HEMOLYTIC ACTIVITY OF BACITRACIN-A AND ITS DERIVATIVES

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INVESTIGATIONS of the relationships between biological functions and structure of proteins and peptides are feasible by chemical modification of reactive groups on these molecules. With peptide antibiotics, such studies can provide a basis for modifications in which the desirable activities are retained or enhanced and undesirable activities are suppressed or eliminated. Chemical modification studies from this laboratory, have been successful in establishing structure-activity correlations for linear gramicidins[1], and polymyxins[2].

Bacitracins are antibacterial peptides produced by certain strains of *Bacillus licheniformis*, the main component of which is bacitracin-A. Like the other polypeptide antibiotics, bacitracin-A is toxic, which limits its systemic use. Earlier studies[3,4] on bacitracin-A have revealed that (i) the single side chain amino group of D-ornithine residue is necessary for the antibacterial activity of the molecule, since acetylation, formylation, carbamylation and deamination of the antibiotic caused 90–92% loss of antibacterial activity of the molecule; (ii) the single imidazole nucleus of L-histidine residue is also important for the antibacterial activity of the molecule, since iodination, carboxymethylation and coupling of diazobenzene sulphonic acid to the histidine residue in the antibiotic caused 90–94% loss of antibacterial activity of the molecule; and (iii) the α- and γ-carboxyl groups of D-asparagine and D-glutamic acid residues of the molecule are not required for the antibacterial activity of the antibiotic, because esterification, amide formation and acid-chloride formation resulted in nearly 10% loss of the antibacterial activity of the antibiotic. Also, the bacitracin sulphone and sulphoxide derivatives are as active as the parent antibiotic. The present study deals with the hemolytic activity of bacitracin-A and its derivatives[5].

The bacitracin derivatives used in this study were those prepared earlier[3,4]. The assay for hemolytic activity of bacitracin-A and its derivatives was carried out according to the method of Dimick[6], where the rate of disappearance of absorption at 660 nm in washed suspensions of red blood cells from the rat was measured. The decrease in optical density for various time intervals for various concentrations of the antibiotic derivative was plotted on graph paper to yield a family of curves. The percentage hemolytic activity for the modified antibiotic is derived by comparison of the relevant slopes of the plotted curves for 2 min of hemolysis. Where hemolytic activity was low, higher concentrations of modified antibiotic were used for the assay.

The results on the hemolytic activity of bacitracin and its derivatives are shown in table 1. Our studies have emphasized the essentiality of the single sulphur atom in the thiazoline ring of bacitracin-A in its hemolytic activity, since the oxidation of the sulphur atom to the level of sulphoxide and sulphone resulted nearly 97% loss of the hemolytic activity, while retaining about 95% of the antibacterial activity of the parent antibiotic. Also, the free α- and γ-carboxyl groups of D-asparagine and D-glutamic acid residues of bacitracin-A are important in its hemolytic activity of the molecule, because bacitracin methyl glycolate, bacitracin anilide and bacitracyl chloride have lost nearly 91% of the hemolytic activity, while retaining nearly 90% of the antibacterial activity of the parent antibiotic. On the other hand, amino group modified bacitracins and histidine residue modified bacitracins have retained nearly 80–92% of the hemolytic activity, while having lost nearly 95% of the antibacterial activity of the parent antibiotic. The hemolytic activity of bacitracin-A is nearly 30% compared to the hemolytic activity of gramicidin-A.

Table 1 *Hemolytic activity of bacitracin and its derivatives.*

Sample	Hemolytic* activity (%)
Bacitracin	100
Formyl bacitracin	88
Acetyl bacitracin	92
Carbamyl bacitracin	78
Deamino bacitracin	90
Iodo bacitracin	82
Carboxymethyl bacitracin	88
Diazobenzene sulphonic acid coupled bacitracin	92
Bacitracin methyl glycolate	11
Bacitracin anilide	9
Bacitracyl chloride	12
Bacitracin sulphone	4
Bacitracin sulphoxide	7

* Average of three experiments.

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A NOTE ON THE DIFFERENTIATION OF ORE BODIES EXHIBITING SIMILAR ANOMALY PATTERNS

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ORE bodies which can be approximated to single poles and thin sheets exhibit similar anomaly shapes. In such a situation it causes ambiguity in deciding the actual geometrical disposition of the ore body. Any magnetic anomaly can be split into symmetric and asymmetric components. The plot of the ratio of the symmetric and asymmetric components, versus the distance x in both the cases gives a straight line. In such a circumstance, another alternative procedure is to be adopted to discern the nature of the source of the magnetic field. A method is suggested to determine whether the anomaly is due to a single pole or a thin sheet.

The analytical expressions for the total field anomaly due to a single pole and a thin sheet are given as follows:

$$\Delta T(X)_{sp} = m.f(x).\ \sin(i-\phi(x)) \tag{1}$$

$$\Delta T(X)_{TS} = M.F(x)\ \sin(Q-\phi(x)) \tag{2}$$

where $f(x) = (x^2+h^2)^{-1}$, $F(x) = (f(x))^{1/2}$, $\phi(x) = \tan^{-1}(x/h)$, $M = A.b$, $A = 2KTb$, $b = (1-\cos_i^2\cos^2\alpha)^{1/2}$, $Q = 2I-\delta$, and $I = \text{arc tan}(\tan i/\sin\alpha)$

In this, i is the goemagnetic field inclination, δ dip of the body, α is the strike angle measured clockwise w.r.t. the magnetic north. K is the susceptibility contrast and T is the base level of the total magnetic field intensity. These are shown in figure 1.

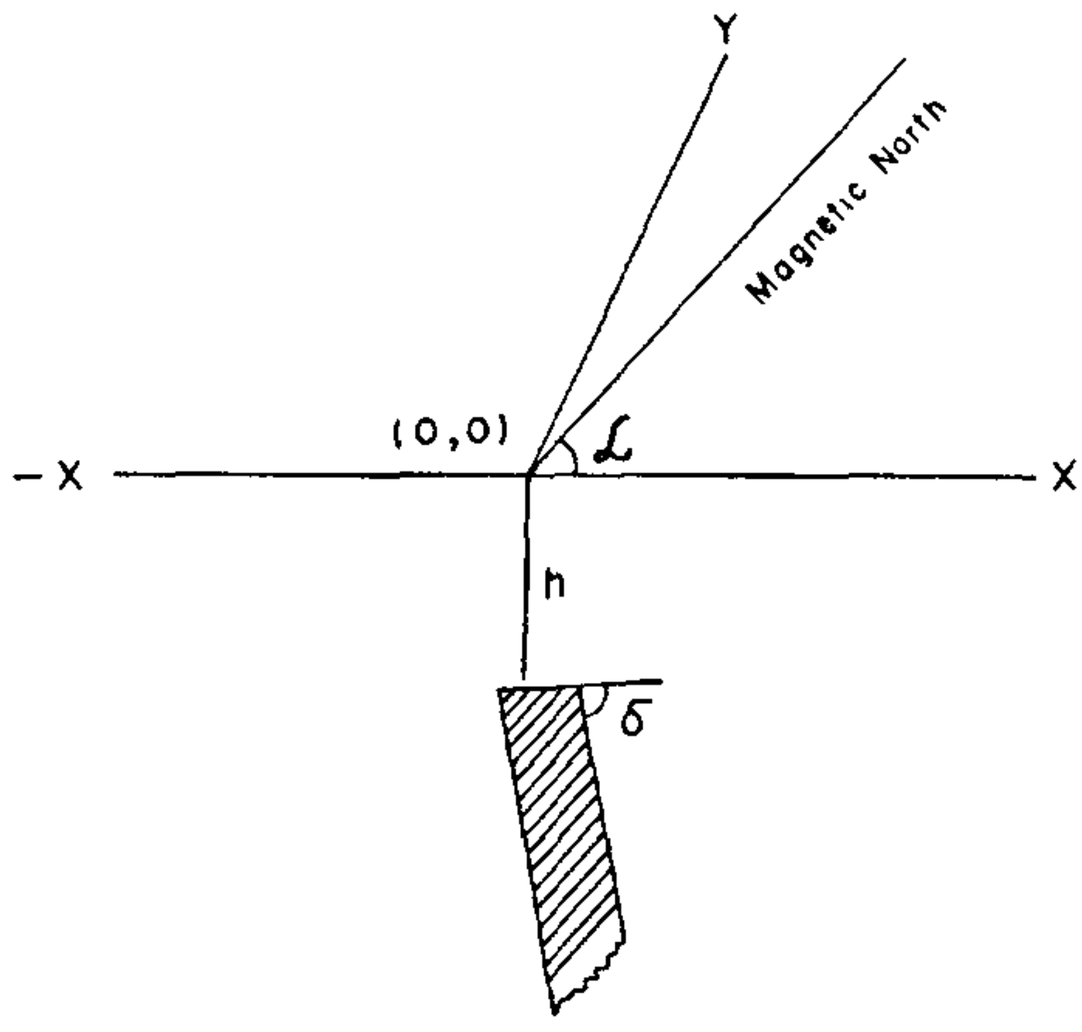

Figure 1. Cross sectional view of a Thin Sheet.

The ratio of the symmetric and asymmetric components in the case of single pole and thin sheet define straight line with differnt slopes.

$$Y_{sp} = (h/x)\tan i \tag{3}$$

$$Y_{TS} = (h/x)\tan Q. \tag{4}$$

Even these two identities do not result in identification of the individual sources.

The horizontal and vertical derivatives of the anomalies in both the above cases can be written as follows. In the case of single pole,

$$\Delta T_H = m.f(x).\ (3\cos(2\phi(x)-i)-\cos i) \tag{5}$$

$$\Delta T_V = m.f(x).\ (3\sin(2\phi(x)-i)-\sin i) \tag{6}$$

where $f(x) = (x^2+h^2)^{-3/2}$ and $\phi(x) = \tan^{-1}(x/h)$. In the case of a thin sheet

$$\Delta T_H = M.F(x).\ \cos(\phi(x)+Q) \tag{7}$$

$$\Delta T_V = M.F(x).\ \sin(\phi(x)+Q) \tag{8}$$

where $F(x) = (x^2+h^2)^{-1}$; $\phi(x) = \tan^{-1}\left(\dfrac{2xh}{x^2-h^2}\right)$

In the case of thin sheet, the resultant plot of ΔT_H and ΔT_V is a cardioid satisfying the equation of the form $(m^2+n^2-am)^2 = a^2(m^2+n^2)$, where $m = F(x).\cos\phi(x)$, $n = F(x)\sin\phi(x)$ and $a = (M/2h^2)$. But in the case of single pole it is not a cardioid.

Two theoretical examples have been shown in figures 2 and 3. The horizontal derivative can be calculated easily by the relation: $\Delta T_H = (\Delta T(x+\Delta x)$