C A L C I T O N I N

ESSAY

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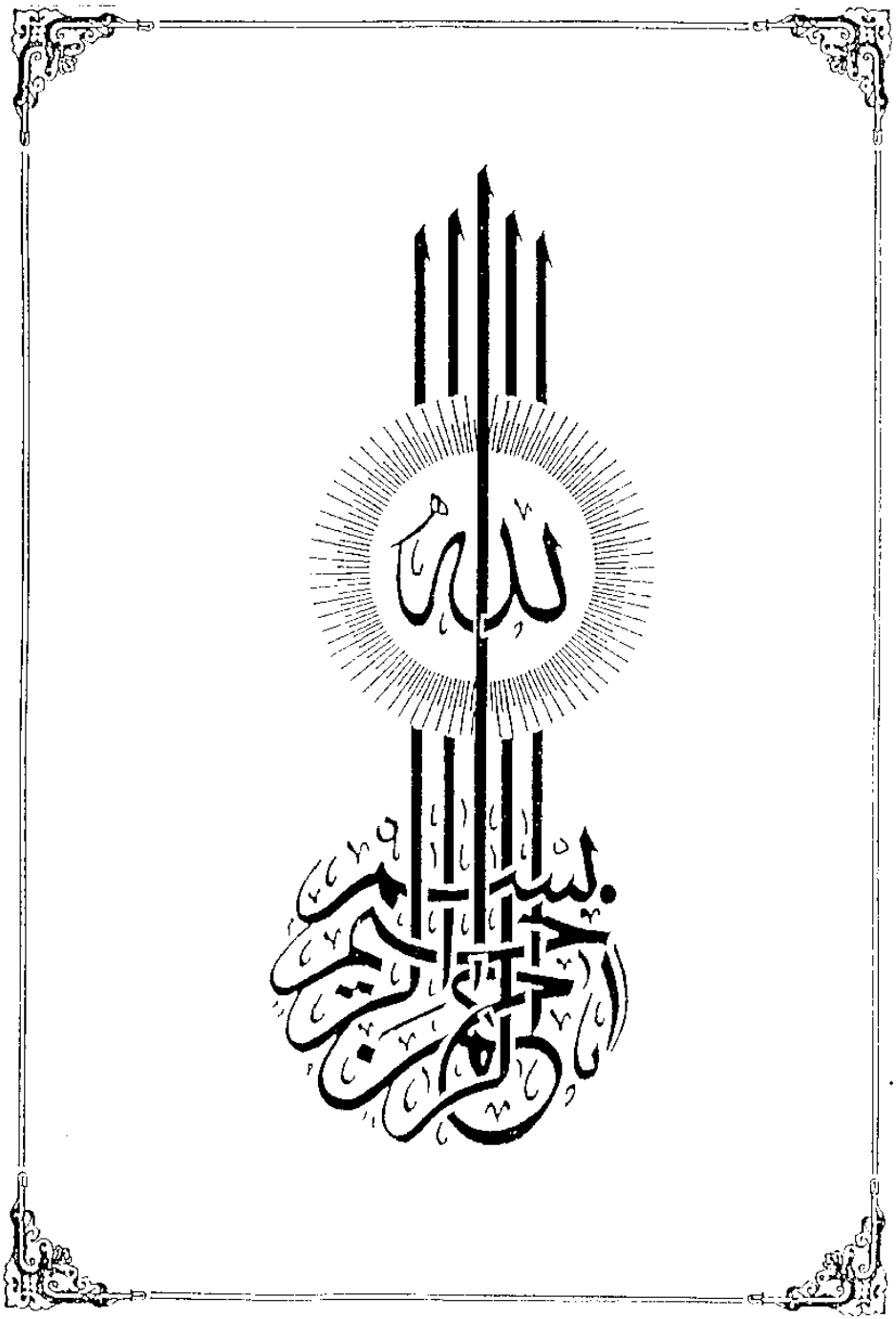
بسم الله الرحمن الرحيم

" وأنزل عليك الكتاب والحكمة وعلمك ما لم تكن
تعلم وكان فضل الله عليك عظيما "

صدق الله العظيم



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INTRODUCTION

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About 20 years ago, a new hormone that has effects on blood calcium opposite to that of parathyroid hormone was discovered in several lower animals and at first was believed to be secreted by the parathyroid glands.

This hormone was named calcitonin because it reduces the blood calcium ion concentration.

Soon after its initial discovery, it was found to be secreted in the human being not by parathyroid glands but instead by the thyroid gland, for which reason it has also been called thyro-calcitonin.

Still more recently it was discovered that calcitonin is secreted by the ultimobranchial glands of fish, amphibia, reptiles and birds and that it plays an especially important role in helping to control the blood calcium ion concentration when these animals change their habitat from fresh water to sea water, where there are great excesses of calcium.

Furthermore, its concentration in these ultimobranchial glands is extremely great.

In the human being, ultimobranchial glands do not exist as such but have been incorporated into the thyroid gland.

The so called parafollicular cells or (C) cells, in the interstitial tissue between the follicles of the human thyroid glands are remnants of the ultimobranchial glands of lower animals and it is these cells that secrete the calcitonin **(Guyton, A.C. 1981).**

Calcitonin was discovered through its hypocalcemic effects, subsequently shown to be due to inhibition of bone resorption.

These two effects of calcitonin provided the rationale for its use as a drug in diseases characterized by hypercalcemia and increased bone resorption.

Three major types of calcitonin can be defined by amino acid sequence patterns.

These are teleost calcitonin, artiodactyl calcitonin and human calcitonin.

For historical reasons, synthetic salmon calcitonin (Calcimar) is most widely used in the United States, whereas natural porcine calcitonin is widely used in Europe and synthetic human calcitonin in England.

This hypocalcemic effect may be difficult to show in normal human subjects but becomes evident in bone hyperresorptive states such as Paget's disease and metastases to bone **(Deftos, L.J. and First, B.P. 1981).**

CHEMISTRY OF CALCITONIN

CHEMISTRY OF CALCITONIN
(Fig. 1,2,3)

In most species including humans, calcitonin is a polypeptide of molecular weight 3600 (32 amino acids). In comparison with parathyroid hormone, the complete structure of calcitonin is required for biological activity, although there are variations in the amino acid composition occuring in different species **(Martin, D.W. et al., 1983).**

Human calcitonin joins an increasing number of small polypeptide hormones such as vasopressin, neurophysin, somatostatin, glucagon, melanotropin, adrenocorticotropin and endorphins which are synthesized as high molecular weight polyproteins, subsequently cleaved by proteolytic enzymes to the mature hormone form i.e., synthesized as precursor polyprotein **(Allison, J. et al., 1981).**

Calcitonin polypeptides have been isolated from at least nine different species and uniformly consist of a 32 amino acid polypeptide with an N-terminal 7-membered disulfide ring and C-terminus of prolineamide. The structures are remarkable in that as many as 19 of the 32 amino acids differ in the most diverse (Human versus ovine) forms of the polypeptide.

There are, however, a number of features common to the molecules.

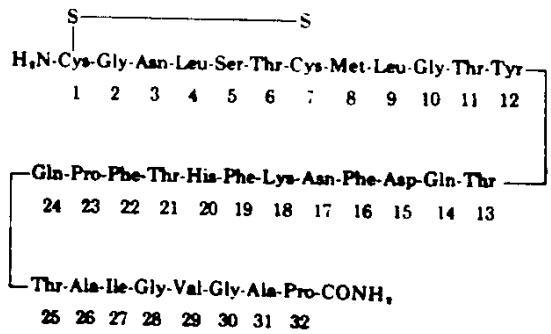

Fig. 1 Human Calcitonin
(Martin, C.R., 1976).

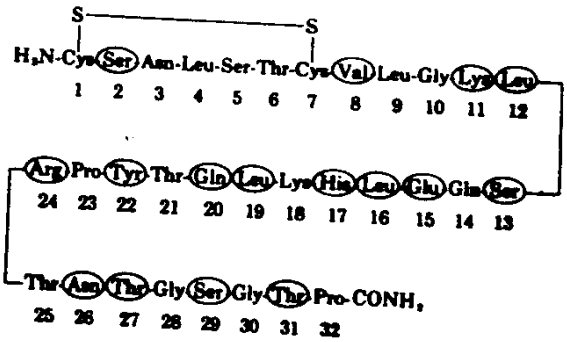

Fig. 2 Salmon Calcitonin
(Martin, C.R., 1976)

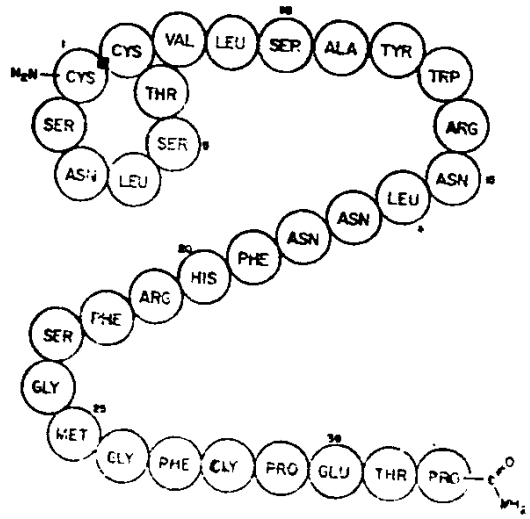

Fig. 3 Porcine Calcitonin
(Hirsch, P.F. and Munson, P.L., 1969)

In addition to the aminoterminal disulfide bridge and constant chain length terminated in prolineamide, six of the seven amino acid terminal residues are identical in all calcitonins and the sequence variability of the middle region of the molecule (residues 10-27) is more apparent than real.

Although no single amino acid in this region is constant in all of the known congeners, they show similar types of side chain function in this region.

An acidic residue (aspartic acid or glutamic acid) is found uniformly at position 15, and the only other acid residue is found at position 30.

Basic residues are also limited to a few positions. When substitutions for basic residues occur, asparagine or glutamine is the most common replacement.

Aromatic residues may exist at positions 12, 13, 16, 19, 22, or 27 but have never been found at the aminoterminal 11 residues.

All variants contain at least one aromatic amino acid, but some contain neither tryptophane or tyrosine.

The ovine molecule is unique in that it contains 3 tyrosines. The human and rat hormones are closely related in amino acid

sequence and also show a high degree of immunologic cross reactivity (**Williams, R.H., 1981b**).

The first correct report of the amino acid composition Arg 2, His, Asn 4, Thr 2, Ser 4, Glu, Pro 2, Gly 3, Ala, Val, Met, Leu 3. Phe 3, Tyr, Trp, Cys 2.

Porcine thyrocalcitonin has distinctive structural features in addition to the absence of isoleucine and lysine. The single tyrosine and tryptophan are adjacent. The proportion of charged amino acids is low. There is a 1-7 interchain disulfide bridge, providing a 23 membered ring at the amino terminus.

The carboxyl terminal amino acid is prolineamide.

Calcitonin from a single large medullary carcinoma of the thyroid gland has many differences in amino acid sequence e.g.

Arginine and tryptophan are absent whereas lysine and isoleucine are found at positions 18 and 27, methionine is found at position 8 (**Hirsch, P.F. and Munson, P.L., 1969**).

Structure function relationships:

The entire 32-amino acid chain appears to be required for significant biologic activity. Even the comparatively long fragments consisting of residues 10-32 or 1-10 joined to residues 20-32 (with ommision of the central nonapeptide) are inactive. In fact,

shortening the chain by ommision of even a few amino acids causes almost complete loss of biologic activity even if the C-terminal prolineamide residue is retained.

Methionine when located at position 8 immediatly adjacent to the heptapeptide ring, represents a site of potential inactivation through oxidation.

Conversion of the methionine to methionine sulfone at this locus destroys the biologic activity.

When methionine is located at position 25, oxidation does not alter biologic activity.

An acidic carboxyl function is not essential for activity and indeed substitution of asparagine for the aspartic acid at position 15 in bovine CT enhances biologic activity.

One possible explanation for enhanced potency of salmon CTI includes an increase in hydrophilicity, however, this property is not seen in salmon CTII or in eel CTs which are also potent.

It is perhaps also important to note that salmon CT shows the highest net positive charge of all CTs.

Deamination of the carboxyl-terminal proline (increase the negative charge) leads to reduced biologic activity. Areas of