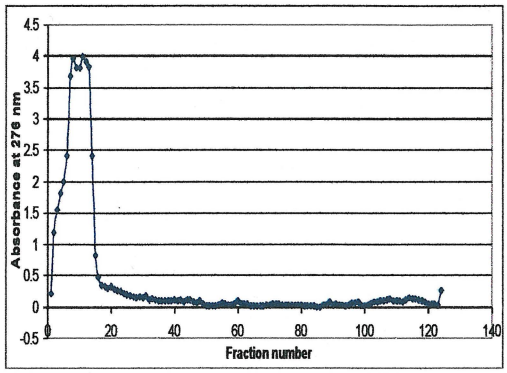

Fig.3 Gel filtration profile of Tilapia brockmann bodies extract. Separation was achieved on a column of Bio-Gel P-30 (89 x 2.5 cm) eluted with 3 M acetic acid. Fractions (5 ml) were pooled as indicated.

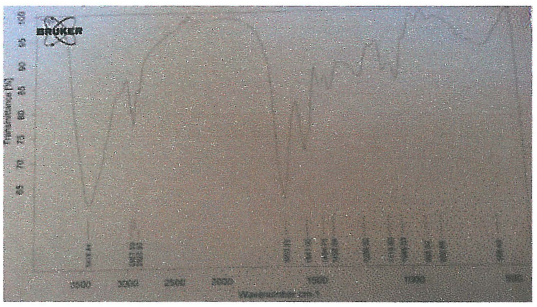

Fig. 4 IR Spectrum of standard insulin.

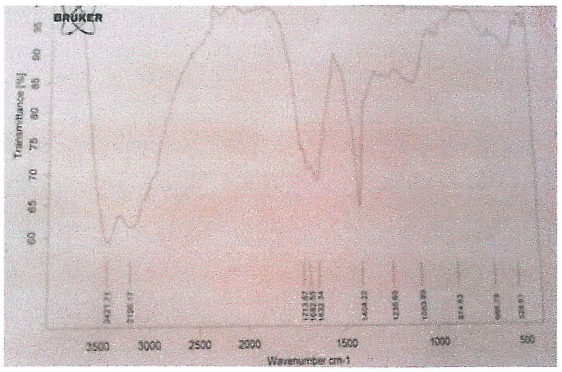

Fig. 5 IR Spectrum of material extracted from Brockmann body of Tilapia Fish.

In present study, there were about 5.063 g islet tissue was obtained from81 fish with approximate weight ranged from 150- 350g. Addition of ammonium acetate and sodium chloride prior to the ethanol/ether step aided precipitation of the slightly oily insulin-containing extract. The gel filtration step (Fig. 1) gave one major peak: an asymmetric peak corresponding to fraction eleven with ultra-violet absorbance of 3.983 nm

Tilapia Brockmann bodies are scattered within the adipose tissue surrounding the common bile duct in a triangular region bounded anteriorly by the edge of the liver, superiorly by the stomach, and inferiorly by the spleen and gall bladder (i.e., "the Brockmann body region") **(5).** The larger islets can be removed simply by excising the entire "region", placing it in a plastic petri dish with Hank's Balanced Salt Solution, and micro-dissecting them from the adipose tissue while visualizing them with a dissecting microscope (6). There is a linear relationship between fish body weight and the number of islet endocrine cells (7); therefore, the sum of the body weights ofmultiple donor fish can be used to predict the total islet cell mass as well as the number of transplants that can be performed (8). It should be noted that in large tilapia, some

Brockmann bodies can measure up to 5 mm in maximum dimension [n.b., tilapia produce new islets and their older islets grow throughout their lifespan and so there is a tremendous range in islet size(9).

Wright *et al.* 2012(5)and Yang*et al.*, 1997 a (10) found that in transplantation work, all large islets are broken up into smaller "mammalian islet" sized fragments).**11 Wright and Yang 1997** (11) have used tilapia BBs as an inexpensive model for studying islet xenograft rejection between discordant species. When transplanted into immunocompetent diabetic mice, tilapia BBs reject in roughly 7-8 days. Results to date suggest that tilapia islets are very immunogenic and that encapsulation is necessary to achieve long-term function in euthymic recipients. Tilapia islets currently represent an excellent, inexpensive donor source for discordant islet xenotransplantation studies. In the not distant future, encapsulated islets harvested from transgenic tilapia bearing humanized tilapia insulin genes may also play a role in establishing clinical islet xenotransplantation as a useful treatment modality for type I diabetes mellitus.**Yang and Wright 1995 (12)** performed a series of transplants using mass-harvested BBs to determine whether BBs harvested in this manner functioned in a manner similar to those harvested by micro-dissection. Long-term normo-glycemia was achieved in streptozotocin-diabetic nude mice and mean graft survival time was not altered in streptozotocin-diabetic euthymic balb/c mice. However, the total weight of donor fish required per recipient was decreased by 50% in both strains (12).

The Brockmann body of the teleost fish, the tilapia (Oreochromisnilotica) has been considered as a potential source of islet xenograft tissue for patients with insulin-dependent diabetes. The primary structure of tilapia insulin is similar to insulins from other teleosts (particularly the anglerfish, Lophiusamericanus) except that the strongly conserved glutamine residue at position 5 in the A-chain, a residue that is important in the binding of insulin to its receptor, is replaced by glutamic acid. In common with other teleosts, the tilapia Brockmann body expresses two non-allelic glucagon genes. Alternative pathways of post-translational processing lead to glucagons with 29 and 36 amino acid residues derived from proglucagon I and glucagons with 29 and 32 residues derived from proglucagon II. Glucagon-like peptides with 30 and 34 residues derived from proglucagon II were

also isolated. In each case, the longer peptide is a C-terminally extended form of the shorter. Tilapia peptide tyrosine-tyrosine (PYY) was isolated in a C-terminally alpha-amidated from with 36 amino acid residues that is structurally similar (89% sequence identity) to anglerfish PYY. A 30-amino acid peptide, representing the C-terminal flanking peptide of PYY, was also isolated that shows only 53% sequence identity with the corresponding anglerfish peptide. Tilapia somatostatin-14 is identical to mammalian somatostatin but the [Tyr7, Gly10] somatostatin-containing peptide derived from prosomatostatin II contains the additional substitution (Phe11-->Leu) compared with the corresponding peptide from other teleosts (13).

After extensive characterization, transgenic tilapia could become a suitable, inexpensive source of islet tissue that can be easily mass-produced for clinical islet xenotransplantation. Because tilapia islets are exceedingly resistant to hypoxia by mammalian standards, transgenic tilapia islets should be ideal for xenotransplantation using immunoisolation techniques. (14)

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