Journal of Peptide Science
J. Peptide Sci. **11**: 97–103 (2005)
Published online 15 September 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.604

JOURNAL OF PEPTIDE SCIENCE

Modelling of anti-HSV activity of lactoferricin analogues using amino acid descriptors

HåVARD JENSSEN,[a] TORE J. GUTTEBERG[a] and TORE LEJON[b]*

[a] Department of Medical Microbiology, University Hospital of North Norway, N-9038 Tromsø, Norway
[b] Department of Chemistry, University of Tromsø, N-9037 Tromsø, Norway

Received 24 April 2004; Accepted 7 June 2004

Abstract: Herpes simplex virus (HSV) causes a number of diseases and new therapies are being pursued vigorously. Earlier studies have shown that modified peptides based on lactoferricins reduce HSV-1 and HSV-2 infection, and structure–activity studies indicate that the anti-viral activity correlates with the binding affinity for heparan sulphate and chondroitin sulphate. In this study it is shown that theoretically derived amino acid descriptors can be used to model the anti-viral activity of peptides, as well as other peptide properties, even more accurately. Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: lactoferricin; anti-viral activity; herpes simplex virus; heparan sulfate; quantitative structure–activity relationships; projections to latent structures

INTRODUCTION

Herpes simplex virus (HSV) infections cause clinical symptoms ranging from periodic skin lesions to encephalitis. Latent virus may be reactivated in response to various types of physiological stress [1]. Most of the treatment for HSV is based on acyclovir and acyclovir-like drugs. However, the diseases are usually self-limiting in the immunocompetent host. Among HIV-positive patients the incidence of resistant HSV-2 isolates is slowly increasing [2].

The entry of HSV occurs at the cell surface due to fusion of the viral envelope with the plasma membrane of the cell [3]. The initial attachment of HSV to cells is through binding of the viral glycoprotein gC to heparan sulfate (HS) on the cell surface [4]. If gC is lacking, glycoprotein gB will replace this function and bind to HS. In the absence of HS, virus can bind to chondroitin sulfate (CS), although with lower efficiency [5]. The entry process involves additional viral glycoproteins which all are possible targets for new anti-viral drugs.

The development of novel compounds with alternative mechanisms of anti-viral action is important. A common feature of anti-viral peptides is their net positive charge and their propensity for forming highly ordered amphipathic conformations, such as α-helices or β-sheets. α-Helical peptides (magainins, cecropins, clavanins and LL-37) have been shown to cause little HSV inactivation [6–8], while β-sheet peptides (defensins, tachyplesin and protegrins) show higher activity towards HSV [9,10]. Lactoferricin peptides have also shown anti-HSV activity [11,12]. Lactoferricin (Lfcin) is generated by pepsin cleavage from the *N*-terminal part of milk protein, lactoferrin (LF) [13], which has shown anti-viral activity against several viruses [14]. In the LF molecule the Lfcin sequence makes an amphipathic alpha-helical [15,16] but after pepsin cleavage the peptide changes into a distorted β-sheet between a C-C bridge [17]. Whether the activity of LF is solely influenced by the Lfcin segment or is the combination of the Lfcin segment in concert with other structural features is unknown.

In order to decide which part is responsible for the activity it would be necessary to synthesize peptides expressing only the α-helix or β-sheet structure. The difficulty in designing such peptides caused us look into using other parameters for modelling anti-viral activity. The peptide's affinity for heparan sulfate as a probe for activity was considered based on the theory that some anti-viral peptides need to bind to negatively charged residues on the cell surface. Since the parameter set, using HS-affinity, CS-affinity or other parameters related to charge of the peptides, did not fully model the biological activity, the use of other parameters as descriptors was considered. Having used amino acid descriptors successfully in modelling both antibacterial [18,19] and anti-cancer activities [20] of peptides, this appeared to be a natural choice for the study of anti-viral activity. There have been a few reports on the use of QSAR and PLS in HIV studies [21–23] and as a general method in peptide research [24], but to the best of our knowledge this approach is new in HSV research.

The advantages of this approach are clear, since no measurements of peptide properties are needed and the results can be used directly for designing

* Correspondence to: Tore Lejon, Department of Chemistry, University of Tromsø, N-9037 Tromsø, Norway;
e-mail: tore.lejon@chem.uit.no

Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

new peptides. As in the earlier studies, a parameter set (Table 1) describing each naturally coded amino acid by three theoretically derived variables, denoted z_1, z_2 and z_3, was chosen [25,26]. These variables were originally derived from a set of physico-chemical parameters describing lipophilicity — hydrophilicity, size and charge related properties of the amino acids, in addition to NMR- and HPLC-data.

METHODS

Jenssen *et al.* characterized 12 peptides with six descriptors, either measured or calculated (Table 2) [12]. Affinities were measured against three different sepharose columns using heparan sulfate (HS), chondroitin sulfate (CS) type A or C as ligands. The grand average of hydropathicity (GRAVY) [27], aliphatic index [28] and net charge at pH 7.0 were calculated. The anti-viral activity against HSV-1 and HSV-2 was measured by infection assay using MRC-5 cells, serial dilutions of HSV-1 or HSV-2 and a checkerboard titration with different peptide concentrations. The calculations of IC_{50} values were based on the median effect principle of Chou and Talalay [29]. To evaluate the cytotoxic effect of the peptides against the MRC-5 cells, the MTT reduction assay was employed [30].

The program package Simca-P 10.0 from Umetrics, Umeå, Sweden was used for all calculations. The theoretically derived *z*-scales were used without scaling, since the variables used for the original analysis had been scaled, and activity data were used as the logarithm. All the data were centred prior to calculations.

A subset of the original 25-mer peptides was chosen for the study [12]. The omitted peptides included those in which the hexane moieties had been incorporated to mimic lipophilic sectors. However, peptide B3, in which amino acids 1 and 25 were left out, was included, as was B4 which is the only linear peptide, in order to test the robustness of the model.

Table 1 Descriptor Scales z_1, z_2 and z_3 for Amino Acid

Amino acid	z_1	z_2	z_3
A	0.07	−1.73	0.09
V	−2.69	−2.53	−1.29
L	−4.19	−1.03	−0.98
I	−4.44	−1.68	−1.03
P	−1.22	0.88	2.23
F	−4.92	1.30	0.45
W	−4.75	3.65	0.85
M	−2.49	−0.27	−0.41
K	2.84	1.41	−3.14
R	2.88	2.52	−3.44
H	2.41	1.74	1.11
G	2.23	−5.36	0.30
S	1.96	−1.63	0.57
T	0.92	−2.09	−1.40
C	0.71	−0.97	4.13
Y	−1.39	2.32	0.01
N	3.22	1.45	0.84
Q	2.18	0.53	−1.14
D	3.64	1.13	2.36
E	3.08	0.39	−0.07

RESULTS

In the original data set each of the peptides was described by 75 descriptors, i.e. 25 amino acids, each described by three *z*-values. The calculated model resulted in six significant components (according to cross validation or large eigenvalue) explaining all the variation in the *x*-variables and more than 95% of the variation in the *y*-variables. Most of the information is contained in the first two components, which explain more than 70% of the variation in both the *x*-variables and the *y*-variables.

In order to visualize the results from the calculation, predicted vs observed values for selected *y*-variables were plotted (Figures 1a,b,2a–c). As seen from the r^2-values included in the plots, the correlations between the calculated values and those obtained from measurements, or other types of calculations, varied from good to excellent. This indicates that the variables employed provide a good model for both the anti-viral activities and other properties of the peptides.

In Figure 3a,b the loadings for the calculation were plotted, describing the relative importance of the variables z_1, z_2 and z_3, with variables far from the origin being those most important for the modelling.

DISCUSSION

General Discussion

From the data it is clear that binding to HS (Figure 2a) and CS-C (Figure 2b) as well as the calculated GRAVY parameter (Figure 2c) are well modelled by the amino acid descriptors as judged from the experimentally derived values compared with what the model predicts. This is not surprising since the amino acid descriptors are derived using parameters dealing with both charge and lipophilicity. Still, this is further evidence that information about charge, size and lipophilicity are reflected in the amino acid descriptors.

The plots of activity against HSV-1 (Figure 1a) and HSV-2 (Figure 1b) of the peptides should be treated with some caution, since only seven peptides are included in the study. Also, it should be noted that the activity has not been measured above a certain threshold value. Peptides with no detectable anti-viral activity within

Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

Table 2 Overview of the Bovine and Human Analogue Peptides, Affinity, Toxicity and Anti-viral Activity

Peptide[a]	Amino acid sequence (single letter code)																								GRAVY[c]	AI[d]	Net Charge[e]	HS [mM][f]	CS-A [mM][f]	CS-C [mM][f]	MRC-5 IC_{50} [μM][g]	HSV-1 IC_{50} [μM][h]	HSV-2 IC_{50} [μM][h]
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25								
B1	F	K	C	R	R	W	Q	W	R	M	K	K	L	G	A	P	S	I	T	C	V	R	R	A	F	57.6	50.80	7.84	313	224	145	245	14.6	12.3
B2	—	—	—	—	—	—	—	—	—	—	—	—	—	R	—	—	—	—	—	—	—	—	—	—	—	74.0	50.80	8.84	326	80	90	239	13.5	122.2
B3		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		87.0	55.22	7.84	309	90	120	311	N.D.[i]	N.D.
B4[b]	R	F	L	V	C	—	K	Q	K	I	W	G	K	A	R	—	—	M	C	T	R	—	A	R	—	57.6	50.80	7.84	227	200	065	240	74.9	67.2
H1	T	—	—	F	Q	—	—	R	N	—	R	—	V	R	G	—	P	V	S	—	I	K	—	D	S	111.6	38.80	5.85	143	90	40	274	N.D.	N.D.
H2	T	—	—	—	—	—	—	R	N	—	R	—	V	R	G	—	P	V	S	—	I	K	—	D	S	144.8	38.80	7.85	219	105	95	273	N.D.	N.D.
H3	T	—	—	F	Q	—	—	—	N	—	R	—	V	R	G	—	P	V	S	—	I	K	—	D	S	97.2	38.80	4.85	150	131	125	249	N.D.	N.D.

[a] Bovine lactoferricin shortened B1, and the homologue from the human lactoferricin sequence shortened H1.
[b] Acetamidomethyl (Acm)-protecting groups on the cysteines.
[c] Grand average of hydropathicity.
[d] Aliphatic index.
[e] Net charge at pH 7.0.
[f] Concentration of NaCl required to elute the peptide from the affinity column, mean value of 3–5 experiments.
[g] Peptide concentration giving 50% cell death, mean value of four experiments.
[h] Concentration required for 50% reduction in virus amplification. Values are expressed as a result of 4–8 repeated experiments.
[i] N.D., Not detectable antiviral activity within tested concentration range (3–160 μM).

Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

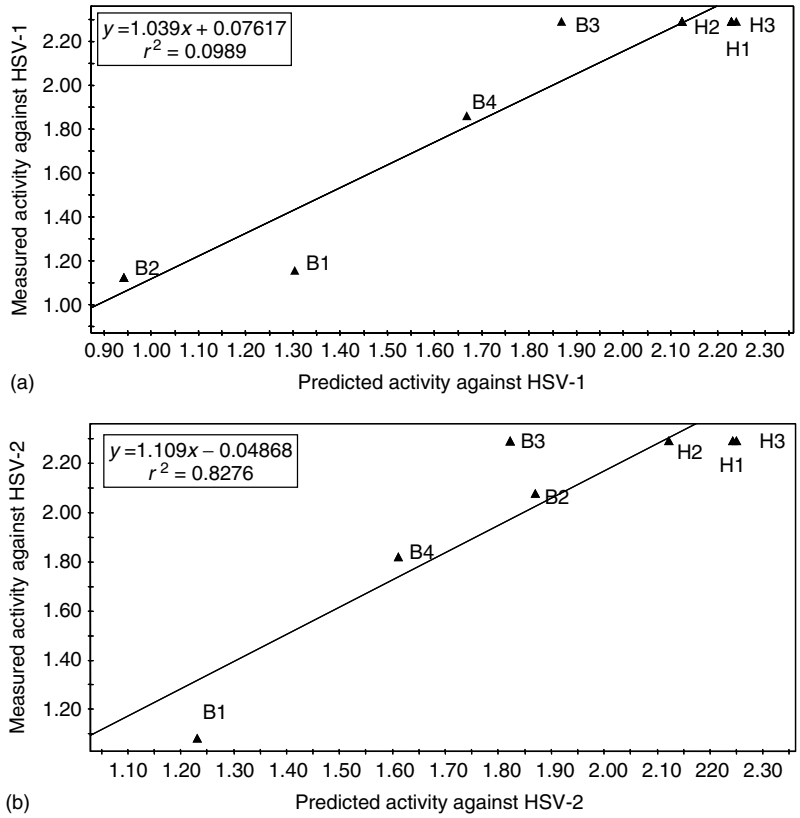

Figure 1 (a) Predicted vs measured activity against HSV-1, (b) Predicted vs measured activity against HSV-2.

the tested concentration range were given an anti-viral activity value equal to the threshold value, obviously not the same as the model would predict. Different amino acid contents in the peptides will give different activities but it is only one of the peptides (B3) that is predicted to have a better activity than that which is actually the case. Peptides H1 and H3 are both predicted to have an anti-viral activity above the tested concentration, which is in accordance with experimental data.

Comparing predicted HS affinity and anti-HSV activity it is clear that the correlation for binding is better than for the anti-viral activity. This is in accordance with the data from the previous study [12] where it was shown that there is information about anti-viral activity in the affinity data, but that anti-viral activity cannot solely be explained by the affinity. Binding to the cell surface is believed to be the mode of action of lactoferricin, thus preventing virus attachment, followed by subsequent penetration of the cell [31]. If this were to be the only mode of action of anti-viral peptides, the activity of a peptide would depend upon the combined effects of concentration and the number cationic residues participating in binding, as in the antibacterial peptide carpet model [32]. Since this does not seem to be the case, this indicates that the mode of action is more complicated than solely binding.

In order to further investigate this, more peptides will have to be included in the study, and work is in progress to address this. It should, however, be noted that the peptides should be of similar length so that they may be used successfully in the same calculations.

Analysis of Amino Acid Content

From the loading-plots it is possible to deduce those variables that are the most important for modelling the activity and it is also possible to see how variables are correlated to each other. The variables having the largest influence on the model are found furthest from the origin. Examination of Figure 3a reveals that the most important amino acids are those in positions (1), 4, 8, 9, 14, 15 and 25, but it is the different properties of the amino acids that are responsible for this. In amino acids 4, 8 and 25 it is z_1, which contains information about hydrophilicity/hydrophobicity, which is the most important while the others are dominated by the size-related variable, z_2, *vide infra*. In Figure 3b the *y*-variables are extracted from Figure 3a. By examining the position of the variables it is possible to see which *y*-variables are positively/negatively correlated with *x*-variables, and/or *y*-variables. It is clear that the anti-viral activity is negatively correlated with all other *y*-variables in the first dimension while HSV-1 and HSV-2 activities are negatively correlated to each

Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

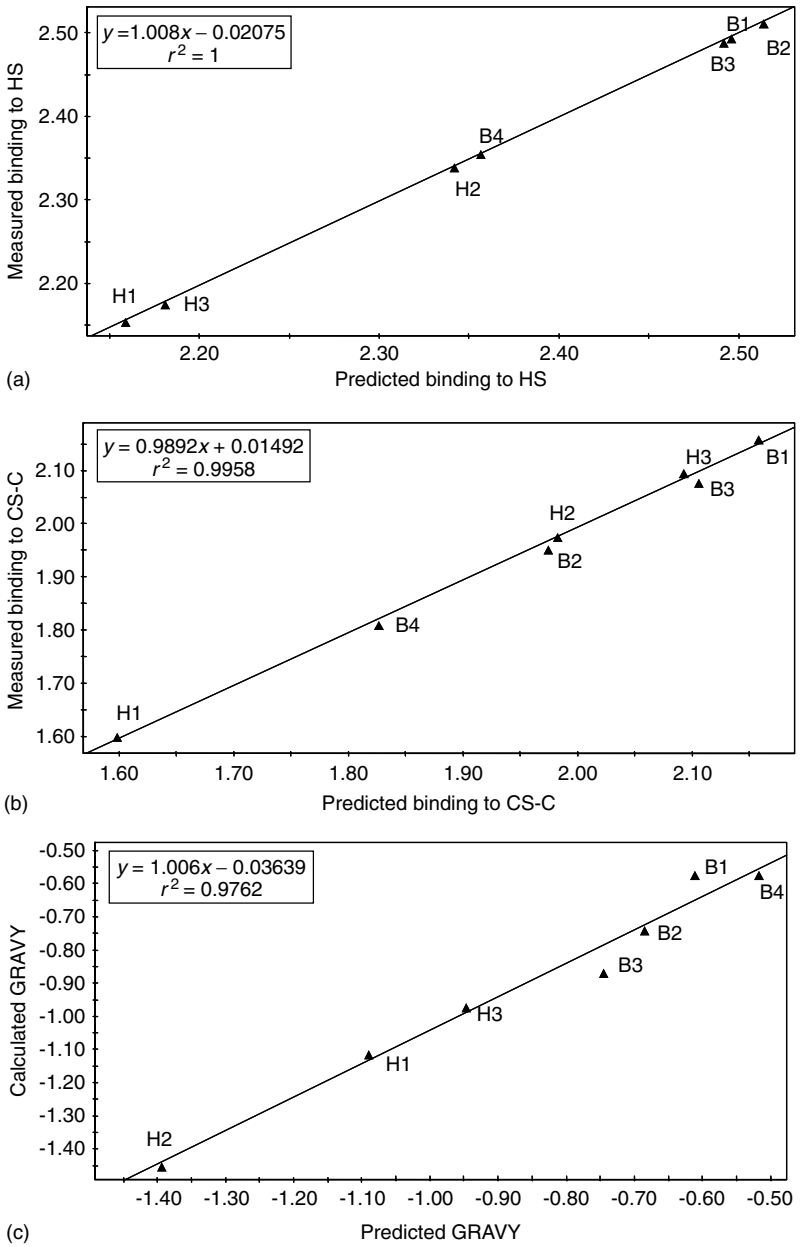

Figure 2 (a) Predicted vs measured binding to heparan sulfate, (b) Predicted vs measured binding to chondroitin sulfate C, 2(c) Predicted vs calculated grand average of hydropathicity.

other in the second dimension. Since the activities are not modelled equally by the amino acids included in the calculations it should be possible to use this fact for designing peptides with specificity for either strain of virus, in addition to designing peptides with higher activity. Amino acids that are to be included in new peptides are chosen from the information contained in the plots. For example, amino acid 25 has a large influence on the activity and since z_1 for this amino acid is positively correlated to the anti-viral activities, amino acids that have large negative z_1 values should be included in this position (Tables 1 and 2). For the amino acid in position 15 it is seen that it is z_2 that is important for modelling and that this property is negatively correlated with HSV-activity. Thus, amino acids with large z_2 values are to be incorporated into this position in order to increase HSV-activity. Since z_1 for amino acid 25 and z_2 for amino acid 15 are only present in the first dimension, incorporating new amino acids with the desired values should in theory only affect activity and not specificity. As a change of amino acid will cause the desired effect in one descriptor (z_1, z_2 or z_3) accompanied by a change in the other descriptors simultaneously, this approach is only valid in theory. Another problem arises when substitutions are made in, for example, position 4 (z_3) and 14 (z_2). This should ideally result in better selectivity without significantly influencing the activity. As discussed before, this is

Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

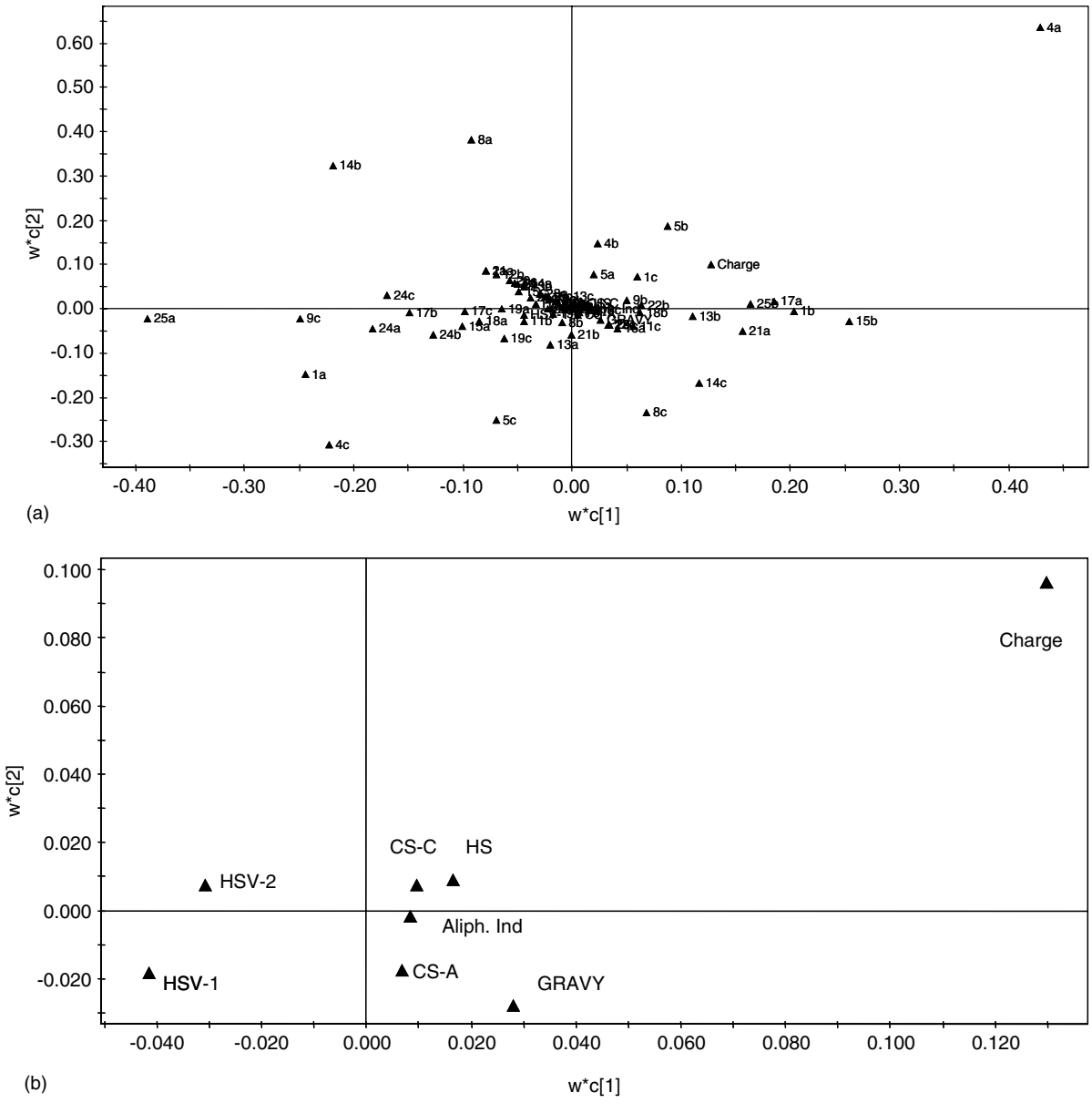

Figure 3 (a) Loading plot for entire data set (b) Loading plot for *y*-variables.

only an ideal case, but one of the strengths of the described approach is that before any peptides are synthesized, they can be 'tested' in the model. If the model does not predict a better result it is probably better to design a new peptide before proceeding with synthesis. Once more peptides have been synthesized and tested these can be included in the test set and new and more accurate models can be developed in an iterative fashion. Work is in progress in our laboratories on refining the model and synthesizing new peptides.

CONCLUSIONS

Theoretically derived amino acid descriptors are well suited for modelling activity against HSV, as well as other peptide properties such as HS/CS-affinity and GRAVY. From the data it appears clear that the mechanism(s) for the action of peptides is more complicated than simply binding to the cell surface.

Acknowledgement

Alpharma AS is greatly acknowledged for financial support to Håvard Jenssen and Frederick Leeson for linguistic assistance.

REFERENCES

1. Whitley RJ, Kimberlin DW, Roizman B. Herpes simplex viruses. *Clin. Infect. Dis.* 1998; **26**: 541–553.
2. Reyes M, Shaik NS, Graber JM, Nisenbaum R, Wetherall NT, Fukuda K, Reeves WC. Acyclovir-resistant genital herpes among

Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.

persons attending sexually transmitted disease and human immunodeficiency virus clinics. *Arch. Intern. Med.* 2003; **163**: 76–80.
3. Spear PG, Shieh MT, Herold BC, WuDunn D, Koshy TI. Heparan sulfate glycosaminoglycans as primary cell surface receptors for herpes simplex virus. *Adv. Exp. Med. Biol.* 1992; **313**: 341–353.
4. WuDunn D, Spear PG. Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. *J. Virol.* 1989; **63**: 52–58.
5. Banfield BW, Leduc Y, Esford L, Visalli RJ, Brandt CR, Tufaro F. Evidence for an interaction of herpes simplex virus with chondroitin sulfate proteoglycans during infection. *Virology* 1995; **208**: 531–539.
6. Aboudy Y, Mendelson E, Shalit I, Bessalle R, Fridkin M. Activity of two synthetic amphiphilic peptides and magainin-2 against herpes simplex virus types 1 and 2. *Int. J. Pept. Protein Res.* 1994; **43**: 573–582.
7. Ourth DD, Lockey TD, Renis HE. Induction of cecropin-like and attacin-like antibacterial but not antiviral activity *in Heliothis virescens* larvae. *Biochem. Biophys. Res. Commun.* 1994; **200**: 35–44.
8. Yasin B, Pang M, Turner JS, Cho Y, Dinh NN, Waring AJ, Lehrer RI, Wagar EA. Evaluation of the inactivation of infectious herpes simplex virus by host-defense peptides. *Eur. J. Clin. Microbiol. Infect. Dis.* 2000; **19**: 187–194.
9. Lehrer RI, Daher K, Ganz T, Selsted ME. Direct inactivation of viruses by MCP-1 and MCP-2, natural peptide antibiotics from rabbit leukocytes. *J. Virol.* 1985; **54**: 467–472.
10. Daher KA, Selsted ME, Lehrer RI. Direct inactivation of viruses by human granulocyte defensins. *J. Virol.* 1986; **60**: 1068–1074.
11. Andersen JH, Osbakk SA, Vorland LH, Traavik T, Gutteberg TJ. Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. *Antiviral Res.* 2001; **51**: 141–149.
12. Jenssen H, Andersen JH, Uhlin-Hansen L, Gutteberg TJ, Rekdal O. Anti-HSV activity of lactoferricin analogues is only partly related to their affinity for heparan sulfate. *Antiviral Res.* 2004; **61**: 101–109.
13. Tomita M, Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K. Potent antibacterial peptides generated by pepsin digestion of bovine lactoferrin. *J. Dairy Sci.* 1991; **74**: 4137–4142.
14. van der Strate BW, Beljaars L, Molema G, Harmsen MC, Meijer DK. Antiviral activities of lactoferrin. *Antiviral Res.* 2001; **52**: 225–239.
15. Baker EN, Anderson BF, Baker HM, Day CL, Haridas M, Norris GE, Rumball SV, Smith CA, Thomas DH. Three-dimensional structure of lactoferrin in various functional states. *Adv. Exp. Med. Biol.* 1994; **357**: 1–12.
16. Haridas M, Anderson BF, Baker HM, Norris GE, Baker EN. X-ray structural analysis of bovine lactoferrin at 2.5 Å resolution. *Adv.Exp.Med.Biol.* 1994; **357**: 235–238.
17. Hwang PM, Zhou N, Shan X, Arrowsmith CH, Vogel HJ. Three-dimensional solution structure of lactoferricin B, an antimicrobial peptide derived from bovine lactoferrin. *Biochemistry* 1998; **37**: 4288–4298.
18. Lejon T, Strøm MB, Svendsen JS. Antibiotic activity of pentadecapeptides modelled from amino acid descriptors. *J. Pept. Sci.* 2001; **7**: 74–81.
19. Lejon T, Stiberg T, Strøm MB, Svendsen JS. Prediction of antibiotic activity and synthesis of new pentadecapeptides based on lactoferricins. *J. Pept. Sci.* 2004; **10**: 329–335.
20. Yang N, Lejon T, Rekdal Ø. Antitumour activity and specificity as a function of substitutions in the lipophilic sector of helical lactoferrin-derived peptide. *J. Pept. Sci.* 2003; **9**: 300–311.
21. Joao HC, De Vreese K, Pauwels R, De Clercq E, Henson GW, Bridger GJ. Quantitative structural activity relationship study of bis-tetraazacyclic compounds. A novel series of HIV-1 and HIV-2 inhibitors. *J. Med. Chem.* 1995; **38**: 3865–3873.
22. Kulkarni SS, Kulkarni VM. Structure based prediction of binding affinity of human immunodeficiency virus-1 protease inhibitors. *J. Chem. Inf. Comput. Sci.* 1999; **39**: 1128–1140.
23. Buolamwini JK, Assefa H. CoMFA and CoMSIA 3D QSAR and docking studies on conformationally-restrained cinnamoyl HIV-1 integrase inhibitors: exploration of a binding mode at the active site. *J. Med. Chem.* 2002; **45**: 841–852.
24. Strøm MB, Haug BE, Rekdal Ø, Skar ML, Stensen W, Svendsen JS. Important structural features of 15-residue lactoferricin derivatives and methods for improvement of antimicrobial activity. *Biochem. Cell Biol.* 2002; **80**: 65–74.
25. Hellberg S, Sjöström M, Wold S. The prediction of bradykinin potentiating potency of pentapeptides. An example of a peptide quantitative structure-activity relationship. *Acta Chem. Scand. B* 1986; **40**: 135–140.
26. Hellberg S, Sjöström M, Skagerberg B, Wold S. Peptide quantitative structure-activity relationships, a multivariate approach. *J. Med. Chem.* 1987; **30**: 1126–1135.
27. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 1982; **157**: 105–132.
28. Ikai A. Thermostability and aliphatic index of globular proteins. *J. Biochem. (Tokyo)* 1980; **88**: 1895–1898.
29. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.* 1984; **22**: 27–55.
30. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 1983; **65**: 55–63.
31. Andersen JH, Jenssen H, Sandvik K, Gutteberg TJ. The anti-HSV activity of lactoferrin and lactoferricin is dependent on the presence of heparan sulfate at the cell surface. *J. Med. Virol.* 2004; **74**: 262–271.
32. Pouny Y, Rapaport D, Mor A, Nicolas P, Shai Y. Interaction of antimicrobial dermaseptin and its fluorescently labeled analogues with phospholipid membranes. *Biochemistry* 1992; **31**: 12416–12423.

Copyright © 2004 European Peptide Society and John Wiley & Sons, Ltd.