Antibiotics from within: antibacterials from human and animal sources

Peter Elsbach

The isolation of potent antibacterial proteins and peptides from human and animal phagocytes has raised the possibility that these 'antibiotics from within' can be used as therapeutic agents. Problems in delivery to and toxicity towards the host must be faced, but rapid progress in defining the structural determinants of the cytotoxic action of these naturally occurring cytotoxins provides a basis for biotechnological development of new classes of antibiotics.

All living organisms exist in an environment teeming with potentially harmful microbes. Nevertheless, there are only relatively few major disruptions (epidemics) in the natural balance between a given animal species and its microbial pathogens, and this reflects a highly effective host-defense system against serious infections. In humans, this is also evident from the fact that most of us escape major infectious disease, after early childhood and before the last stages of our lives, if our resistance is not impaired by other illness.

During the past decade, proteins and peptides that are potent antimicrobial agents have been isolated in pure form from multicellular organisms, including man. These 'antibiotics from within' bear little or no structural resemblance to the commercial available antibiotics. In this review I consider:

- the salient structural and functional features of the best-defined of these naturally occurring antimicrobial proteins and peptides; and
- the prospects for their use as pharmacological agents, or as model compounds upon which the design and construction of new classes of antibiotics can be based.

Endogenous antimicrobials in the context of host defense

Endogenous antimicrobials operate in an extraordinarily complex setting of integrated anti-infection host-defense, consisting of both cellular and extracellular (humoral) systems (broadly categorized in Table 1).

Among the cellular elements are the phagocytes, cells that have a highly developed endocytic apparatus, enabling the host to sequester foreign invaders intracellularly in phagocytic vacuoles. These vacuoles fuse with cytoplasmic granules (Fig. 1), which store a broad mix of bioactive substances, mostly cytotoxic proteins and peptides, and digestive enzymes that contribute to the destruction of ingested microorganisms. In higher organisms, different types of phagocytes can be distinguished; polymorphonuclear leukocytes (PMN), many types of macrophages and eosinophils.

One component of the granule-associated proteins that has little or no cytotoxic activity by itself, but that plays a major role in the phagocyte's antimicrobial functions is myeloperoxidase (MPO). MPO amplifies the effects of the cytotoxic O_2-derivatives produced during the respiratory burst that accompanies phagocytosis, presumably by aiding their delivery and that of Cl^- or other halide ions to target cells with binding affinity for MPO (Ref. 1). In addition, the MPO-mediated formation of highly reactive hypohalous acids creates a toxic environment.

Two other essential elements of defense against infection are parts of the immune systems and are expressed in the circulating fluids:

- antibodies contribute greatly to the specificity of the phagocytic cellular response;
- the components of the complement system that produce protein mediators which direct mobile phagocytes to their targets, promote phagocytosis (opsonization) and form a cytotoxic complex that participates in microbial destruction.

Table 1

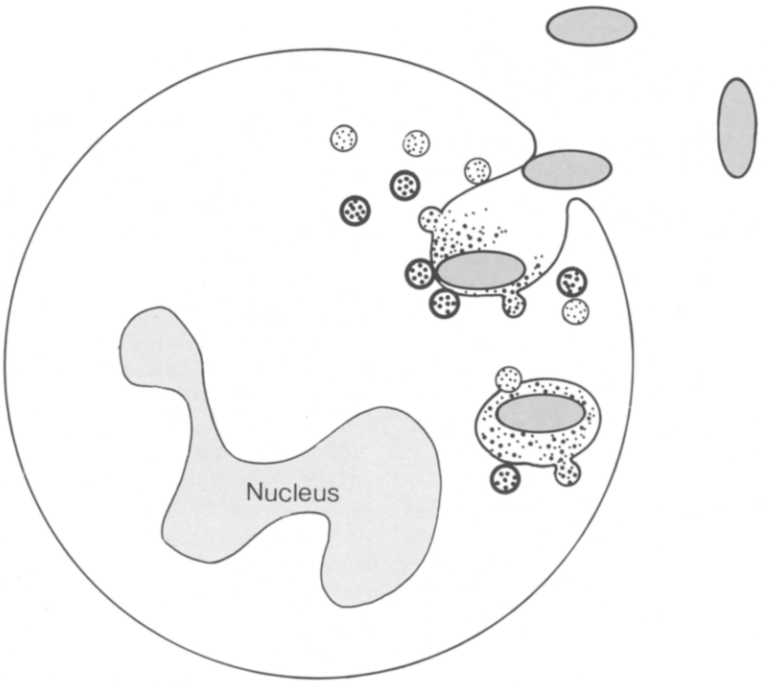
Host defense against microbial invaders

Main components	Equipment	Function
Phagocytes	Endocytic apparatus	Intracellular sequestration
Polymorphonuclear leukocytes (PMN), Macrophages, Eosinophils	Respiration producing toxic O_2-derivatives; Proteins and peptides stored in granules	Microbicidal action
Polymorphonuclear leukocytes (PMN), Macrophages, Eosinophils	Granule-associated degradative enzymes; Activation of microbial degradative enzymes	Microbial disassembly
Lymphocyte system	Immunoglubulins, Perforins	Recognition cell-mediated immunity
Cells producing complement components	Complement cascade	Amplification or modulation of host defense (including phagocyte function)
Cells producing other mediators	e.g. Interleukins	

P. Elsbach is at the Department of Medicine, New York University School of Medicine, New York, NY 10016, USA.

 0167 – 9430/89/$2.00

Fig. 1



Phagocytosis and the role of the phagocyte's granules. Ingested bacterial invaders, ⬭ are enclosed in phagocytic vacuoles. The vacuoles fuse with cytoplasmic granules which contain cytotoxic proteins and digestive enzymes. Various types of granules may be present, including primary (azurophil) granules, ◉ and specific granules, ◌ .

Finally, the functions of this defense network are further modulated by messengers such as the interleukins, tumor necrosis factors, interferons and other signals.

One corollary of the complexity of these interactive antimicrobial defense systems is that the action(s) of an isolated component *in vitro* need not reflect a similar role *in vivo*, where biological effects may be the outcome of competition, potentiation or inhibition among many factors.

Despite this caveat, enough has now been learned about the structure and function of a range of naturally occurring antimicrobial proteins and peptides to justify exploring the application of these endogenous antibiotics as pharmacologic agents.

Cytotoxic (antibiotic) proteins and peptides have been isolated from organisms all along the evolutionary scale. This seems to imply an important defensive/offensive function. However, the size, structure and effects of the many proteins and peptides are so variable that common molecular determinants of cytotoxic action have not become apparent (except that amphipathy may bear on the membrane-perturbing effects of many, if not all, cytotoxins). Some of the agents show very narrow target-cell specificity, and many are indiscriminate in their cytotoxicity.

I will now review, in the order of the degree of their molecular characterization, the defined proteins and peptides isolated from animal sources that can be viewed as antibiotics from within. More comprehensive reviews have appeared recently[2,3].

Defensins

Like all the phagocyte's antimicrobial proteins and peptides, the defensins are stored in the cytoplasmic granules where they are, by far, the most abundant protein. The defensins are a highly conserved family of peptides; they have an M_r <4000 and have been isolated from PMN of humans, rabbits, guinea pigs and rats, and from the lung macrophages of rabbits[4]. The amino acid sequences of eleven of these basic, arginine- and cystine-rich compounds have been determined. The cDNA and genomic sequences of two human and two rabbit defensins have been reported recently[5,6]. One of the rabbit PMN defensins has been crystallized[7] and the (unusual) disulfide array in a human PMN defensin has been determined, revealing a cyclic structure[8]. The three disulfides, one arginine and a glycine in the primary structure are shared by all defensins.

The defensins vary in their potency and breadth of cytotoxicity, both increasing with higher arginine content. They are toxic at relatively high concentration (10^{-5} M) *in vitro* towards bacteria (both Gram-negative and Gram-positive), fungi, some viruses, and mammalian cells. Their toxicity is highest at neutral pH and at low salt concentrations. However, physiological salt concentrations are inhibitory, raising questions about their action *in vivo*. Because the defensins are so abundant in the PMN and the lung macrophages (the only cells known to carry them), it has been proposed that the high concentrations needed for their action do not contradict their making an important contribution to the antimicrobial function of these cells. The antibacterial action of the defensins, on *E. coli* at least, apparently rests on their interaction with both outer and inner envelope layers[9].

Very recently, it has become evident that the defensins or related molecules are not confined to myeloid cells, but are also present in other cells, including the Paneth cells of the small intestinal crypts[10]. This is interesting for at least two reasons. First, this is not the first demonstration of the presence of antibacterial peptides in non-myeloid cells: peptides called magainins were identified in frog skin and these form part of an epithelial barrier against microbial invaders[11]. Second, there is growing evidence that the functions of defensin-like molecules are not limited to antimicrobial action[10].

The broad cytotoxicity of the defensins (extending to the host's own cells) may limit their potential use as pharmacological agents. The construction of biologically active defensin analogues may be complicated by the strict dependence of activity on the proper alignment of the disulfide bridges[8].

Bactericidal/Permeability Increasing protein (BPI)

BPI is a 50–60 kDa basic (pI > 9.6), lysine-rich, apparently non-catalytic protein which has been purified from

human and rabbit PMN. The two BPI variants have very similar structures and functions. The human BPI gene has been cloned and the amino acid sequence of the molecule is now known[12]. In combination with fragmentation of human BPI by limited proteolysis[13], the initial dissection of BPI into functional domains has now been completed. BPI appears to be encoded by a single gene that is expressed only in cells of the myeloid series (i.e. precursors of PMN)[2,12].

On a molar basis, BPI is the most potent of the known mammalian cytotoxins. Its target cell specificity is also remarkable: BPI inhibits the growth of numerous Gram-negative bacterial species as soon as the protein is added *in vitro* but seems to have no damaging effects on Gram-positive bacteria or eukaryotic cells. This cytotoxic specificity of BPI is due to its high binding affinity for the outer envelope lipopolysaccharides (LPS) which are unique to Gram-negative bacteria. This preferential binding of BPI to LPS of intact *E. coli*, which essentially excludes the binding of the other cationic granule proteins in crude PMN extracts, has allowed us to purify BPI in a single-step process. LPS-bound BPI can then be (roughly) quantitatively eluted with high concentrations of Mg-salts[14].

The actions of BPI can be divided into two categories: nearly immediate, sublethal (reversible, i.e. bacteriostatic) effects and later, irreversible growth arrest (i.e. bactericidal effects)[15]. The former category includes discrete outer envelope alterations (increased permeability to normally impermeant hydrophobic substances, and selective activation of bacterial enzymes hydrolysing envelope phospholipids and peptidoglycans) and cessation of growth. The transition from reversible to irreversible growth arrest correlates closely with the impairment of biochemical functions that are linked to an intact cytoplasmic (inner) membrane[15].

All these biological effects of BPI are reproduced by a 25 kDa N-terminal fragment. The C-terminal portion of BPI is devoid of antibacterial activity, but may serve as an anchor into the granule membrane (BPI is more tightly bound to the membrane than other granule proteins)[16].

The action of BPI on its target cells is strikingly similar to that of intact PMN. This, and other findings, suggests that BPI has a prominent role in the antimicrobial functions of PMN against BPI-sensitive, Gram-negative bacteria[2,3,17].

Other BPI-like proteins have been isolated from human PMN, though it is unclear whether these are BPI or distinct proteins (reviewed in Refs 2 and 3).

Antimicrobial proteins of eosinophils

The chief function of eosinophils is in the defense against multicellular parasites such as helminths. Two extremely cationic proteins, Major Basic Protein (MBP) and Eosinophil Cationic Protein (ECP), are considered to be critical for this role.

Major Basic Protein

MBP is another arginine and cystine-rich basic (pI > 10) small protein ($M_r \sim 13$ kDa) that comprises almost half of the protein in the large specific granules of human, guinea pig, horse and rat eosinophils. Two of the six cystine residues have free sulfhydryls that apparently are not involved in biologic activity. The amino acid sequence of MBP has been determined both by direct analysis and from the nucleotide sequence of cDNA clones[18,19]. At high concentrations (10^{-4} M–10^{-6} M) *in vitro*, MBP is toxic to many helminths, and also to mammalian cells. Contrary to previous reports, MBP also inhibits bacterial growth (*E. coli* and *Streptococcus faecalis*)[20,21]. The nature of the toxic effects on parasites is unknown, but includes envelope damage that develops slowly (up to several days). Generally speaking, the anti-parasitic action of MBP *in vivo* may not cause host-tissue damage because the eosinophil delivers the protein very locally, to ingested or attached target organisms. On the other hand, in localized or general states of hypereosinophilia, immunoreactive MBP is abundantly present in various tissues and this could cause host-tissue damage.

Eosinophil Cationic Protein

ECP is an arginine-rich Zn-containing protein, the carbohydrate content of which varies, giving rise to heterogeneity in apparent M_r (17–21 kDa) during SDS-PAGE electrophoresis. The cytotoxicity of ECP towards parasites and mammalian cells is evident at concentrations as low as 10^{-7} M and is manifested as membrane alterations that are more discrete than those caused by MBP[21,22]. No antibacterial activity of ECP has been reported. One striking feature of ECP and of a related eosinophil neurotoxin is its RNAse activity and the corresponding structural homology with RNAses from various sources[23,24]. However, the demonstration of prominent channel-forming activity of ECP towards artificial and natural membranes[25] leaves the biological implications of the nuclease activity uncertain.

Lysozyme

This well-characterized and abundant ~ 14 kDa protein, capable of digesting bacterial peptidoglycans, is a major constituent of the granules of phagocytes. By itself, lysozyme can only reach its substrate in the cell walls of non-pathogenic Gram-positive bacteria. Whether lysozyme contributes greatly to the destruction of ingested pathogens as part of the complete antibacterial apparatus of the PMN is not clear. The absence of lysozyme in some animal species is not associated with decreased resistance to infection.

Bactenecins

A new group of cationic bactericidal polypeptides, the bactenecins, has been isolated from the granules of bovine PMN[3,26]. They occur only in myeloid cells and, so far, have only been found in cows. The first one isolated was a dodecapeptide with four arginines, six hydrophobic residues and a disulfide bridge between the two cysteine residues. Two additional arginine-rich (20%) polypeptides (5 kDa and 7 kDa; called Bac5 and Bac7) have been purified and are unrelated to the dodecapeptide. Both Bac5 and Bac7 contain an extraordinarily high proline content (> 45%) but, although their amino acid composition is similar, their amino acid sequences differ substantially[3]. Bac5 and 7 are both synthesized as higher M_r pre-propeptides.

The dodecapeptide kills both Gram-negative and Gram-positive bacteria *in vitro* at 10^{-5}–10^{-6} M,

resembling the defensins in potency. Bac5 and 7, in a dose range of 1–50 $\mu g\,ml^{-1}$ apparently kill only Gram-negative bacteria. Growth inhibition may be due to cytoplasmic membrane damage that is manifested as decreased rates of respiration and macromolecular synthesis within 5 minutes[3]. Bac7 neutralizes herpes simplex virus, but not a rhinovirus[3,26].

Cathepsin G

The granules of phagocytes contain substantial amounts of different proteases (M_r 24–30 kDa range) with distinct substrate specificities. One of these, cathepsin G exhibits relatively weak antibacterial activity that is independent of the protease activity (reviewed in Refs 2 and 3). The amino acid sequence of cathepsin G has been determined from isolated cDNA[27]. Its rather broad antibacterial activities (against Gram-positive as well as Gram-negative bacteria, including gonococci) may be mediated by cytoplasmic membrane damage[2,3]. Recent evidence[30] indicates that cathepsin G also alters mammalian cells.

Lactoferrin

The gene of this 80 kDa iron-binding protein has recently been cloned[28]. In contrast to most of the antibacterial proteins of PMN that are located in the azurophil granules, lactoferrin is associated with the specific granules (Fig. 1). Its roles in host defense against infection appear complex because it may inhibit bacterial growth both by competing for iron, an essential bacterial growth factor, and also by a mechanism independent of its iron-binding properties. Further, lactoferrin may also act by modulating PMN function (reviewed in Ref. 2).

Azurocidin

Very recently, a novel major 29 kDa protein constituent of azurophil granules of human PMN, azurocidin, has been purified[29]. It is distinct from previously identified proteins in its N-terminal sequence. It resembles BPI in its preferential cytotoxicity towards Gram-negative bacteria (*E. coli*), but its specific activity is less by 10-fold[29]. Azurocidin also shows growth inhibitory activity toward *S. faecalis*, but only under conditions of low ionic strength.

Towards pharmacological agents?

What does this brief synopsis of antimicrobial proteins and peptides of mammalian phagocytes, cells that are crucial for host defense against infection, reveal about their potential usefulness as pharmacologic agents?

The most critical issue facing the therapeutic use of these 'antibiotics from within' is their target-cell specificity. Many, if not most, of these cytotoxins from mammalian (and from more primitive) cells[2,15] lack specificity and can damage host cells. Because the granule-associated antimicrobials usually only reach cytotoxic concentrations within the confines of the phagocytic vacuole, the host cells are usually protected from damage. Systemic administration of these substances may pose a serious threat, however.

Another major issue, related to target cell specificity, is delivery to the desired site of action. Even if the protein or peptide administered orally or intravenously escapes destruction in the gut and in the circulation, and is not rapidly removed by the liver or kidney, or prevented from action by complexing to serum constituents, is the attraction to the microbial target sufficient for effective competition with sites in the host?

Even for BPI, the most target-specific mammalian cytotoxin yet recognized which is apparently devoid of cytotoxicity towards cells other than Gram-negative bacteria, it is uncertain whether the complete protein or biologically active fragments would be effective as administered agents. In the whole animal, BPI's function appears to be solely an intracellular one and the survival and biological effectiveness of BPI in the circulating fluids remains to be determined. BPI might be useful as a topical antibiotic against Gram-negative infections of skin and urinary tract.

While it is not possible to predict whether the recent rapid progress in the identification of endogenous antibiotics will lead to their pharmacological use, there should be no doubt that the further analysis of the structural determinants of their action, coupled with state-of-the-art biotechnology, will be the basis on which the design of new classes of peptide antibiotics can be built.

Acknowledgements

My research on the Bactericidal/Permeability-Increasing protein is supported by USPHS grants 5R37DK05472 and AI 18571.

References

1 Klebanoff, S. J. (1988) in *Inflammation: Basic Principles and Clinical Correlates* (Gallin, J. I., Goldstein, I. M. and Snyderman, R., eds), pp. 391–444, Raven Press
2 Elsbach, P. and Weiss, J. (1988) in *Inflammation: Basic Principles and Clinical Correlates* (Gallin, J. L., Goldstein, I. M. and Snyderman, R., eds), pp. 445–470, Raven Press
3 Gennaro, R., Romeo, D., Skerlavaj, B. and Zanetti, M. (1989) in *Subcellular Biochemistry: Blood Cells* (Vol 17) (Harris, J. R., ed.) Plenum Press
4 Ganz, T., Selsted, M. E. and Lehrer, R. I. (1988) in *Bacteria–Host-Cell Interaction, UCLA Symposia on Molecular and Cellular Biology New Series* (Vol 64) (Horwitz, M. A., ed.), pp. 3–14, Alan R. Liss
5 Daher, K. A., Lehrer, R. I., Ganz, T. and Kronenberg, M. (1988) *Proc. Natl Acad. Sci. USA* 85, 7327–7331
6 Ganz, T., Rayner, J. R., Valore, E. V. *et al.* (1989) *J. Immunol.* (in press)
7 Stanfield, R. L., Westbrook, E. M. and Selsted, M. E. (1988) *J. Biol. Chem.* 263, 5933–5935
8 Selsted, M. E. and Harwig, S. S. L. (1989) *J. Biol. Chem.* 264, 4003–4007
9 Lehrer, R. I., Barton, A., Daher, K. A. *et al.* (1989) *J. Clin. Invest.* 84, 553–561
10 Ouellette, A. J., Greco, R. M., James, M. *et al.* (1989) *J. Cell Biol.* 108, 1687–1695
11 Zasloff, M. A. (1987) *Proc. Natl Acad. Sci. USA* 84, 5449–5453
12 Gray, P. W., Flaggs, G., Leong, S. R. *et al.* (1989) *J. Biol. Chem.* 264, 9505–9509
13 Ooi, C. E., Weiss, J., Elsbach, P., Frangione, B. and Mannion, B. A. (1987) *J. Biol. Chem.* 262, 14891–14894
14 Mannion, B. A., Kalatzis, E. S., Weiss, J. and Elsbach, P. (1989) *J. Immunol.* 142, 2807–2812
15 Mannion, B. A., Weiss, J. and Elsbach, P. (1989) *Clin. Res.* 37, 435A
16 Weiss, J. and Olsson, I. (1987) *Blood* 69, 652–659
17 Elsbach, P. and Weiss, J. (1988) in *Bacteria–Host-Cell Interaction, UCLA Symposia on Molecular and Cellular Biology New Series* (Vol 64) (Horwitz, M. A., ed.), pp. 47–60
18 Wasmoen, T. L., Bell, M. P., Loegering, D. A. (1988) *J. Biol. Chem.* 263, 12559–12563
19 Barker, R. L., Gleich, G. J. and Pease,

L. R. (1988) *J. Exp. Med.* 134, 907–934
20 McGrogan, K. M., Simonsen, C., Scott, R. *et al.* (1988) *J. Exp. Med.* 168, 2295–2308
21 McLaren, D. J., McKean, J. R., Olsson, I., Venge, P. and Kay, A. B. (1981) *Parasite Immunol.* 3, 359–373
22 Ackerman, S. J., Gleich, G. J., Loegering, D. A., Richardson, B. A. and Butterworth, A. E. (1985) *Amer. J. Trop. Med. Hyg.* 34, 735–745
23 Gleich, G. J., Loegering, D. A., Bell, M. P. (1986) *Proc. Natl Acad. Sci. USA* 83, 3146–3150
24 Barker, R. L., Loegering, D. A., Ten, R. M. (1989) *J. Immunol.* 143, 952–955
25 Young, J. D. E., Peterson, C. G. B., Venge, P. and Cohn, Z. A. (1986) *Nature* 321, 613–616
26 Romeo, D., Skerlavaj, B., Bolognesi, M. and Gennaro, R. (1988) *J. Biol. Chem.* 263, 9573–9575
27 Joseph, L. J., Chang, L. C., Stamenkovich, D. and Sukhatme, V. P. (1988) *J. Clin. Invest.* 81, 1621–1629
28 Rado, T. A., Wei, X. P. and Benz, E. J. Jr (1987) *Blood* 70, 989–993
29 Gabay, J. E., Scott, R. W., Campanelli, D. *et al.* (1989) *Proc. Natl Acad. Sci. USA* 86, 5610–5614
30 Clark, R., Olsson, I. and Klebanoff, S. J. (1976) *J. Cell Biol.* 70, 719–723

□ □ □ □ □ □ □ □ □ □ □ □

Books received

Review copies of the following books have been received. Books which have been reviewed in *Trends in Biotechnology* are not included. The appearance of a book in the list does not preclude the possibility of it being reviewed in the future.

Atherton, E. and Sheppard, R. C. *Solid Phase Peptide Synthesis* IRL Press, 1989. £18.00 pbk, £27.00 spiralbound (ix + 203 pages) ISBN 0 19 963067 4 (pbk), ISBN 0 19 963066 6 (sp)

Baumberg, S., Hunter, I. S. and Rhodes, P. M. (eds) *Microbial Products: New Approaches* Cambridge University Press, 1989. £42.50 (v + 383 pages) ISBN 0 521 37065 5

Beynon, R. J. and Bond, J. S. (eds) *Proteolytic enzymes: a practical approach* Oxford University Press, 1989. £29.00 hbk, £19.00 pbk (v + 259 pages) ISBN 0 19 963058 5 (hbk), ISBN 0 19 963059 3 (pbk)

Campbell, I. and Duffus, J. H. (eds) *Yeast: A Practical Approach* IRL Press, 1988. £18.00 (xv + 289 pages) ISBN 0 947946 79 9

Campbell, R. and Macdonald, R. M. (eds) *Microbial Inoculation of Crop Plants* Oxford University Press, 1989. £40.00 hbk, £25.00 pbk (v + 118 pages) ISBN 0 19 963016 X

Cantarelli, C. and Lanzarini, G. (eds) *Biotechnogy Applications in Beverage Production* Elsevier Applied Science, 1989. £40.00 (x + 257 pages) ISBN 1 85166 328 2

Da Costa, M. S., Duarte, J. C. and Williams, R. A. D. (eds) *Microbiology of Extreme Environments and its Potential for Biotechnology* Elsevier Science Publishers, 1989. £56.00 (v + 429 pages) ISBN 1 85166 361 4

Dechow, F. J. *Separation and Purification Techniques in Biotechnology* Noyes Publications, 1989. $72.00 (x + 490 pages) ISBN 0 8155 1197 3

Fish, N. M., Fox, R. I. and Thornhill, N. F. (eds) *Computer Applications in Fermentation Technology: Modelling and Control of Biotechnological Processes* Elsevier Applied Science, 1989. £52.00 (xix + 471 pages) ISBN 1 85166 397 5 hbk

Giddings, J. C., Grushka, E. and Brown, P. R. (eds) *Advances in Chromatography – Biotechnological Applications and Methods* Marcel Dekker, 1989. $125 USA and Canada, $150 all other countries (iii + 267 pages) ISBN 0 8247 8095 7

Haaland, P. D. *Experimental Design in Biotechnology* Marcel Dekker, 1989. $107.50 (288 pages) ISBN 0 8247 7881 2

Harwood, C. R. (ed.) *Bacillus* Plenum Press, 1989. $71.40 (xvii + 414 pages) ISBN 0 306 43137 8

Hathway, D. E. *Molecular Mechanisms of Herbicide Selectivity* Oxford University Press, 1989. £32.50 (v + 214 pages) ISBN 0 19 857642 0

Heap, R. B., Prosser, C. G. and Lamming, G. E. (eds) *Biotechnology in Growth Regulation* Butterworths, 1989. £35.00 (iii + 286 pages) ISBN 0 407 01473 X

Hoffmann, G. *The Chemistry and Technology of Edible Oils and Fats and Their High-Fat Products* Academic Press, 1989 £49.50 (xv + 384 pages) ISBN 0 12 352055 X

Jacobson, G. K. and Jolly, S. O. (eds) *Biotechnology: A Comprehensive Treatise in 8 Volumes (Volume 7B)* VCH Verlagsgesellschaft, 1989. DM 495, £198.00 (xv + 587 pages) ISBN 3 527 26517 1

Keren, D. F. (ed.) *Flow Cytometry in Clinical Diagnosis* American Society of Clinical Pathologists, 1989. $75.00 (xvi + 343 pages) ISBN 0 89189 274 5

Lindsey, K. and Jones, M. G. K. *Plant Biotechnology in Agriculture* Open University Press, 1989. £15.95 (x + 241 pages) ISBN 0 335 15817 X

Murrell, J. C. and Roberts, L. M. (eds) *Understanding Genetic Engineering* Ellis Horwood, 1989. £19.95 (132 pages) ISBN 0 7458 0453 5

Quash, G. A. and Rodwell, J. D. (eds) *Covalently Modified Antigens and Antibodies in Diagnosis and Therapy* Marcel Dekker, 1989. $99.75 (x + 236 pages) ISBN 0 2847 8107 4

Rein, R. and Golombek, A. (eds) *Computer-Assisted Modeling of Receptor–Ligand Interactions, Progress in Clinical and Biological Research, Vol. 289* Alan R. Liss, 1989. $96.00 (540 pages) ISBN 0 8451 5139 8

Sanders, J. K. M., Constable, E. C. and Hunter, B. K. *Modern NMR Spectroscopy* Oxford University Press, 1989. £9.95 (vi + 118 pages) ISBN 0 19 855287 4

Setlow, J. K. (ed.) *Genetic Engineering Principles and Methods Vol. 11* Plenum Press, 1989. $52.50 (ix + 235 pages) ISBN 0 306 43339 7

Sharp, D. H. *Bioprotein Manufacture: A Critical Assessment* John Wiley and Sons, 1989. £34.95 (5 + 140 pages) ISBN 0 470 21470 8

Steinkraus, K. H. (ed.) *Industrialization of Indigenous Fermented Foods* Marcel Dekker, 1989. $119.50 (456 pages) ISBN 0 8247 8074 4

Swaminathan, B. and Prakash, G. (eds) *Nucleic Acid and Monoclonal Antibody Probes Applications in Diagnostic Microbiology (Infectious Disease and Therapy Series/2)* Marcel Dekker, 1989. $150.00 USA and Canada, $180.00 all other countries (752 pages) ISBN 0 19 963127

Takaku, H. (Symposium organizer) *Sixteenth Symposium on Nucleic Acids Chemistry* IRL Press, 1989. £30.00 (vii + 142 pages) ISBN 0 19 963127 1

Vandamme, E. J. (ed.) *Biotechnology of Vitamins, Pigments and Growth Factors* Elsevier Applied Science, 1989. £68.00 (xii + 439 pages) ISBN 1 85166 325 8

Walker, P. M. B. (ed.) *Chambers Biology Dictionary* Cambridge University Press, 1989. £8.95 pbk (v + 324 pages) ISBN 1 85296 153 8

Wolbarsht, M. L. (ed.) *Laser Applications in Medicine and Biology* Plenum Press, 1989. $75.00 (xiii + 295 pages) ISBN 0 306 43074 6

Wu, R., Grossman, L. and Moldave, K. (eds), *Recombinant DNA Methodology* Academic Press, 1989. $49.95 (xxxi + 760 pages) ISBN 0 12 765560 3