Retention of Histidine-Containing Peptides on a Nickel Affinity Column

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Abstract

The retention of histidine-containing peptides in immobilized metal-affinity chromatography is studied using several hundred modeled peptides. Retention is driven primarily by the number of histidine residues; however, the amino acid composition in the immediate vicinity plays a significant role. Specifically, the arginine and tryptophan content has to be taken into consideration. During the course of this study, an alternative tag that can be used similarly to a polyhistidine tag is discovered.

Introduction

Immobilized metal-affinity chromatography (IMAC) is an important tool for the purification of proteins containing residues that form metal complexes (histidine, cysteine, and tryptophan) (1–4). The polyhistidine tag is extremely useful in molecular biology where it serves to facilitate the isolation of genetically engineered proteins from complex mixtures and can be used for the targeted immobilization of these proteins (4,5).

A high-throughput method for the purification of peptide-oligonucleotide conjugates is developed. One of our strategies is to place three histidines at the amino terminus of the peptide and three histidines at the 5' end of the oligonucleotide. When joined together, the six histidines should form a tag that can be bound to a Ni-Sepharose affinity column. After washing away the unreacted components, the purified peptide-oligo conjugate can be eluted with a gradient of increasing imidazole concentration. For this strategy to be successful, the concentration of imidazole that elutes three histidines (His3) must be significantly less than the concentration that elutes six histidines (His6). In addition, the elution concentration for His6 should be relatively insensitive to the identity of amino acids surrounding the His6 tag.

Surprisingly, there is little information available in the literature on the relative affinity for Ni-Sepharose, in the presence of imidazole gradients, of polyhistidine-containing peptides and the influence of surrounding amino acids. Consequently, an array of model compounds containing different numbers of histidines in various sequential arrangements and in combination with various other amino acids is synthesized. This manuscript presents comprehensive information that should facilitate the design and purification of engineered peptides and proteins.

Experimental

Fluorenylmethyloxycarbonyl (Fmoc) amino acids, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) reagent, and Rink resin (0.42 mmol/g) were purchased from Novabiochem (EMD Biosciences, San Diego, CA). Solvents were from VWR International, Inc. (West Chester, PA). 4-Methylpiperidine was from Sigma-Aldrich (Milwaukee, WI).

Rink resin (300 mg) was added into a mixture of dimethylformamide (DMF) and dichloromethane (DCM) (10 mL total) to form a non-sedimenting suspension, which was distributed into the wells of flat bottom polypropylene microtiterplates (Evergreen Scientific, Los Angeles, CA). The plates were placed into a centrifugal synthesizer (6,7). An additional 100 µL of DMF was added into the plate wells (beads sedimented), and the plate was centrifuged with a tilt of 6 degrees. A standard protocol was used for the synthesis to remove the Fmoc protecting group. 4-Methylpiperidine was used instead of piperidine (8). Individual Fmoc protected amino acids (0.3M solution in 0.3M HOBt in DMF) were pipetted to the wells, and a solution of BOP (0.6M in DMF) and 1.2M diisopropylethylamine (DIEA) in DMF was delivered to each well. Plates were oscillated five times and allowed to rest for 50 s. During oscillation, the plates were rotated at a speed at which the liquid does not overflow over the wall of the well and solid support moves towards the outer side of the well. When the rotation was stopped, liquid returned to the horizontal position and beads distributed at the well bottom, thus mixing the well content. This procedure was repeated 30 times. The plate was centrifuged, and the addition of amino acids and reagents was repeated. After another 30 cycles of oscillation and pausing, the reagents were removed by centrifugation and washing, and deprotection was repeated to prepare the plate for the next cycle of synthesis.

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Table I. Structure of Model Peptides and Their Retention (Eluting Imidazole Concentration) on Nickel Column

#	Peptide	Im concentration (M)	#	Peptide	Im concentration (M)	#	Peptide	Im concentration (M)
			46	GAHHGAGHHAY	0.150	96	RREHHHHEEEY	0.180
1	GAGAHGAGAGY	0.083	47	GHHAGAGHHAY	0.155	97	AAKHHHHAEAY	0.180
2	GAGAHHGAGAY	0.098				98	DDHHHHDDKY	0.180
3	GAGAHHHGAGY	0.138	48	RRHHGGHHRRY	0.215	99	DDKHHHHKDDY	0.180
4	GAGAHHHHGAY	0.190	49	RRHHGGHHEEY	0.128	100	AKAHHHHAADY	0.180
5	GAGHHHHHGAY	0.240	50	RSHHRSHHRSY	0.220	101	SSSHHHHSSSY	0.183
6	GAHHHHHHGAY	0.275	51	RSHHESHHRSY	0.168	102	EKHHHHKEKY	0.183
7	GHHHHHHHGAY	0.328	52	ESHHRSHHESY	0.135	103	AAKHHHHAAEY	0.183
8	GHHHHHHHHAY	0.370				104	RDDHHHHDDRY	0.185
9	HHHHHHHHHAY	0.408	53	GAHHEEHHGAY	0.115	105	RSRHHHHESEY	0.188
10	HHHHHHHHHHY	0.460	54	GAHHEGHHGAY	0.133	106	EERHHHHREEY	0.188
			55	GAHHIIHHGAY	0.138	107	AKAHHHHAAEY	0.188
11	HGAGAGAGAGY	0.090	56	GAHHLLHHGAY	0.143	108	KKHHHHDDKY	0.188
12	HHAGAGAGAGY	0.085	57	GAHHERHHGAY	0.145	109	KKHHHHKDDY	0.188
13	HHHGAGAGAGY	0.120	58	GAHHGGHHGAY	0.150	4	GAGAHHHHGAY	0.190
14	HHHHAGAGAGY	0.165	59	GAHHSGHHGAY	0.150	110	ILVHHHHVLIY	0.190
15	HHHHHGAGAGY	0.210	60	GAHHIGHHGAY	0.150	111	KEHHHHEKKY	0.193
16	HHHHHHAGAGY	0.243	61	GAHHLGHHGAY	0.150	112	RAAHHHHAADY	0.193
17	HHHHHHHGAGY	0.288	62	GAHHFGHHGAY	0.163	113	AARHHHHADAY	0.193
18	HHHHHHHHAGY	0.325	63	GAHHFFHHGAY	0.173	114	AARHHHHDAAY	0.193
			64	GAHHRRHHGAY	0.190	115	KKHHHHDKDY	0.193
2	GAGAHHGAGAY	0.098				116	AAHHHHAAKY	0.193
19	GAGHAHGAGAY	0.108	65	HHHAGAGHHHY	0.263	117	AARHHHHEAAY	0.195
20	GAHGAHGAGAY	0.098	66	HHHAGAHHHGY	0.255	118	KKHHHHEEKY	0.195
21	GAHGAGHAGAY	0.095	67	HHHAGHHHAGY	0.258	119	RRDHHHHDDDY	0.195
22	GAHGAGAHGAY	0.098	68	HHHAHHHGAGY	0.263	120	AARHHHHAADY	0.195
			69	HHHGHHHGAGY	0.273	121	GGGHHHHGGGY	0.198
3	GAGAHHHGAGY	0.138	16	HHHHHHAGAGY	0.243	122	AARHHHHAEAY	0.198
23	GAHHGHAGAGY	0.128				123	KKHHHHKEEY	0.198
24	GAHHGAHGAGY	0.123	4	GAGAHHHHGAY	0.190	124	KKHHHHEKEY	0.198
25	GAHHGAGHAGY	0.120	70	EEEHHHHEEEY	0.127	125	ARAHHHHAADY	0.198
26	GAHHGAGAHGY	0.121	71	ESEHHHHESEY	0.128	126	AARHHHHAAEY	0.200
			72	EEHHHHEEEY	0.128	127	RRRHHHHDDDY	0.200
4	GAGAHHHHGAY	0.190	73	EEHHHHEEKY	0.140	128	RAAHHHHAAEY	0.203
27	GAHHGHHAGAY	0.155	74	REEHHHHEEEY	0.145	129	KAAHHHHAAKY	0.203
28	GAGHHGHHGAY	0.165	75	KEHHHHEEEY	0.150	130	AAKHHHHAAKY	0.205
29	GAGHHAHHGAY	0.163	76	EEKHHHHKEEY	0.163	131	AAKHHHHKAAY	0.205
30	GAHHAHHGAGY	0.165	77	KDHHHHDDDY	0.163	132	DKHHHHKDKY	0.205
31	GAGHHPHHGAY	0.158	78	DDHHHHDDDY	0.165	133	AAKHHHHAKAY	0.206
32	GAGHHEHHGAY	0.088	79	FFLHHHHESEY	0.168	134	ARAHHHHAAEY	0.208
33	GAGHHDHHGAY	0.088	80	KKKHHHHEEEY	0.168	135	DDRHHHHRDDY	0.208
34	GAGHHWHHGAY	0.203	81	KSKHHHHESEY	0.168	136	KDHHHHDKKY	0.208
35	GAGHHKHHGAY	0.173	82	REEHHHHEERY	0.168	137	RRRHHHHEEEY	0.210
36	GAGHHMHHGAY	0.163	83	DDDHHHHDDDY	0.168	138	AKAHHHHAAKY	0.212
37	GAGHHNHHGAY	0.168	84	AAHHHHAADY	0.168	139	KKHHHHDKKY	0.218
38	GAGHHQHHGAY	0.160	85	KKHHHHDDDY	0.170	140	DKKHHHHKKDY	0.218
39	GANHHAHHNAY	0.155	86	KKHHHHEEEY	0.175	141	KSKHHHHKSKY	0.225
40	GAQHHAHHQAY	0.143	87	KAAHHHHAAEY	0.175	142	KKHHHHEKKY	0.225
41	GAGHHIHHGAY	0.158	88	AAKHHHHAADY	0.175	143	EKKHHHHKKEY	0.228
42	GAGHHLHHGAY	0.155	89	AAKHHHHADAY	0.175	144	AARHHHHAARY	0.242
43	GAGHHFHHGAY	0.170	90	AAKHHHHDAAY	0.175	145	RAAHHHHAARY	0.244
44	GAGHHRHHGAY	0.180	91	AAAHHHHAAAY	0.178	146	AARHHHHARAY	0.244
			92	AAKHHHHEAAY	0.178	147	AARHHHHRAAY	0.244
4	GAGAHHHHGAY	0.190	93	RDDHHHHDDDY	0.178	148	ARAHHHHAARY	0.249
27	GAHHGHHAGAY	0.155	94	PPPHHHHPPPY	0.180	149	RERHHHHRERY	0.253
45	GAHHGAHHGAY	0.150	95	FFLHHHHKSKY	0.180	150	RRRHHHHDDRY	0.253

Table I. (continued) Structure of Model Peptides and Their Retention (Eluting Imidazole Concentration) on Nickel Column

#	Peptide	Im concentration (M)	#	Peptide	Im concentration (M)	#	Peptide	Im concentration (M)
151	RRRHHHHHRDDY	0.253	183	GAGAGRRRRRY	0.070	213	RRGASGASGASGHHHHY	0.200
152	RRRHHHHHDRDY	0.255	184	GAGARRRRRRY	0.085	214	EEGASGASGASGHHHHY	0.175
153	RREHHHHERRY	0.260	185	GAGAWWGAGAY	0.120	215	RREESGASGASGHHHHY	0.183
154	FFLHHHHRSRY	0.263	186	GAGAWGAGAGY	0.000			
155	RRRHHHHHEERY	0.265	187	GAGAHWGAGAY	0.095	216	PRREEGGRWGY	0.080
156	KKKHHHHHKKKY	0.267	188	GAGAHWWGAGY	0.138	217	PRREEPGRWGY	0.080
157	FFLHHHHLFFY	0.270	189	GAGHHWGAGAY	0.135	218	PGDYDDDRRQY	0.000
158	RRRHHHHHEREY	0.270	190	GAGHHWWGAGY	0.213	219	PGDYDDKRRQY	0.000
159	RRRHHHHHREEY	0.273	191	GAGARWGAGAY	0.085	220	QPRKIRPEGRY	0.000
160	RDRHHHHRDRY	0.280	192	GAGARWWGAGY	0.135	221	QADKGEPEGRY	0.000
161	RRDHHHHDRRY	0.283	193	GAGRRWGAGAY	0.098	222	QPRMIRPEGRY	0.000
162	DRRHHHHRRDY	0.288	194	GARRWWRRGAY	0.223	223	FNAEFNEIRRY	0.000
163	RSRHHHHRSRY	0.295				224	GNAEPNEIRRY	0.000
164	ERRHHHHRREY	0.313	195	HHASGASGASGASGHHY	0.140	225	NNFGKLFEVKY	0.000
165	RRRHHHHDRRY	0.315	196	HASGASGASGASGHHHY	0.172	226	NNFGKDKEVKY	0.000
166	RRRHHHHERRY	0.340	197	ASGASGASGASGHHHHY	0.197	227	EQKLISEEDLY	0.000
167	RRRHHHHHKKKY	0.368	198	ASGASGASGASHHGHHY	0.143	228	YPYDVPDYAY	0.000
168	RRRHHHHRRRY	0.426	199	ASGASGASGAHHSGHHY	0.133	229	LEHDGY	0.000
			200	ASGASGASGHHASGHHY	0.135	230	LEHDGGY	0.000
169	HRAGAGAGAGY	0.108	201	ASGASGASHHGASGHHY	0.133	231	ALEHDGGY	0.000
170	HRHGAGAGAGY	0.148	202	ASGASGAHHSGASGHHY	0.128	232	LLEHDGGY	0.000
171	HRHRAGAGAGY	0.168	203	ASGASGHHASGASGHHY	0.130	233	KLEHDGGY	0.000
172	HRHRHGAGAGY	0.220	204	ASGASHHGASGASGHHY	0.130	234	ELEHDGGY	0.000
173	HRHRHRAGAGY	0.243	205	ASGAHHSGASGASGHHY	0.130	235	SLEHDGGY	0.000
174	HGAGAGAGARY	0.100	206	ASGHHASGASGASGHHY	0.133	236	PLEHDGGY	0.000
175	HHGAGAGAGRY	0.133	207	ASHHGASGASGASGHHY	0.130	237	HRIFLAGDKDY	0.098
176	HHAGAGAGRRY	0.140	208	AHHSGASGASGASGHHY	0.130	238	HRIFLAGDEDY	0.093
177	HHHGAGAGRRY	0.190	209	HAHSHGHASGASGASGY	0.173	239	KRKGDEVDGVY	0.000
178	HHHAGAGRRRY	0.210	210	HASHGAHSGHASGASGY	0.150	240	RKGDEVDGVDY	0.000
179	AGAGAGAGARY	0.000	211	HASGHASGHASGHASGY	0.148	241	KGDEVDGVDEY	0.000
180	AGAGAGAGRRY	0.000	212	HASGAHSGASHGASGHY	0.148	242	GDEVDGVDEVY	0.000
181	GAGAGAGRRRY	0.000	197	ASGASGASGASGHHHHY	0.197	243	DEVDGVDEVAY	0.000
182	GAGAGARRRRY	0.000						

At the end of the synthesis, the plate was dried in vacuo and 150 µL of mixture K (9) (trifluoroacetic acid–thioanisol–water–phenol–ethanedithiol, 82.5:5:5:5:2.5, v/v) was added. The plate was capped and shaken on the plate shaker for 3 h. The suspension was transferred by multi channel pipettor to a filter plate (Orochem Technologies, Lombard, IL). The filtrate was collected in a deep well plate (VWR) and precipitated with ether (600 µL). After standing in a refrigerator for 2 h, a pellet was formed by centrifugation. The supernatant was removed by a surface suction device and the pellet was resuspended in ether (600 µL) and centrifuged again. The process of supernatant removal and resuspension was repeated three times. The product was dried in a Speedvac (ThermoSavant, Waltham, MA), dissolved in 200 µL of H_2O or 50% dimethylsulfoxide (DMSO)–50% H_2O, and samples of 20 µL were taken into 180 µL of water. Twenty microliters were injected onto high-performance liquid chromatography (HPLC) column (Waters, Milford MA, µBondapak, C18, 10 µ particle, 125 Å pore, 3.9 × 150 mm, gradient 0.05% TFA in H_2O to 70% acetonitrile, 0.05% TFA in 15 min, flow rate, 1.5 mL/min, detection by UV at 217 nm). Retention on the reversed-phase column was measured for all studied peptides. No significant deviation from the predicted retention based on the amino acid composition of the peptide have been observed, suggesting that there is no steric or conformational effect on the retention. MS was performed at HT-Labs (San Diego, CA).

The retention of peptides using IMAC was evaluated using an HPLC equipped with a 1-mL volume HisTrap column (Amersham Biosciences) with the detection at 260 nm. The peptides were injected in 0.02M sodium phosphate buffer pH 7.4 containing 0.5M NaCl. The concentration of imidazole was increased linearly from 0 to 0.5M during 20 min.

Results

Synthesized model peptides and the concentration of imidazole needed for their elution (extrapolated from the retention time in gradient elution) are given in Table I. All sequences were synthesized on tyrosine-modified resin to simplify UV detection (280 nm) of peptides eluted with increasing imidazole concentrations.

The first issue to be addressed was the dependency of the

retention on the content of histidines in the sequence. Figure 1 illustrates that the concentration of imidazole needed for elution of polyhistidine peptides depends linearly on the number of consecutive histidines (peptides 1 to 18). There is a notable difference in the elution of peptides containing amino terminal histidine versus peptides with all histidines in nonterminal positions. A free amino group on the *N*-terminal histidine residue reduces the affinity of these peptides for the nickel column. In longer sequences, this effect may be equivalent to having one fewer histidine in a nonterminal position.

Next, the arrangement of histidines within a series of undecapeptides were studed. As can be seen (Table I), separation of two histidines by one amino acid residue improves the retention slightly (peptides 2 and 19); separation by more than one amino acid residue does not have a significant effect (peptides 20–22).

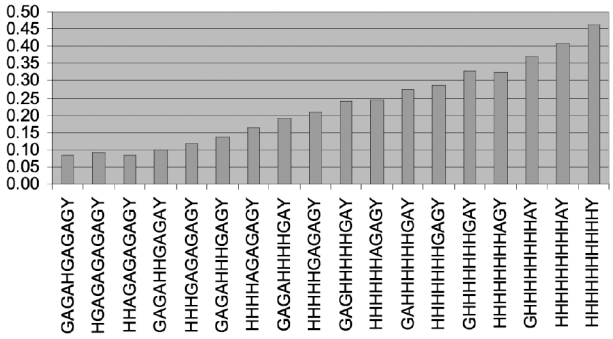

Figure 1. Concentration of imidazole needed for elution of histidine decapeptides with various numbers of histidines: X-axis, peptide sequence; Y-axis, concentration (M) of imidazole needed for peptide elution.

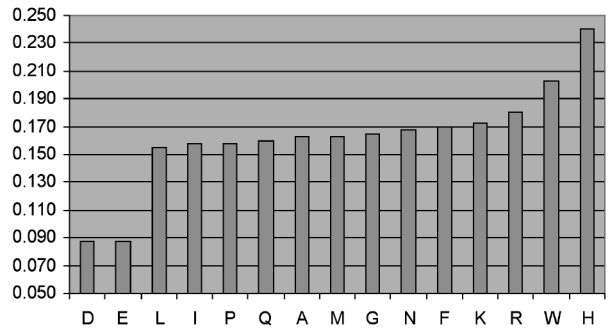

Figure 2. Retention of peptides GAGHHXHHGAY: X-axis, amino acid X; Y-axis, concentration (M) of imidazole needed for peptide elution.

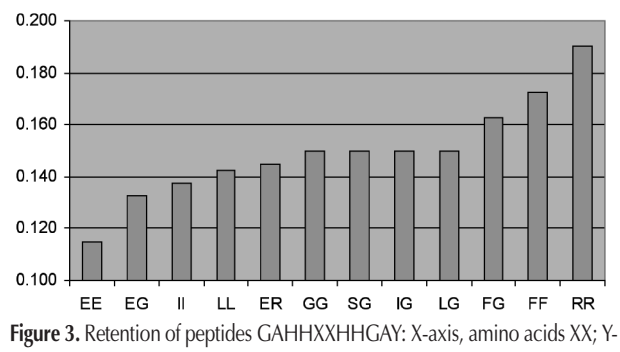

Figure 3. Retention of peptides GAHHXXHHGAY: X-axis, amino acids XX; Y-axis, concentration (M) of imidazole needed for peptide elution.

Separation of two and one histidine residue by one amino acid residue decreases the retention; however, separation by two to four amino acid residues does not decrease the retention further (peptides 3, 23–26). Separation of four histidine residues into two doublets decreases the retention; the number of intercalating residues does not make a significant difference (peptides 4, 27, and 45–47). The separation of two histidine doublets in the frame of a 17-mer was also studied, and it was found that the separation by two amino acids has the same effect as separation by 12 residues (peptides 197–208). Four histidine residues distributed regularly throughout the 17-mer (separated by two, three and four amino acid residues) have approximately the same retention, though a peptide with four histidines separated by one amino acid residue is slightly more retained (peptides 209–212). This result is easily explainable by the better availability of imidazole side chains for interaction with metal ions when histidine residues are separated by one amino acid residue. The preferred *trans* conformation of the amide bond brings amino acid side chains in 1–3 positions closer than side chains in the 1 and 2 positions.

A series of peptides with six histidine residues separated into two triplets by one to four amino acid residues do not have significant differences in their retention, although any separation slightly increases retention compared with the peptide with six adjacent histidines (peptides 16 and 65–69). This result was the most promising for our idea of combining two fragments, each containing a histidine triplet. This result permitted the use a variety of linking chemistries without concern for the spacing between the two triplets. As long as the initial components are released from the nickel column at a concentration of imidazole < 0.14M (peptide 3 or 13), and the conjugate is eluted by an imidazole concentration > 0.25M, the purification scheme might be very simple: (*i*) introduce the conjugation mixture to the column in a solution containing 0.14M imidazole; (*ii*) wash out the unreacted starting materials; (*iii*) and elute the purified His6 product with a solution containing 0.3M imidazole. To be able to apply this concept successfully, elution of the conjugation product should be relatively insensitive to the composition of the components surrounding the His6 tag. In the case of peptide conjugation, this requires relative insensitivity to the surrounding amino acids.

Consequently, over a hundred peptide sequences were designed and synthesized to determine the effect of amino acids surrounding the polyhistidine tag on its affinity to the nickel column. The effect of one or two amino acids joining two histidine doublets was evaluated in detail. Figure 2 shows the effect of an amino acid in the sequence GAGHHXHHGAY. The negative effect (decreased retention) of aspartic and glutamic acids (D and E) is very significant, and there is a slight positive effect (increased retention) of basic residues lysine (K) and especially arginine (R). A tryptophan (W) residue increased retention by approximately 50% of the increase achieved by addition of another histidine. This is in agreement with the effect of tryptophan observed in IMAC studies of various proteins (3,4).

Figure 3 shows the effect of a two amino acid linkage in the sequence GAHHXXHHGAY (peptides 53 to 64). The influence of glutamic acid and arginine is confirmed, and phenylalanine (F) appears to contribute to increased retention. It is more the effect of the aromaticity of the phenylalanine moiety than just the

hydrophobicity of the dipeptide, that is two leucines (L) or two isoleucines (I) do not increase retention significantly. Two isoleucines actually decrease the retention, probably due to steric factors (sequence HHIIHH is significantly less flexible than HHGGHH and/or HHIGHH). The combination of arginine and glutamic acid residues was studied with sequences 48 to 52. Compensation of the effects of basic and acidic residues was independent of the relative proximity of these residues.

The effect of surrounding a polyhistidine sequence with a negative, positive, hydrophilic, and hydrophobic residue was studied by constructing an additional 98 peptides. Sequences of the form (X)XXHHHHXXXY (peptides 70 to 168) are sorted in Table I by increasing retention. Obviously, charged amino acids have the largest impact on the retention of the model peptides. Arginine and lysine residues increase retention, and glutamic acid and aspartic acid residues decrease retention. The effect of arginine compares with that of two lysine residues, and the effect of glutamic acid is slightly stronger than the effect of aspartic acid. The opposing effect of basic and acidic residues is illustrated by the finding that retention of RSRHHHHESE is the same as GAGAHHHHGA. Hydrophobic residues increase retention significantly: FFLHHHHLFF has almost the same retention as GAHHHHHHGA, although it has two fewer histidine residues. The 17-mer peptides 197 and 213–215 reveal an interesting effect of placing arginine and/or glutamic acid residues at a long distance from the polyhistidine cluster. Surprisingly, the effect of arginines is diminished significantly, becoming almost negligible, though the effect of glutamic acid still persists.

The combination of histidine and arginine residues was studied with peptides 169–178. When the retention of these peptides was compared with the retention of peptides with histidine alone (peptides 11–16), it was found that HR is retained better than HH, and that HRH is more effective than HHH. In addition, HHHH is very similar to HRHR, and HHHHHH and HRHRHR are retained identically. The pentamer sequence HRHRH has slightly more affinity than HHHHH. Peptides 174–178 show that histidines and arginines work cooperatively in the frame of a decapeptide as well. With peptides 179–184, the effect of polyarginine sequences and their affinity for the nickel column was studied. As can be seen, retention is observed only at the level of pentaarginine, showing that retention of the HR-containing peptides is caused by a cooperative effect of histidine and arginine.

Peptides 185–194 were designed to study the effect of tryptophan (W) on retention to the nickel column. A single W in the sequence did not facilitate retention; however a doublet of tryptophans is equivalent in its effect to a triplet of histidines. The HHH sequence is retained almost identically to sequences HWW and HHW. Furthermore, RW is retained as well as RRW, and RWW is equivalent in retention to the sequence HHH. Finally, RRWWRR has nearly the affinity of five histidines in a row. This result may be useful in the genetic engineering of proteins, where tags alternative to pentahistidine may be desirable.

Using our high-throughput peptide synthesizer, several thousand peptides were recently synthesized for use in our protease and protein kinase assays. We tested some of these (peptides 216–243), each containing a terminal tyrosine, for their retention on the nickel column (Table I). Only peptides containing RW or HR showed any affinity. Retention of peptides with one histidine residue in proximity of acidic residue (D or E) was completely eliminated.

Conclusion

An exhaustive study of the affinity of histidine-rich peptide sequences for a nickel Sepharose column was performed, using elution by a gradient of increasing imidazole concentration as a measure of affinity. Retention of histidine-containing peptides depends on the arrangement of histidines within the sequence, with consecutive histidines not necessarily the best arrangement, and on the type of amino acid connecting histidine clusters. Affinity also strongly depends on the amino acid composition of neighboring sequences. Tryptophan and arginine, and to a lesser extent lysine and phenylalanine, increase affinity, while affinity is decreased by glutamic and aspartic acids. The findings indicate that it is possible to construct an affinity tag (e.g., RRWWRR) that performs with the same efficiency as pentahistidine, but does not contain any histidines.

Acknowledgments

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