Other Activities

6

6.1. Introduction:

Several biophysical studies have indicated that host defense antimicrobial peptides interact with model membranes with preference for anionic lipids (Matsuzaki et al., 1989; Matsuzaki et al., 1991; Cruciani et al., 1991; Sitaram and Nagaraj, 1993; Liu and Deber, 1997). Differential scanning colorimetry studies suggest that these peptides have an effect on bilayer organization (Lohner and Prenner, 1997; Lohner et al., 1997). Enantiomeric peptides of cecropins and magainins, composed of all D-amino acids have exhibited the same potency as naturally occurring L-peptides indicating that chiral recognition is not involved in antimicrobial action (Bessale et al., 1990; Wade et al., 1990). This would rule out the involvement of protein "receptors" in the membrane binding and permeabilization process.

The cytotoxic profiles of host-defense peptides can be broadly categorized into following types- i) peptides that are cytotoxic only to
bacteria. Examples include cecropins (Steiner et al., 1981), magainins (Zasloff, 1987), dermaseptins (Mor et al., 1991) and several other peptides isolated from amphibians; ii) peptides selectively cytotoxic to mammalian cells such as δ-hemolysin isolated from *Staphylococcus aureus* (Dhople and Nagaraj, 1993); iii) peptides that are not cell selective. Examples include the bee venom melittin (Habermann and Jentsch, 1967) and the neurotoxin pardin (Lazarovici et al., 1986; Shai et al., 1988). Several host-defense antibacterial peptides like indolicidin (Subbalakshmi et al., 1996) and brevenins also exhibit hemolytic activities at concentrations comparable to their antimicrobial activities. Several “designed” peptides with antibacterial activities (Blazyk et al., 2001) also have hemolytic activities and there have been several efforts to engineer peptides to have selective antibacterial activity (Sitaram and Nagaraj, 1990; Sitaram et al., 1992). Since defensins exert their activities *in vivo* in the milieu of tissues, it was of interst to examine the cytotoxic effects of defensins and their analogs against a variety of eukaryotic cells. Defensins have been reported in the saliva of patients with oral cancer or diseases (Mizukawa et al., 1998; Mizukawa et al., 1999). Activities against transformed cells were investigated as there have been reports that magainins selectively lyse cancerous cells in addition to possessing antibacterial activity (Ohsaki et al., 1992; Baker et al., 1993). In this chapter, results of studies on the cytotoxicity profiles of α-defensin HNP-1 and β-defensin BNBD-12 and their analogs against mammalian cells such as erythrocytes and various cancerous as well as non-cancerous cell lines are described. The different cell lines used in this study are – COS-1 which is a fibroblast like cell line (SV40 transformed African Green monkey kidney); K562, a human leukaemia cell line; HeLa, a human cervical carcinoma cell line and BC8, a well established rat histiolytic cell line (Khar A, 1986) of this laboratory.
6.2. Hemolytic activity:

HNP-1 and its analogs were tested for their lytic activity against human and rat erythrocytes as described in Experimental Procedures, section 2.11. The data is presented in Fig.6.1. The activities were marginal against the human erythrocytes except for HNP-1'. However, against rat erythrocytes all the peptides, except cy21, showed nearly 45% lysis. The single disulfide bridged analog cy21 showed marginal activity against both rat and human red blood cells. Mellitin has been included for comparison. The peptide concentrations at which hemolytic activities were determined (shown as ball and sticks) are much higher than the lethal concentrations observed against bacterial cells, *E.coli*.

For BNBD-12 and analogs, hemolytic activities were determined against human erythrocytes at lethal and twice lethal concentrations. The data is shown in Fig.6.2. All the peptides demonstrated concentration dependent activities. Maximum activity (-15%) was observed for cy28' the non-native two disulfide analog, followed by non-native BNBD-12' and native BNBD-12. The single disulfide containing peptides cy26 and cy22 exhibited very little hemolytic activity, < 2%.

6.3. Activities against transformed cells:

The activities of α-defensin HNP-1, β-defensin BNBD-12 and their analogs were tested against various cell lines in presence and absence of 10% FCS. Cytotoxicity was assessed by MTT colorometric assay as described in Experimental Procedures, section 2.12. Peptide concentrations corresponded to their lethal doses observed against bacterial cells, *E.coli*. The data for HNP-1 and analogs is shown in Fig.6.3. The peptides exhibited varying extent of lysis which was substantially less than melittin. The single disulfide containing peptide cy21 showed
Fig. 6.1. Hemolytic activity of HNP-1 and analogs

HNP-1  HNP-1'  cy26'  cy21  Mellitin

% hemolysis

Human RBC  Rat RBC  concentration (µM)

concentration (µM)
Fig. 6.2. Hemolytic activity of BNBD-12 and analogs

![Graph showing hemolytic activity of BNBD-12 and analogs. The x-axis represents different compounds (BNBD-12, BNBD-12', cy 28, cy 28, cy 26, cy 22, Mellitin) and the y-axis represents % hemolysis. The graph includes data for % hemolysis at lethal concentration and % hemolysis at twice lethal concentration, as well as lethal concentration (μM).]
Fig. 6.3. Cytotoxicity of HNP-1 and analogs against various cell lines

- COS 1 cell line
- K562 cell line
- HeLa cell line
- BC8 cell line

- % cytotoxicity
- DMEM + 10% FCS (blue)
- DMEM (red)
maximum activity against K562 and HeLa cell lines, but low activity against BC8, in absence of FCS. However, the non-native two disulfide containing analog cy26' and non-native three disulfide analog HNP-1' as well as native HNP-1 showed 20-30% activity. In all the cases, FCS seemed to provide protection to the cells against the damaging effect of peptides as evident by their decreased activities. However, against non-cancerous COS-1 cell line, all the peptides showed minimum activity irrespective of the presence of serum.

The data for β-defensin BNBD-12 and analogs are shown in Fig. 6.4. The single disulfide analog cy22 was active against all the cancer cell lines tested. Another single disulfide analog cy26 showed activities comparable to cy22 except in BC8, where the activity was low. The non-native two disulfide containing peptide cy28' showed slightly lower activity as compared to cy28. BNBD-12' with non-native disulfide connectivities exhibited pronounced cytolytic activities against K562 and BC8 cell lines as compared to BNBD-12. In case of β-defensins too, diminished activity was observed in presence of FCS. Against COS-1 cell line, all the peptides exhibited lower activity as compared to cancer cell line.

6.4. Discussion:

The bacterial and mammalian cell membranes differ in the composition and topological arrangement of lipids (Graham and Higgins, 1997). Bacterial membranes generally contain high amounts of phosphatidylglycerol (PG) and cardiolipin (CL) moieties whereas the outer leaflet of the mammalian cell membrane is rich in zwitterionic lipids with the anionic lipids like phosphatidylserine (PS) being asymmetrically distributed to the inner leaflet. Thus, the surface of the lipid bilayer is anionic whereas the outer leaflet of the lipid bilayer in mammalian cells is zwitterionic. Tumour cells, on the other hand appear to have
Fig. 6.4. Cytotoxicity of BNBD-12 and analogues against different cell lines

COS 1 cell line

K562 cell line

Hela cell line

BC8 cell line

DMEM + 10% FCS

DMEM
lost part of their lipid asymmetry and exhibit a more anionic character at the outer leaflet (Utsugi et al., 1991). This could enable the cationic peptides to selectively bind the anionic membranes of bacterial as well as tumour cells, owing to charge-charge interactions. The selective toxicity of linear antibacterial peptides towards cancer cells have been explained based on these differences in lipid composition (Matsuzaki et al., 1995; Blondelle et al., 1999).

The technique of differential scanning calorimetry have been used to study the effects of the human neutrophil peptide, HNP-2 (Lohner et al., 1997) on the thermotropic behaviour of liposomes mimicking bacterial and erythrocyte membranes. The study revealed that addition of HNP-2 to liposomes mimicking bacterial membranes i.e., PE/PG mixtures resulted in phase separation into peptide-rich and -poor domains. On the other hand, HNP-2 did not affect the phase behaviour of membranes mimicking erythrocyte membranes (equimolar mixtures of PC and SM) indicating preferences for anionic lipids.

Defensins and analogs in addition to possessing antibacterial activity can also lyse cancer cells. There appears to be selectivity towards cancer cells as erythrocytes and COS cells are not lysed. In particular, the analogs with a single disulfide bridge can discriminate efficiently between a healthy and a tumour cell in vitro, and at the same time exhibits a high antimicrobial potency. There had been studies indicating that defensins may also interact with components of adaptive immunity as they have chemotactic activities for monocytes (Territo et al., 1989) and T cells (Chertov et al., 1996) which would help in recruiting these cells at the site of infection. It would be of interest to examine if the shorter single disulfide bridged peptides have these activities. Peptides with multiple biological activities, without toxic effect on normal cells would be good lead candidates for use as therapeutic agents.