Particles as Protein Markers: Nanoscale Microscopy Towards Picoscale

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ABSTRACT

We proposed the proteins-nanogel particles as potential nanogel adducts for their commercial value in measurement of enhanced metabolic energy mechanism by muscle contractile protein conformational changes at picoscale. Picoscale (at the down-side level of 10^{-12}) is the minutest limit of molecular detection till date. In muscle, tropomyosin-calcium bound nanogels trigger the conformational changes at picoscale (10^{-12} meters or 10^{-2} ºA) and offer a promise of safe and rapid modality to increase the energy holding capacity of muscle tropomyosine-calcium protein assembly. Till date, electron microscopy and biophysical techniques can predict these submolecular physical dimensions without any information of metabolic energy mechanism or dynamicity. The proposed picoscale measurement of in vivo protein concentration and molecular dynamic events in ***Heteropneustes fossilis*** fish muscle may open a window to predict subphysiological, submolecular conformation to design hyperenergetic marine diets to get high quality fish food. The present report shows the emerging trend of picotechnology in bioengineering, ***Heteropneustes fossilis*** fish protein characterization by PAGE-gel electrophoresis, 10-100 nm nanoadducts by electron microscopy with possibility of nanogel-peptide(polyacylate-polyethylene-iron oxide) adducts as predictors of fish muscle proteins participating to enhance the capacity of tropomyosin-calcium hyperexcitation to measure it at picoscale in muscle tissue. In conclusion, the nanogel-protein adduct as bio-technology has tremendous commercial potentials to design marine diets, aquaculture, nanogel-drug carriers.

Key words: Tropomyosin-calcium, fish, protein, nanoparticles

1. INTRODUCTION

In fish muscle, the Ca^{2+} microdomains generated around the mouth of open ion channels represent the basic building blocks from which cytosolic Ca^{2+} signals in muscle tropomyosin are constructed. Recent improvements in optical imaging techniques using nanoparticles allow these calcium microdomains as single channel calcium fluorescence transients (SCCaFTs) to speculate channel properties and replace patch-clamp recordings. We report use of protein marker paramagnetic nanoparticles with a review of recent advances in single channel Ca^{2+} and other multimodal imaging by MRI, PET, total internal reflection fluorescence microscopy (TIRFM) as the technique of choice for recording SCCaFTs from voltage- and ligand-gated plasmalemmal ion channels. The old technique of 'optical patch-clamp possibly permits simultaneous imaging of hundreds of channels and provides millisecond resolution of calcium gating kinetics and sub-micron spatial resolution of channel locations; and seems applicable to evaluate the effect of seasonal changes on fish muscle membrane channels that display partial permeability to Ca^{2+} ions [1,2]. Picoscope has given hope of studying further intricacies of these molecules in muscle. To confirm the role of calcium and proteins in fish at different seasonal temperatures, we performed PAGE electrophoresis to distinguish several muscle protein candidates on scan in the electrophoretic mobility range of 0.5-2.5 eV/min.

2 PREPARATION OF NANOPARTICLES

A. Synthesis and Modification of Maghemite Nanoparticles

Iron oxide ($\gamma Fe_{2\alpha}$) particles of size 5-10 nm were synthesized using a three-step process of (i) coprecipitation of iron-oxide by mixing 0.1 M ferrous chloride and 0.1 M ferric chloride (1:2) with 10 M sodium hydroxide with continuous stirring for one hour at 20° C at 90°C, (ii) peptidization with 2M nitric acid, and (iii) sonication for 10 min. at 90° C. at 50% amplitude. For efficient binding with polyethylene, iron-oxide powder was stirred with 30% w/w sodium oleate at 1000 rpm speed for 2 hours. The mixture of iron oxide and sodium oleate was washed and dried under vacuum to yield fine 5-10 nm iron oxide particles.

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B. Formation of the Polymer Composite Particles

A batch process was developed for preparing composite particles. A 0.05% w/w; 10 ml solution of polyethylene wax (number average MW of 700 g/mole (Honeywell Corp.) was prepared using decaline or octamethylcyclotetrasiloxane OMCTS (Dow Chemical Company) at 150°C. To this solution, iron oxide powder was added with 30%-50% w/w polyethylene, and sonicated at 50% amplitude for 30 seconds. Then, 10 ml of tetraglyme ("TG") (obtained from Sigma-Aldrich) was added to the iron-oxide polyethylene mixture at 150°C, and sonicated at 50% amplitude for about 30 seconds. Next, the mixture was immediately cooled to about 0°C in ice water held at 0°C. Within three to four minutes, the polyethylene-iron-oxide mixture transformed into an emulsion with microdroplets made of supercooled polyethylene wax solution and iron oxide dispersed in a continuous phase of nonsolvent. The emulsion was warmed to room temperature 27°C for 45 minutes to make polyethylene and maghemite particles suspended in the emulsion. This emulsion was cooled to -10°C for half an hour to form a macrophase separated system made of thin reddish-brown sandwiched between top solvent layer and non-solvent bottom layer. The reddish-brown layer of polyethylene/iron oxide particles was centrifuged to isolate the particles from solvent mixture as shown in Figure 1 and described elsewhere.

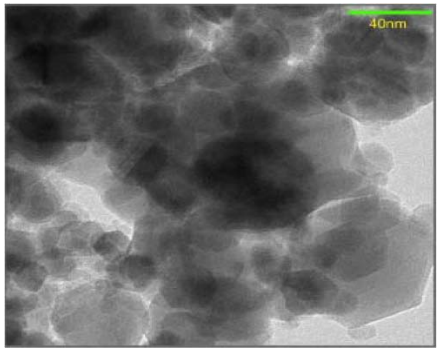

Figure 1: The 35 nm sized poly Ethylene – FeO nanoparticles are shown under Scan Electron Microscope (magnification x 10^5).

3. POLY AGAROSE GEL ELECTROPHORESIS (PAGE) OF FISH PROTEINS

Discontinuous SDS-PAGE analysis was performed under standard Laemmli conditions. The PEG–protein conjugates were loaded with an average of 7.5 mg (15 mL load volume) per well and run at 110 V constant voltage per gel. The gel dimension was 10.167.360.75 cm^3.The running buffer was 0.025 M Tris–0.19 M glycine–0.05% SDS. The gels were silver-stained for the protein portion. Native PAGE characterization of PEG–HAS conjugates and native PAGE analysis of the PEG–protein conjugates was carried out by loading samples with an average of 7.5 mg(15 mL load volume) per well and run at 110 V constant voltage per gel. The stacking gel, separatory gel, and the running buffer were prepared in the same way as the SDS-PAGE, except that no SDS was used. The gels were silver-stained for the protein portion.

4. RESULTS

SDS-PAGE has been a popular method to characterize PEG–proteins. The assumptions made were that the higher molecular weight gel band ladders represented one molar incremental additions of PEG to the protein. Most of the gel bands are smeared or blurred, especially for the PEG 5000. Three standard PEG 5000, 10000, 20000, were used as samples to compare the unknown fish proteins [3, 4]. At 110 eV, HS albumin, 5 kDa, 10 kDa, 20 kDa PEG gave specific spots as shown in Figure 2. However, major proteins were seen at locations of 31 kDa, 45 kDa, 65 kDa, 94 kDa as shown in Figure 2. The *Heteropneustes fossilis* fish muscle protein electrophoresis was used to identify different protein candidates based on their pI values as shown in Figure 2.

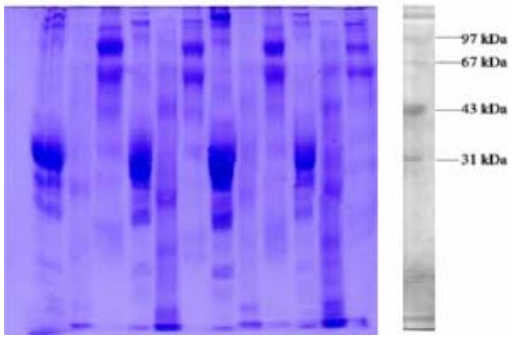

Figure 2: A representative PAGE elctrophoresis scan of fish muscle protein fractions. Notice the different proteins at electrophoretic mobility (pI locations).

5. NANOPROBES FOR MUSCLE PROTEINS AND PICOTECHNOLOGY

Recently, advancements are made in the field of protein markers and enhanced protein detection limits more and more accurate and with high sensitivity upto $1/100^{th}$ fraction (10 picomoles) of nanoscale.

For example the calcium regulatory behavior of calmodulin protein and calcium ion channel in single muscle cell can be observed up to the fraction of nanovolt or less. These outcomes are challenging and depend on the success of high precision and minute size of biosensor probes in use. We focus here on fluoro-sensitive green protein and phosphor sensitive immunospecific protein markers as shown in Table 1. These markers have uniqueness to generate images and measurement capability. The current trend is towards development of multimodal and multifunctional protein markers. Still the art is in infancy. However, the application of proteins and proteomics is expanding in marine biology, fishries, health science, cell biology and molecular biology.

Table 1: Muscle proteins and calcium-Magnesium regulatory markers are shown in muscle by different biochemical, physical, imaging and scanning techniques. Notice that some markers act as picogram level in action(shown by *).

Muscle Protein	Calcium/Mg	Technique used (reference)
Tropomyosin	Mg-Calmodulin	Biochemical (1, 11)
	Conformation	NMR (2)
	MRI Imaging	Nano-FluoroProbe(1)
	Optical Imaging	Nano-FluoroProbe(1)
	FRIT-imaging	Green Protein(1,2)
	Ca^{++} channel imaging	*SCCaFTs (1,2)
	Ca^{++} channel imaging	*TIRFM (1,2)
	Electrophoresis	2D-3D SDS/PAGE(3)
	Myosin conc	Biosensor (1)
Myosin	Mb4-myosin	Nano-Feo-AbMyo(1)
	Electrophoresis	2D-3D SDS/PAGE(3)
Actin	Ca-ATPase	Enzyme/histology(5)
	Filament Protein	*FluoroNanoProbe(2)

Table 2: Protein markers and protein probes in imaging applications of muscle proteins

Muscle Protein	Nanoprobe	Technique used (reference)
Tropomyosin	FETNIM-Imagin	MRI(15)
Myosin-Actin	FTIR Imaging	IR(15)
	CLIO-imaging	Nano-FeO Probe(15)
	MION- imaging	Nano-FeOProbe(15)
	Dendrimer imaging	Poly-nanoprobe(1)
	Cd-S imaging	*Quantum dots (1)
	Multimodal imaging	*Zn-Gd-Fe probe (15)
	Nanogel imaging	Polymer composits
	Mb4-FeO-CNT	Biosensor (15)
	Nanochips	Ion channel sensor(2)

6. EFFECT OF TEMPERATURE AND SEASON ON FISH MUSCLE PROTEIN AND CALCIUM CONTENT

Proteins with all other cellular constituents are in a state of continuous turnover. Protein turnover is offcourse a function of the rate of synthesis and the rate of degradation, both of which are under separate control (5).This phenomenon may significantly enhance the organism's ability to readily adapt to the changes in the environment (6). The energy associated with spawning is derived from liver (18%) somatic tissue (33%) and gonad (48%) for males in adult pacific cod (10). During maturation both protein synthesis (7, 13) and phosphorylation of proteins (5, 10) may occur. Fish muscle Protein content ranged from 15.9 to 16.5 percent during November2000 to March 2001. Maximum Protein content was recorded in summer months when water temperature was 23.5 degree centigrade (March 2001)and minimum proteins was in winter months i.e. December 2000 and January 2001 when the water temperature was 14.8 degrees Centigrade and 10.9 degree centigrade. It was also observed that in winter months the fishes consumed less feed which resulted in poor growth. Therefore, protein content was decreased in winter months. In summer months when the temperature started increasing, fishes also started taking more feed in comparison to winter months resulting in higher protein percentage. When the temperature became less than 15 degree centigrade the protein percentage decreased, while at higher temperatures of 20 degree centigrade and above the protein percentage have also increased. The protein content was significantly affected by the season in rohu. (3). Similar types of results were observed in cyprinus carpio. Variation in calcium content recorded in different months. Calcium content in rohu ranged from 620 to 650(mg/100g) in our experiments, from November 2000 to March 2001. Maximum calcium content was recorded in summer and minimum in winter. Like other contents it also increased and decreased with increasing and decreasing temperature. It may be due to the intake of feed in various months (3).Similar type of results were observed by Missima et al. (11).

7.CONCLUSION

In conclusion, the protein detection methods and current use of nanoprobes in muscle proteins is highlighted. The nanotechnology and its application in biology, health science and diagnostics is

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expanding as real-time, sensitive, monitoring rapid method. In marine biology, muscle protein characteristics play significant role in fish growth at optimized fish living water temperature conditions.

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