Peptide QSAR on Substance P Analogues, Enkephalins and Bradykinins Containing L- and D-Amino Acids

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Peptide QSARs are constructed for substance P analogues, enkephalins (two examples) and bradykinins containing both L- and D-amino acids. As descriptors in the QSARs, the previously developed descriptors z_1 (hydrophobicity), z_2 (bulk) and z_3 (electronic effect) are used together with a qualitative variable coding for variation in chirality. Two parametrizations of the peptide sequences are tested. In the first no chiral description is used at all, and in the second chirality is described by the qualitative variable. It is concluded that for the current series of peptides, the biological response to variation in amino acid sequence and chirality can be modelled.

D-Amino acids can be useful in the search for more potent and selective biologically active peptides.[1] Semi-empirical modelling of the relationship between amino acid sequence and biological activity of series of peptides containing D-amino acids might be possible provided that the change in amino acid sequence and chirality can be regarded as a moderate perturbation of the system. If, however, the chirality disturbance is severe, separate models must be used when an L-amino acid is substituted with a D form.

In order to construct peptide quantitative structure–activity relationships (QSARs) for series of related peptides containing both L- and D-amino acids it is necessary to use a scale describing enantiomeric characteristics for amino acids. Previously, we have developed three descriptor scales (z_1, z_2 and z_3; tentatively interpreted as related to hydrophobicity, bulk and electronic effects) for coded[2] (by mRNA) and non-coded[3] amino acids, and applied them in multivariate peptide QSARs.[2–4] Originally, these scales (or 'principal properties' of amino acids) were developed from measurements on L-amino acids mainly in non-chiral systems and thus contain no chiral information.

The main objective of this study is to examine whether the already available z-scales are sufficiently informative for the construction of peptide QSARs for series of peptides with both L- and D-amino acids, or whether they need to be supplemented with an extra scale reflecting some kind of enantiomeric characteristic. Ideally, this should be tested on peptide sets constructed according to an experimental plan[5] so as to probe specifically the effect of multipositional chirality changes. Unfortunately, such sets of peptides are difficult to find. We were able to find three biologically relevant peptide families, namely substance P analogues, enkephalins (two examples) and bradykinins, where the effects of both L- and D-amino acids have been studied. Within these three sets of peptides the chirality was generally changed one position at a time.

Present study. For this study, sequences and biological test data for four sets of peptides were compiled from the literature (see Table 1). A series of 39 antagonists of the undecapeptide substance P, studied by Folkers *et al.*,[6,7] constitutes the first set of peptides. From a review by Morley,[8] two sets of enkephalin-like peptides have been compiled. These peptides have been investigated by measuring their *in vitro* activity in guinea pig ileum (set 2, GPI) and mouse vas deferens (set 3, MVD). The last set of peptides consists of 43 bradykinin analogues (tested on isolated rat uterus), which have been collected from an extensive compilation work by Schröder.[9] All amino acid sequences and test data are listed in Table 1.

To develop peptide QSARs for these four sets of peptides, we use the extended z-scales which cover 20 coded (by mRNA) and 35 non-coded amino acids.[3d] These scales are used to translate the structural variation in each peptide into a vector of numerical values (three numbers per varied amino acid position), by using the values of z_1, z_2 and z_3 corresponding to the amino acid sequence. For each set of peptides, two parametrizations of the peptide sequences are tested. In the first no chiral description is used at all, and in the second, chirality is described by a qualitative $1/-1$ variable. Thus, in the first situation only z_1–z_3 are used, while in the second case they are supplemented by the extra variable that codes L-amino acids as 1, D-amino acids as -1 and achiral amino acids as 0.

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Table 1. Amino acid sequences[a] and biological activities (BA)[b] for four sets of peptides.

No.	Sequence	BA		
		Obs.	Calc.[c]	
1	rPKPQQwFwLL	1.71	0.83	0.95
2	rpKpQQfLWLL	−0.15	−0.39	−0.47
3	rpKPfQwFwLL	0.81	1.56	1.48
4	rpKPqqwFwLL	0.86	0.83	0.91
5	rpKPfQwFwLL	1.86	1.56	1.67
6	rpKPQQwXwLL	1.99	1.20	1.23
7	rpKPQQwFwGL	0.70	0.59	0.86
8	rpKPfQwXwLL	1.79	1.93	1.94
9	rpKPxQwXwLL	2.08	1.97	1.97
10	rPKPQQwFwLO	2.16	0.84	0.96
11	rPKPfQwFwLO	2.23	1.57	1.67
12	rPKPxQwXwLO	1.60	1.98	1.98
13	rPKPlQwXwLO	1.70	1.38	1.53
14	rPKPQQwFwwO	1.68	1.39	1.56
15	rPKPQQwFwxO	1.93	1.28	1.48
16	rPKpQQwFwwO	1.79	1.39	1.43
17	rPKPfQwXwwO	1.87	2.49	2.55
18	rPKPxQwXwwO	2.13	2.52	2.58
19	rPKPQQwFwLI	1.31	0.92	1.02
20	rpkpQQfFwLM	−0.70	−0.41	−0.47
21	rpkpqqwFwLM	0.63	0.58	0.42
22	rPKPQQwFwLM	1.25	0.58	0.76
23	RPKPQQwFxLL	1.03	0.46	0.51
24	RPKPQQwLxLL	0.71	0.23	0.34
25	RPKPQQwYxLL	−0.22	0.49	0.53
26	RPKPQQwIxLL	0.18	0.17	0.29
27	RfKPQQwFwLM	0.26	0.09	0.04
28	RxKPQQwFwLM	−0.10	−0.16	−0.14
29	RpKpQQwlwLM	−0.22	0.35	−0.09
30	rpKPQQwFwLM	0.20	0.58	0.57
31	rpKPQQwFwLL	1.00	0.83	0.76
32	rpKPQQwFwLT	−0.30	0.63	0.61
33	rpKPQQwFwLl	−0.04	0.84	0.54
34	rpKpQQwFwLa	−0.22	0.15	0.04
35	RpKPQQwFwLL	0.84	0.83	0.60
36	RPKPQQwFwLL	0.53	0.83	0.78
37	RPKPQQwFwLV	0.49	0.88	0.82
38	RPKPQQwFwLl	0.15	0.83	0.57
39	RPKPQQwFwLa	−0.70	0.15	0.06
40	FGGFM	−2.68	−2.05	−2.05
41	xGGFM	−2.00	−0.95	−1.71
42	yGGFM	−2.00	−1.35	−2.04
43	YBGFL	−0.89	−0.69	−0.91
44	YAGFM	−1.19	−0.70	−1.34
45	YaGFL	0.39	−0.37	−0.22
46	YaGFM	0.55	−0.70	−0.50
47	YfGFL	−0.27	0.68	0.66
48	YpGFM	−2.00	0.10	0.17
49	YJGFL	−1.85	−0.82	−1.01
50	YJGFM	−1.68	−1.15	−1.30
51	YsGFM	−0.04	−0.68	−0.48
52	YtGFM	−0.25	−1.05	−0.79
53	YvGFM	−0.37	−0.79	−0.57
54	AGGFM	−4.04	−4.02	−3.69
55	aGGFM	−4.04	−4.02	−4.27
56	GGGFM	−4.04	−3.55	−3.59
57	YGAFM	−2.00	−2.03	−2.05
58	YGaFL	−1.74	−1.69	−1.73
59	YGaFM	−1.40	−2.03	−2.01
60	YGFM	−2.96	−1.69	−1.75

contd

Table 1. (contd)

No.	Sequence	BA		
		Obs.	Calc.[c]	
61	YGGFL	−0.55	−1.01	−1.18
62	YGGFl	−0.82	−1.01	−0.84
63	YGGFm	−0.98	−1.35	−1.12
64	YGGFM	0.00	−1.35	−1.46
65	YGGFA	−1.54	−1.56	−1.64
66	YGGFa	−1.19	−1.56	−1.30
67	YGGFG	−1.82	−1.15	−1.12
68	YGGFO	−0.38	−1.00	−1.17
69	YGGFP	−2.29	−2.10	−2.09
70	YaGFl	0.61	−0.37	0.12
71	YaGFm	0.26	−0.70	−0.16
72	YsGFl	0.15	−0.34	0.14
73	YAGFL	−2.03	−0.04	−1.04
74	YaGFL	0.95	−0.04	0.20
75	YaGFM	0.83	−0.29	−0.02
76	YlGFL	−0.70	−0.54	−0.23
77	YJGFL	−2.43	−0.93	−1.18
78	YJGFM	−2.22	−1.19	−1.40
79	YwGFL	−1.30	0.43	0.59
80	YGAFL	−1.60	−2.29	−2.25
81	YGaFL	−2.51	−2.29	−2.42
82	YGaFM	−2.89	−2.55	−2.63
83	YGPFL	−3.51	−3.59	−3.36
84	YGpFL	−2.28	−3.59	−3.52
85	YGJFL	−2.51	−1.68	−1.82
86	YGGfM	−3.21	−1.41	−2.23
87	YGGYL	−1.80	−1.43	−1.61
88	YGGYM	−3.00	−1.69	−1.83
89	YGGWM	−0.57	−1.07	−1.30
90	YGGFL	0.10	−1.16	−1.37
91	YGGFl	0.15	−1.16	−0.82
92	YGGFm	−2.70	−1.41	−1.04
93	YGGFM	0.00	−1.41	−1.59
94	YGGFA	−2.51	−1.88	−1.99
95	YGGFG	−1.52	−1.65	−1.51
96	YGGFI	−0.11	−1.06	−1.29
97	YGGFO	−0.80	−1.15	−1.36
98	YGGFF	−0.96	−1.50	−1.66
99	YGGFP	−3.00	−2.18	−2.24
100	YaGFl	1.41	−0.04	0.75
101	YaGFm	0.77	−0.29	0.54
102	YGGFV	−1.39	−1.10	−1.33
103	YsGFl	1.18	0.00	0.79
104	RPPGFSPFR	−3.08	−5.83	−5.64
105	APPGFSPFR	−6.22	−6.23	−6.07
106	ZPPGFSPFR	−5.85	−6.03	−5.86
107	GPPGFSPFR	−6.23	−6.19	−6.22
108	KPPGFSPFR	−5.76	−5.87	−5.68
109	RAPGFSPFR	−5.54	−5.59	−5.38
110	RpPGFSPFR	−5.77	−5.83	−6.21
111	RVPGFSPFR	−5.08	−5.64	−5.44
112	PRPGFSPFR	−5.77	−6.20	−6.05
113	RPAGFSPFR	−3.07	−3.67	−3.28
114	RPpGFSPFR	−7.08	−5.83	−6.47
115	RppGFSPFR	−7.08	−5.83	−7.04
116	RPPAFSPFR	−6.26	−6.46	−6.64
117	RPPJFSPFR	−5.98	−6.01	−5.84
118	RPPGASPFR	−6.05	−6.81	−6.71
119	RPPGYSPFR	−5.78	−5.77	−5.58

contd

Table 1. (contd)

No.	Sequence	BA		
		Obs.	Calc.[c]	
120	RPPGFAPFR	−4.07	−5.77	−5.57
121	RPPGFGPFR	−3.06	−5.56	−5.08
122	RPPGFFPFR	−6.01	6.08	−5.91
123	RPPGFJPFR	−6.25	−5.60	−5.12
124	RPAGFGPFR	−4.75	−3.40	−2.73
125	RPPGFSAFR	−4.97	−5.28	−5.04
126	RPPGFSGFR	−5.06	−4.80	−4.78
127	RPPGFSpFR	−4.95	−5.83	−6.17
128	RpPGFSpFR	−7.08	−5.83	−6.75
129	RPpGFSpFR	−8.08	−5.83	−7.00
130	RppGFSpFR	−8.38	−5.83	−7.58
131	RPPGFSPAR	−6.22	−6.96	−6.87
132	RPPGUSPUR	−3.58	−5.01	−4.74
133	RPPGFGPUR	−3.67	−5.06	−4.54
134	RPPGFTPLR	−5.06	−5.90	−5.71
135	RPPGLGPLR	−7.38	−6.27	−5.85
136	RPPGLTPLR	−7.57	−6.36	−6.22
137	RPPGUNPUR	−5.42	−5.18	−4.93
138	RPPGUGPUR	−4.27	−4.74	−4.19
139	RFPSFGPPR	−6.77	−7.23	−7.21
140	RPPGFSPFA	−5.74	−6.16	−6.00
141	RPPGFSPFr	−8.40	−5.83	−6.59
142	RPPGFSPFZ	−5.68	−6.00	−5.83
143	RPPGFSPFG	−6.21	−6.10	−6.41
144	RPPGFSPFK	−5.77	−5.86	−5.67
145	ZPPGFSPFZ	−6.78	−6.20	−6.05
146	KPPGFSPFK	−6.23	−5.90	−5.71

[a]The peptide sets are substance P analogues (Nos. 1–39), GPI enkephalins (Nos. 40–72), MVD enkephalins (Nos. 73–103) and bradykinins (Nos. 104–146. Lower case letters denote D-amino acids. The one letter symbols are: A = alanine, B = α-aminoisobutyric acid, F = phenylalanine, G = glycine, I = isoleucine, J = sarcosine, K = lysine, L = leucine, M = methionine, N = asparagine. O = norleucine, P = proline, Q = glutamine, R = arginine, S = serine, T = threonine, U = *O*-methyltyrosine, V = valine, W = tryptophan, X = *p*-chlorophenylalanine, Y = tyrosine, Z = citrulline. [b]The antagonistic activity for the SP analogues (Nos. 1–39) is expressed as a log-fold increase in SP concentration to give 50 % of the maximum response in guinea pig ileum. The activities of the enkephalins are given as log potencies in guinea pig ileum and mouse vas deferens relative to Met enkephalin = 1. The activity of each bradykinin is given as log potency in isolated rat uterus relative to bradykinin = 1. [c]The center column gives the calculated BA for the QSAR models without chirality description, and the right column the corresponding values for models in which chirality has been accounted for.

Data analytical method. To establish a model between the *z*-scales describing the amino acid sequence (matrix X) and the biological activity variable y, i.e. to develop the peptide QSARs, we use the method of partial least-squares projections to latent structures (PLS).[10]

The PLS method relates the systematic information in a matrix X to the systematic information in a matrix Y with the purpose of predicting Y from X. PLS simultaneously calculates multivariate projections (latent variables) of the predictor variables (X) and the dependent variables (Y), so that the projection scores of the two data blocks are (*i*) good approximations of X and Y and (*ii*) well correlated. In this way, a quantitative expression of the relationship between the two matrices is obtained. The cross-validation criterion[11] is used to determine the number of significant model dimensions. For a more comprehensive presentation of PLS, we refer to the literature.[10]

Results

Peptide QSAR on 39 antagonists of SP. In this series of undecapeptides (peptides 1–39), taken from the two studies by Folkers and co-workers,[6,7] only 7 out of 11 positions (Nos. 2, 5 and 7–11) are completely varied, see Table 1. The remaining 4 positions (Nos. 1, 3, 4 and 6) each contain one particular amino acid that is the same in all 39 peptides. Thus, in the first case, the X-matrix consisted of 21 variables (7 varied positions times 3 *z*-scales). The PLS analysis resulted in a significant (cross-validation) one-dimensional model that explained 62 % of the variance in antagonistic activity. The corresponding cross-validated result (hereinafter given in parentheses) was 56 %. In Fig. 1(a), the observed antagonistic activity is plotted versus the calculated activity.

To try and improve the first QSAR model, we extended the structural description with a fourth, qualitative variable, to describe the enantiomeric variation in the data set. In each position, an L-amino acid was coded as 1, a D-amino acid as −1, and an achiral amino acid, such as glycine, as 0. This extra variable was used in all positions where chirality was altered, i.e. in all positions except No. 7. Thus, in this case the X-matrix was extended with 10 variables. The PLS analysis gave one significant model dimension that described 71 % (65 %) of the antagonistic activity variance, i.e. an improvement of 9 %. For a display of the results, see Fig. 1(b).

Peptide QSARs on 33 enkephalins (GPI set). The first series of enkephalins (Nos. 40–72) is taken from a review by Morley[8] and contains peptides originating from at least 11 authors or groups of authors. The fourth position in these pentapeptides is constant, which means that we only need the *z*-scales in four out of five positions to parametrize the variation in amino acid sequence. Thus, the X-matrix contained 12 variables. The first 'non-chiral' PLS analysis of this material resulted in a significant one-dimensional model explaining 62 % (54 %) of the biological activity variance. By adding the qualitative variable in a second PLS calculation accounting for variation in chirality, this result was improved to 71 % (63 %). In Figs. 2(a) and 2(b), the observed biological activities are plotted against the corresponding calculated values.

Peptide QSARs on 31 enkephalins (MVD set). The second set of enkephalins, also taken from the work by Morley, contains 31 peptides (Nos. 73–103). In this set, the amino

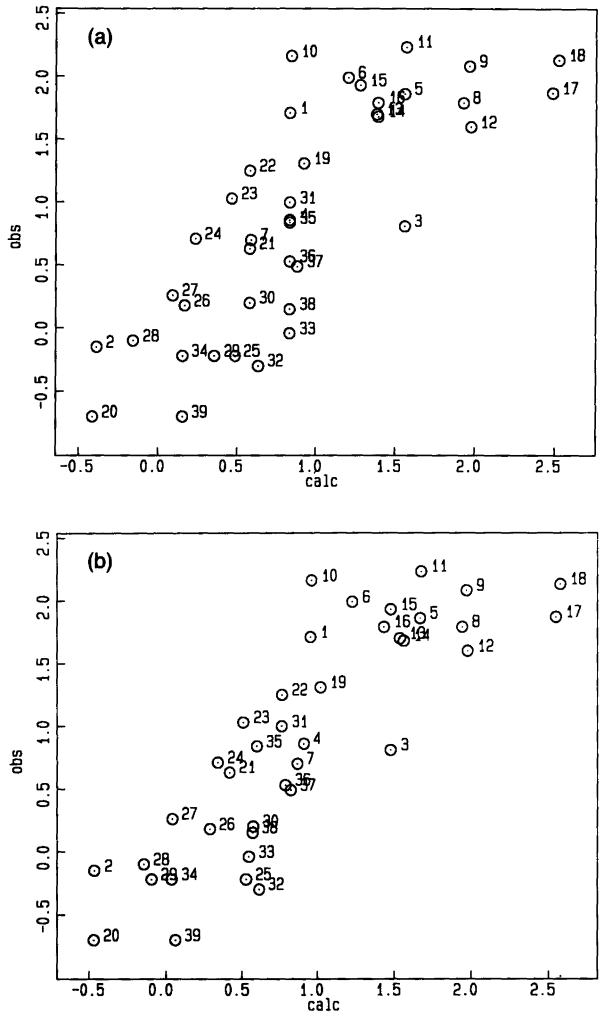

Fig. 1. Observed biological activity for the substance P analogues plotted versus the calculated activity from the QSAR based on (a) z_1, z_2 and z_3 only, and (b) the z-scales plus the qualitative variable. For the numbering of the peptides, see Table 1.

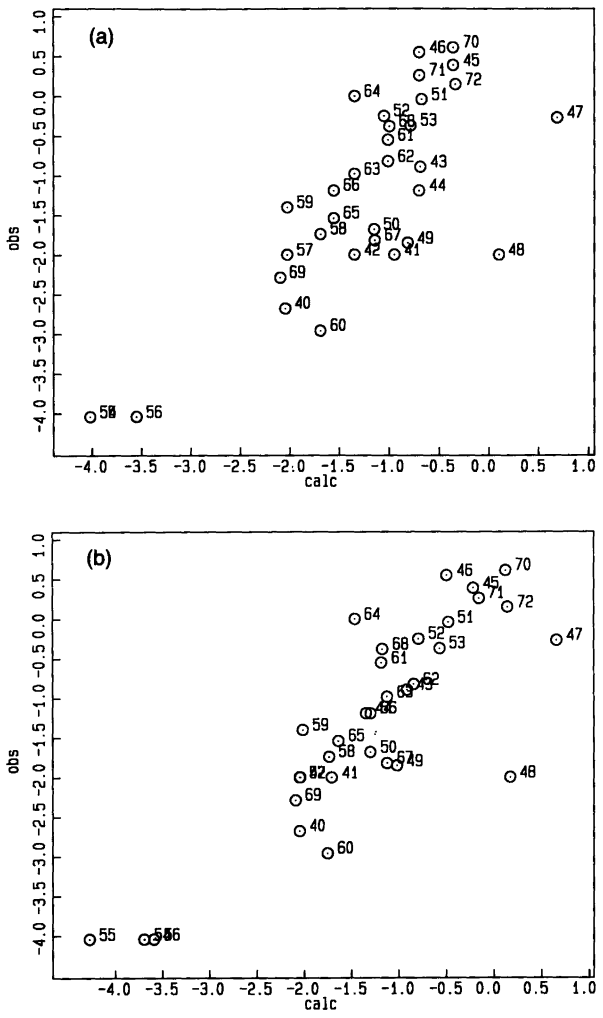

Fig. 2. Observed biological activity for the GPI enkephalins plotted versus the calculated activity from the QSAR based on (a) z_1, z_2 and z_3 only, and (b) the z-scales plus the qualitative variable. For the numbering of the peptides, see Table 1.

acid in the first position is the same in all peptides. Thus, in order to parametrize the structural variation, the z-scales were used in positions 2–5, resulting in an X-matrix with 12 variables. The PLS analysis gave a significant one-dimensional model that described 43 % (32 %) of the variance in biological activity. In the second PLS computation, where the qualitative variable had been included, the amount of explained variance was raised to 60 % (44 %), i.e. a somewhat larger improvement than in the two previous cases. For these two models, the observed and calculated biological activities are plotted against each other in Figs. 3(a) and 3(b).

Peptide QSARs on 43 bradykinins. The last example concerns a set of bradykinins (taken from the compilation work by Schröder[9]) composed of results on 43 peptides (Nos. 104–146) from seven sources. This set of nonapeptides was treated in the same way as the previous data sets. Thus, the z-scales were used to translate the structural variation into numbers, leading to an X-matrix with 27 (9×3) variables. A PLS analysis of this data set gave a barely significant PLS component that accounted for only 26 % (7 %) of the biological activity variance. The limitation of this model, is visualized in Fig. 4(a), where the observed activity is plotted against the calculated activity. However, this poor result was improved by the inclusion of the qualitative variable describing variation in chirality (here chirality was varied in all positions except Nos. 3 and 9, see Table 1). Then a PLS model (significant according to cross-validation) that described 51 % (15 %) of the biological activity variance could be established. As seen from Fig. 4(b), where the observed and calculated activities are plotted against each other, the result is a clear improvement.

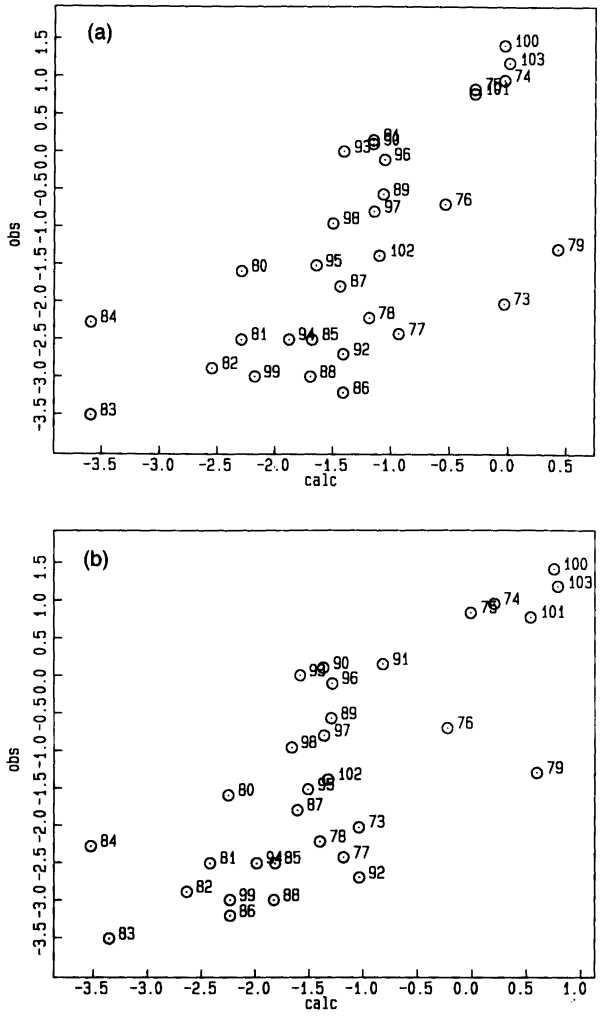

Fig. 3. Observed biological activity for the MVD enkephalins plotted against the calculated activity from the QSAR based on (a) z_1, z_2 and z_3 only, and (b) the z-scales plus the qualitative variable. For the numbering of the peptides, see Table 1.

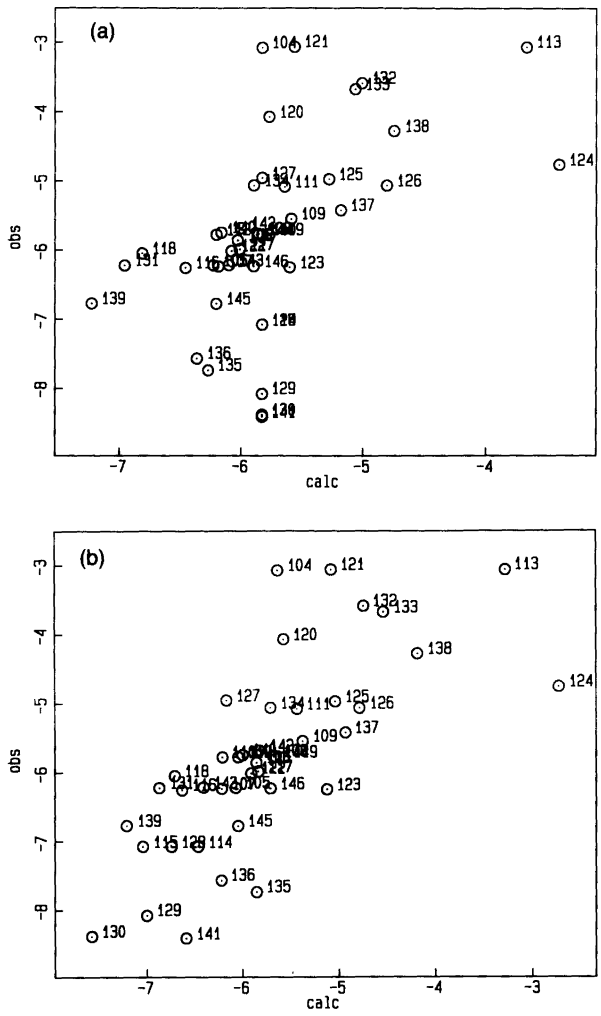

Fig. 4. Observed biological activity for the bradykinins plotted against the calculated activity from the QSAR based on (a) z_1, z_2 and z_3 only, and (b) the z-scales plus the qualitative variable. For the numbering of the peptides, see Table 1.

Discussion

We here have shown the possibility of developing peptide QSARs for four series of peptides with both L- and D-amino acids. Two of the data sets, the substance P analogues and the GPI enkephalins, have been fairly well modelled merely with the z-scales. However, the descriptive ability of both models was improved as a result of the inclusion of the qualitative chirality variable, a fact which implies that the variation in chirality was not negligible (cf. Figs. 1 and 2). In order to construct peptide QSARs for the remaining two data sets (the MVD enkephalins and the bradykinins), a description of variation in chirality was required as a supplement to the z-scales (see also Figs. 3 and 4). Thus, for the current series of peptides, the conclusion is that the variation in chirality makes moderate and, most importantly, modellable contributions to the overall biological activity variation.

It is of principal interest to note that peptides with both L- and D-amino acids can be incorporated in the same model. It seems to be generally believed that the introduction of a D-amino acid in a peptide chain leads to dramatic and unpredictable consequences. However, in the present case at least this is not so; the explanation for this may be the great flexibility of peptide chains which evidently can cope also with L to D changes as a first-order perturbation of similar magnitude as the perturbation caused by changing one L-amino acid to another.

The descriptive abilities of the peptide QSARs obtained are good, although not perfect. One reason for this may be that the data sets consist of contributions from many sources, e.g. systematic differences between different studies are reflected as inconsistencies in the data that negatively influence the descriptive capability of a QSAR. Another explanation, we believe, is that the qualitative 1/−1 variable is a rough and insensitive measure of chirality

effects. It seems reasonable, for example, that a small amino acid, such as alanine, may cause less conformational changes than a bulky, lipophilic amino acid, such as phenylalanine. Such differences would not be properly described by a qualitative variable. Instead, a quantitative variable of some kind would presumably capture such chirality effects in a more efficient way.

We are presently working on the development of a quantitative chiral scale, by studying L- and D-amino acids in chiral chemical and biological test systems. Some preliminary studies with this scale have indicated that it may be possible to develop better QSAR models than those using qualitative 1/−1 variables. We hope to report on these studies in the near future.

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