68 - ANALYSES OF THE PRESENCE OF ALANINE IN THE BLOOD AFTER ANAEROBIC EXERCISE BY FT- RAMAN SPECTROSCOPY

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Introduction

The optic spectroscopy studies the electromagnetic radiation interaction with matter. It has as one of its main objectives the determination of atoms or molecules energy levels (SALA, 1995). At a molecular level, radiation may interact with matter by the process of absorption or scattering, the later can be elastic or inelastic. The elastic scattering of photons is called Rayleigh Scattering, whereas the inelastic is called Raman Scattering (SILVEIRA JR., 2001).

Raman spectroscopy have been largely used in biology and biochemistry as a tool to study the structures and functional dynamics of important molecules, as for example the study of blood components (BAYDEN e McNAUGHTON, 2001; PILOTTO et al., 2001; SATO et al, 2001;). Raman espectroscopy characterizes biological molecular structures from positions and relative intensities of several bands (MAHADEVAN-JANSEN et al., 1992). The spectral region that identifies most biological samples is from 400 to 2500cm^{-1}; this region contains a series of defined bands used to characterize particular molecules, or in some cases, to identify the composition of biological samples (BITAR et al., 2004).

This innovate technique presents innumerous advantages, such as the need of small samples, the reduced invasiveness, and the capacity to offer real time results.

Exercise has some immediate effects on the protein metabolism. Branched chain amino acids oxidation can be an important source of energy to the muscles during exercises, especially if other sources of energy aren't available (oxygen depletion conditions, for example). The amino groups from these broken amino acids are transported to the liver to be used in the urea cycle (MAUGHN et al., 2000)

The branched chain amino acids oxidation results in a deposition of amino groups; some of them are transformed in pyruvate (LEHNINGER et al., 1995). Alanine is formed in the muscle from the combination of pyruvate and ammonia. It works as a transporter, from the muscles to the liver, of the equivalents of ammonia and the carbonic skeleton of the pyruvate. This happens in a non- toxic way through what is called the glucose-alanine cycle (MAUGHN et al., 2000).

The goal of this paper is to compare the presence of alanine in the blood of an athlete before and after a high-intensity anaerobic exercise, using FT-Raman spectroscopy.

Methodology

A small sample of blood was taken from the finger of an athlete at rest and placed in a capillary with heparin. Then, this athlete made 5 sprints of 40 seconds each with 2 minutes of resting time between them. After the last sprint another sample of blood was taken and placed in another capillary with heparin. The use of heparin is important because it keeps the blood from coagulating, and therefore preserves the samples.

For the blood analyses, the capillaries were placed in the Bruker RFS 100 FT-Raman Spectrometer (Figure 1), with a Nd:YAG 1064 nm laser as source of excitation. A germanium detector cooled with liquid nitrogen collected the Raman signals. The output power of the excitation laser was 300mW, the power on the sample was 132mW. It was used 500 scans and spectral resolution of 4cm^{-1}.

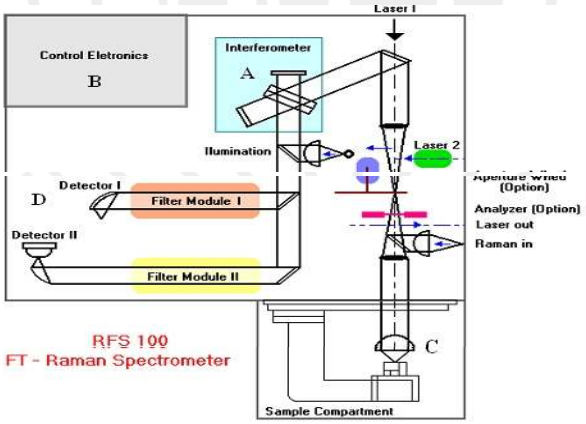

Figure 1: Bruker RFS 100 FT-Raman Spectrometer. The compartments identified by the letters A, B, C e D are, respectively, (A) Interferometer, (B) Control Electronics, (C) Sample Compartment and (D) Detector.

Results

The FT-Raman spectros obtained in this experiment are represented in Figure 2.

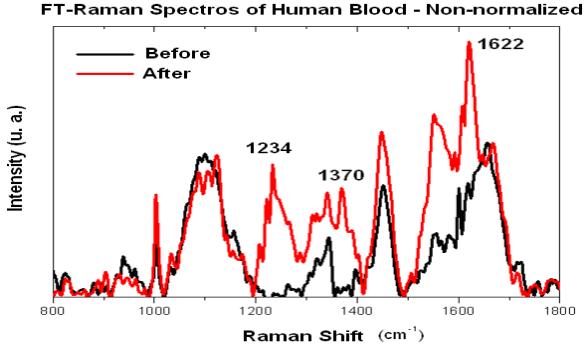

Figure 2: Non-normalized FT-Raman spectros of human blood taken before and after physical exercise.

Discussion

The 1234 pick point (Figure 2) corresponds to the Amide III group, which represents peptide bonds, connections between amino acids (MAHADEVAN-JANSEN, 1996; STONE, 2004). This pick represents an increase of amino acids in the blood stream. The picks points 1370 and 1622 (Figure 2) correspond to the radical CH_3 e to the radicals NH_2 and COOH (LIN-VIEN et al., 1991). Figure 3 shows the chemical structure of the Alanine.

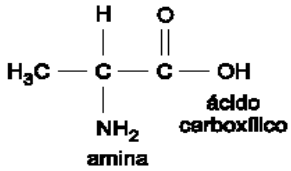

Figure 3: Structural Formula of the Alanine.

The picks clearly indicate a raise in the concentration of alanine in the blood stream after the anaerobic physical activity.

The presence of alanine in the blood stream and its immediate evaluation may be important to characterize a physical activity (aerobic or anaerobic), and specially to evaluate the loss of amino acids during this active.

Although new studies should be done, the intensities of the picks in Figure 2 are significantly high enough to determine that the athlete used amino acids to obtain energy during the exercise protocol.

Conclusion

Using the FT-Raman technique to determine the presence of biochemical substances in the blood is important to athlete's training methods because of its reduced invasiveness and its capacity to offer real time results. The FT-Raman spectroscopy proved to be, in this paper, efficient to identify the lost of amino acids during an anaerobic activity by showing a raise in the concentration of alanine in the blood stream.

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ANALYSES OF THE PRESENCE OF ALANINE IN THE BLOOD AFTER ANAEROBIC EXERCISE BY FT-RAMAN SPECTROSCOPY

Raman spectroscopy has been largely used in biology and biochemistry as a tool to study the structures and functional dynamics of important molecules, as for example the study of the presence of biochemical substances in the blood (BAYDEN e McNAUGHTON, 2001; PILOTTO et al., 2001; SATO et al., 2001;). Exercise has some immediate effects on the protein metabolism. Branched chain amino acids' oxidation can be an important source of energy to the muscles during anaerobic exercises. The presence of the alanine in the blood may indicate the use of amino acids for obtaining energy. The goal of this paper is to compare the presence of alanine in the blood before and after a high-intensity anaerobic exercise, using FT-Raman spectroscopy. It concludes that FT-Raman spectroscopy is efficient to determine and increase in the alanine concentration in the blood.

Keywords: FT-Raman Spectroscopy, Alanine, Anaerobic Exercise

ANALYSE PAR FT- RAMAN DE LA PRESENCE D'ALANINE DANS LE SANG APRES EXERCICE ANAEROBIQUE

La spectroscopie FT-Raman été utilisée principalement en biologie et en biochimie comme un outil pour étudier les structures et la dynamique fonctionnelle de molécules importantes, par exemple pour l'étude des composants du sang

(McNAUGHTON d'e de BAYDEN, 2001 ; PILOTTO et al., 2001 ; SATO et al., le 2001 ;). L'exercice a des effects immédiats sur le métabolisme des protéines. L'oxydation divergée des chaînes d'acides aminés de peut être une source importante d'énergie pour ie muscle pendant les exercices anaérobiques. La présence d'alanine dans le sang peut indiquer l'utilisation d'acides aminés pour obtenir l'énergie. Le bur de cetravail est de comparer la présence d'alanine dans le sang avant et après une un exercice anaérobique de forte intensité, par la spectroscopie FT-Raman. Il conclut que la espectroscopy FT-Raman est efficace pour déterminer la l'augmentation de la concentration d'alanine dans le sang.

Les mots réserve: la Spectroscopie de FT-Raman, Alanine, l'Exercice Anaérobique

ANALIZA DE LA PRESENCIA DE ALANINA EN LA SANGRE DESPUES DEL EJERCICIO ANAEROBIO POR LA ESPECTROSCOPIA FT-RAMAN

La espectroscopia FT-Raman se ha utilizado en gran parte en la biología y la bioquímica como un instrumento para estudiar las estructuras y dinámica funcional de moléculas importantes, como por ejemplo estudios de componentes de la sangre (BAYDEN E McNAUGHTON, 2001; PILOTTO et al., 2001; SATO et al., 2001;). El ejercicio tiene algunos efectos inmediatos en el metabolismo de la proteína. La oxidación de aminoácidos de cadena ramificada puede ser una fuente importante de energía a los músculos durante ejercicios anaerobios. La presencia del alanine en la sangre puede indicar el uso de aminoácidos para obtener energía. Este artículo tienes como objetivo comparar la presencia de alanina en la sangre antes y después de uno ejercicio anaerobio de alto-intensidad, por la espectroscopia FT-Raman. Concluye que la espectroscopia FT-Raman es eficiente para determinar uno aumento en la concentración de alanina en la sangre.

Las palabras clave: la Espectroscopia FT-Raman, Alanina, Ejercicio Anaerobio

ANÁLISE DA Presença de Alanina na corrente sangüínea APÓS ATIVIDADE Física ANAERÓBICA POR ESPECTROSCOPIA FT-RAMAN

Espectroscopia Raman vem sendo freqüentemente usada em biologia e bioquímica para estudar as estruturas e dinâmicas funcionais de importantes moléculas, como por exemplo, o estudo da presença de substâncias bioquímicas no sangue (BAYDEN e McNAUGHTON, 2001; PILOTTO et al., 2001; SATO et al., 2001;). O exercício físico possui alguns efeitos imediatos sobre o metabolismo protéico. A oxidação de aminoácidos de cadeia ramificada pode ser uma importante fonte de energia para o músculo em exercício anaeróbico. A presença da alanina na corrente sangüínea pode ser um indicador do uso de aminoácidos para obtenção de energia durante um exercício. O objetivo deste trabalho é comparar a presença de alanina no sangue de um atleta, antes e após exercício anaeróbico de alta intensidade, através da espectroscopia FT-Raman. Constatou-se que a espectroscopia FT-Raman foi eficaz em mostrar um aumento da concentração de alanina na corrente sangüínea.

Palavras chave: Espectroscopia FT-Raman, Alanina, Exercício Anaeróbico.