RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES
ISSN 2221-1896
www.roavs.com

Amino acid analysis and group function of camel insulin

A. E. A. Baragob[1], O. Y. Mohammed[2], A. F. Mustafa[2], S. M. E. Khojali[3] and H. A. Samia[3]

[1]Faculty of Pharmacy, Omdurman Islamic University, Omdurman, Sudan
[2]Faculty of Pharmacy, Khartoum University, Khartoum, Sudan
[3]Central Veterinary Research Laboratory, Khartoum Sudan

Abstract

In the current study, the claimed amino analysis and groups fraction of Sudanese camels' insulin was obtained by hydrolysis using amino acid analyzer. The result obtained indicated the presence of all amino acids except cystine and tryptophan which were not detected by this method due to destruction during acid hydrolysis.

Keywords: Camel, Insulin, Amino Acids

Introduction

Camel insulin found to be rich with amino acids, the most abundant essential amino acids are measured in direct sample were (Treonine, Valine, Inethioine, Isoleucine, Leucine, Phenylalanine, Histidine, Lysine and Arginine), compared to the other amino acids. However, Aspartic acid, Glutamic acid, Alanine and Glycine appeared to be the main non essential amino acid found in the insulin showed higher concentration than the other amino acids, (Richard et al., 1976; Kane and Rivers, 2000). The objective of this study was to analyze the amino acids of camel insulin and to determine the group functions.

Materials and Methods

Healthy local breed camels with aged 7-9 years and weight 500-600 kg were slaughtered in the slaughter-house to collect pancreatic glands. All chemicals and reagents used in the different stages of insulin extraction, purification amino acid dialysis, function group test and assay were of pharmaceutical or analytical grades. Amino acid analysis is a process to determine the quantities of each individual amino acid in a protein. There were four steps in amino acid analysis, but here we used the hydrolysis method. However, in study of determination of amino acid carried by amino acid analyzer in the preparation of sample by hydrlysates method, amino acid cystine and tryptophane were destroyed completely by this method (Richard, et al., 1976). A known amount of internal standard (norleucine), is added to the sample. Since norleucine dose not naturally occur in proteins, it is stable to acid hydrolysis and can be chromato-graphically separated from the protein amino acid. The molar amount of internal standard should be approximately equal to that of most of the amino acids in the sample. The sample, containing at least 1-5 moles of each amino acid (i.e10µ G of protein) is transformed to hydrolysis tube and dried under vacuum. The tube is placed in a vial containing 6 NGD and a small amount of phenol and the protein is hydrolyzed by the HCL vapors under vacuum. The hydrolysis is carried out for 65 minutes at 150°C. Following hydrolysis the sample is dissolved in distilled water containing EDTA (to chelate medallions).

Both standard and internal controls were analyzed with each sample. Sample preparation for insulin hydrlysates was carried out as follow. Two test samples weighing 0.04g were dissolved in 1 ml (6N Hd) Incubate at 110°C for 24 hours. Filtered the solution using 125 mm filter paper. Evaporated the filtrate at 140°C for about an hour. Added 1 ml of sample diluting buffer to the dried sample. Sample is now ready for analysis. The buffer used in this process was sodium citrate.

Results and Discussion

The quantity and peak of each amino acid in insulin was shown in table 1 and 2. In table (1) leucine concentration was the highest (703.975µg/100mg) followed by argnine (601.17µg/100mg) as essential amino acids. In case of non-essential amino acids glutamic acid was the highest (441.125µg/100mg) followed by aspartic acid (396.97µg/100mg). Cystine and tryptophan were not detected by this method, the

reason due to destruction during acid hydrolysis. In table (2) leucine concentration was the highest (871.875µg/100mg) followed by Argnine (823.023 µg/100mg) as essential amino acids.

In case of non-essential amino acids glutamic acid was the highest (696.137µg/100mg) followed by aspartic acid (558.05µg/100mg).

The Amino Acids Analysis uses a blood or urine sample to assess the levels of 40 amino acids. It provides the most precise measurement of the "essential" arnino acids, the ones we cannot make in our bodies and must get from nutritional sourees. In the case of protein such as Insulin, the free monomeric form can be studied only at very low concentration. However at presesnt there are conciderable technical dificultes in the study of ptotein at vey low concentratons by phisical methods. Our result showed that, the described modification to the competative labelling procedure make it possible to determine the chemical properties and, from these, to infer structural insulin (Harvey et al., 1995).

In many cases an exact knowledge of protein quantities is required for further protein chemistry applications. Analysis is the suitable tool for precise determination of protein quantites, but also provides detailed information relative amino acid composition and free amino acids. The relative amino acid composition gives a characteristic proteins, which is often sufficient for identification of a protein. It is often used as decision support for choice of protein fragmentation.

Table 1: characteristic of amino acid analyzer of test sample ($C_{1)}$ of insulin

Name of amino acid	Amino acid class	Concentration (µg/100mg)
Aspartic acid	NS	396.975
Threonine	ES	214.265
Serine	NS	129.65
Glutamic	NS	414.125
Glycine	NS	74.125
Alanine	NS	351.463
Cystine	No peak found NS	-
Valine	ES	339.325
Methionie	ES	64.313
Isoleucine	ES	316.965
Leucine	ES	703.975
Tgrosine	NS	87.225
Phenylalanine	ES	203.375
Histidine	ES	126.635
lysine	ES	260.1
Argnine	ES	601.17

NS: non-essential; ES: essential

Table 2: characteristic of amino acid analyzer of test sample (C_2) of insulin

Name of amino acid	Amino acid class	Concentration (µg/100mg)
Aspartic acid	NS	558.05
Threonine	ES	278.863
Serine	NS	178.537
Glutamic	NS	696.137
Glycine	NS	102.787
Alanine	NS	421.163
Cytine	NS: No peak found	-
Valine	ES	419
Methionie	ES	80.5
Isoleucine	ES	387.875
Leucine	ES	871.875
Tgrosine	NS	111.6
Phenylalanine	ES	259.237
Histidine	ES	146.6
lysine	ES	341.313
Argnine	ES	823.025

Table 3: Identification of functional groups in insulin samples using FTIR (Forier Transmittance Infra Red)

Band No	Position cm-1	Heights %	Type of Bands
1	3326.83	4.769	N-H stretching vibration
2	3162.42	4.843	O-H stretching vibration
3	1655.10	1.137	C=H stretching vibration
4	1611.09	2.675	C-O stretching vibration
5	1565.03	1.780	N-H bending vibration
6	1506.99	1.387	N-H bending vibration
7	1442.08	2.148	C = C stretching in benzene ring
8	1370.08	8.623	C = C stretching in benzene ring
9	1327.18	1.158	C = C stretching in benzene ring
10	1259.51	3.051	C - C stretching vibration
11	837.25	10.135	C –H bending vibration
12	808.34	11.086	Para sub-situation
13	685.74	13.114	Para sub-situation

References

Havrey, L., Berk, A., Ziqursky, S.L. Matsudaira, P., Baltimore, D. and Darnell, J. 1995. Molecular and cellular Biology, 3rd ed. New Yourk. Pp: 907-911.

Richard, J.S., Neuberger, M.R. and Llu, T.Y. 1976. Complete amino acid analysis of proteins from a single hydrolysate. *Journal of Biological Chemistry*, 251: 1936-1940.

Kane, R. and Rivers, K. 2000. Insulin: structure and function. Medline.Pp: 1-3.