azurins and stellacyanin, six to seven of the nine peaks in this region shift to lower energy upon deuteration.[12b,14] Although few of the peaks in Cu^{II}-LADH or Cu^{II}-LADH/pyrazole show any observable deuterium isotope dependence, in the NADH complex (which is most like a blue copper protein), six of the eight peaks have significantly altered frequencies or intensities in D_2O. The deuterium isotope effects in the blue copper proteins have previously been attributed to the coupling of Cu–L modes with imidazole ring vibrations.[13] However, the large changes in peak intensities and upward shifts in frequency observed for the Cu^{II}-LADH/NADH complex requires a more extensive explanation. For example, these deuterium isotope effects could arise from hydrogen-bonding interactions between the ligating cysteine sulfur atom and hydrogen donor groups of other protein residues. We have observed such anomalous, deuterium-induced intensity and frequency changes in the hydrogen-bonded Fe–O–Fe center of hemerythrin.[40]

Hydrogen bonding of metal-coordinated sulfur atoms is a general phenomenon in sulfur-containing metalloproteins. In the crystal structures of mononuclear, binuclear, and tetranuclear iron–sulfur proteins, almost every sulfur ligand is involved in at least one NH···S or OH···S hydrogen bond to the protein, with the majority arising from peptide NH groups.[41,42] Variations in the extent of hydrogen bonding may explain differences in redox potentials for different iron–sulfur clusters.[41,43] Similarly, in the crystal structures of blue copper sites, each of the cysteine ligands appears to be involved in at least one hydrogen bond between the sulfur atom and the protein backbone. Thus, in plastocyanin there is a hydrogen bond between Cys-84 and the amide NH of residue 38,[5a] in azurin from *A. denitrificans* Cys-112 is hydrogen bonded to the NH of residue 47,[5c] and in azurin from *Pseudomonas aeruginosa* a hydrogen bond is also likely to occur between Cys-112 and the NH of residue 47.[5b,d] Copper-substituted alcohol dehydrogenase may well be an analogous case since the crystal structure of the zinc enzyme indicates that Cys-46 is hydrogen bonded to the amide NH of residue 48.[44] These hydrogen bonds undoubtedly play a role in enforcing the unusual geometry of the blue copper sites and may also explain the unusual deuterium isotope dependences in their resonance Raman spectra.

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Registry No. LADH, 9031-72-5; NADH, 58-68-4; NAD, 53-84-9; Cu, 7440-50-8; Ni, 7440-02-0; Co, 7440-48-4; Fe, 7439-89-6; S, 7704-34-9; $HO(CH_2)_2OH$, 60-24-2; CN^-, 57-12-5; pyrazole, 288-13-1; imidazole, 288-32-4.

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An Approach toward the Complete FAB Analysis of Enzymic Digests of Peptides and Proteins

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Abstract: A current limitation in the use of FAB mass spectrometry for mixture analysis is that some components of the mixture, dissolved in matrices such as glycerol, are not normally observed. Three enzymic digestions (one of a polypeptide and two of small proteins) are used to show that it is the hydrophilic peptides in a mixture that are suppressed. By determination of the FAB spectra of both pure hydrophilic and hydrophobic peptides, and of mixtures of these, it is shown that (i) hydrophilic peptides alone give a relatively poor signal response and (ii) hydrophilic peptides are further suppressed in the presence of hydrophobic peptides that initially occupy the surface of the matrix. A hydrophilicity/hydrophobicity index (ΔF values) can be used to indicate which peptide may be suppressed. Suppression may be reduced by HPLC partial fractionation or the conversion of polar carboxyl groups to more hydrophobic ester derivatives.

The determination of the molecular weights of relatively large polar molecules has been facilitated enormously since the discovery of fast atom bombardment (FAB) mass spectrometry.[1] Additionally, since the advent of genetic engineering and rapid DNA-sequencing techniques, there is an increased demand for efficient methods to check the accuracy of an assumed protein sequence. This demand arises because mistakes can be made in reading DNA-sequencing gels or because posttranslational modifications of proteins that are normally present may be absent when the product is engineered in a bacterial cell. It has been demonstrated that such rapid checking of protein sequences can be achieved by digesting the protein enzymically and determining by FAB mass spectrometry the molecular weights of the peptides in the resulting mixture.[2-7] However, a limitation of this powerful method has been that, in a complex mixture, not all the peptide fragments are seen. In view of the importance of the technique,

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Table I. Bull and Breese Indices and FAB Analysis of Peptides Obtained from Tryptic Digestion of Glucagon (**1**)

frag	B and B indices[a]	MH^+, m/z	pres (+) or abs (-) in FAB MS[b,c]
1–12	+203	1357	-
13–17	-272	653	+
19–29	-131	1352	+
18–29	-63	1508	+

[a] From addition of ΔF values taken from ref 12, each sum being divided by the number of amino acids in the peptide. [b] Spectrum obtained in glycerol containing 1 M trichloroacetic acid; similar results obtained in glycerol containing 1% HCl, glycerol containing 5% H_2O, thioglycerol/diglycerol (1:1), and 1,2,6-trihydroxyhexane. [c] A peptide is described as being "absent" if, for a loading of ~5 nm of digest, the anticipated MH^+ signal had an intensity ≤1.2 units, where 1.0 unit is the average signal for all other peaks in the range m/z (MH^+) ± 10 (corresponding to the coherent background normally seen in FAB spectra). The same criterion is applied in Tables II and III.

we have searched for a general property of those peptides that are suppressed in the FAB analysis and for a simple chemical method to modify that property so that all peptides in a digest may be observed. We report that there is a striking correlation between peptide hydrophilicity and suppression in the commonly used FAB matrices glycerol and thioglycerol/diglycerol (1:1). Additionally, we show that increasing the hydrophobicity of such peptides, by converting them to their isopropyl esters, allows all the components to be detected in both a very simple and two more complex enzymic digests.

Experiments in this laboratory,[8] and others,[7,9] have demonstrated that the surface activity of the compound being monitored is related to the signal seen in FAB mass spectrometry. In particular, Clench et al.[7] have recently shown that when a tryptic digest of the peptide glucagon (**1**) is analyzed by FAB, a signal due to residues 19–29 (MH^+, m/z 1352) is seen in preference to a signal due to residues 1–12 (MH^+, m/z 1357).

^{1}His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-^{12}Lys-
^{13}Tyr-Leu-Asp-Ser-^{17}Arg-Arg-^{19}Ala-Gln-Asp-Phe-Val-
Gln-Trp-Leu-Met-Asn-^{29}Thr

1

We have independently made this observation, and also some others on the same system. First, a signal due to residues 1–12 is not observed, or difficult to detect, in a variety of matrices [glycerol containing ca. 1% HCl, glycerol containing 5% H_2O, thioglycerol/diglycerol (1:1), 1,2,6-trihydroxyhexane (Table I)]. Second, when the above two peptide fragments were separated by analytical HPLC and then independently analyzed by FAB mass spectrometry, each gave an easily detected MH^+ ion (see, for example, Figure 1A). Third, we have correlated the response of the mixture to FAB analysis in terms of a hydrophilicity/hydrophobicity scale for peptides.[10-12] We have chosen the scale of Bull and Breese,[12] which relates to the preference or reluctance of an amino acid to transfer from aqueous solution to an air–water interface. The more positive the index, the more hydrophilic the peptide. The peptide that is suppressed in the analysis of the mixture has a positive value, whereas the ones that are observed are more hydrophobic and have negative values (Table I). Last, we note that although the more hydrophilic peptide is suppressed at the beginning of the FAB analysis, the signal due to the hydrophobic peptide decreases as a function of time, and after the mixture has been in the beam of fast xenon atoms for 4.0 min, MH^+ due to the hydrophilic peptide was more abundant than

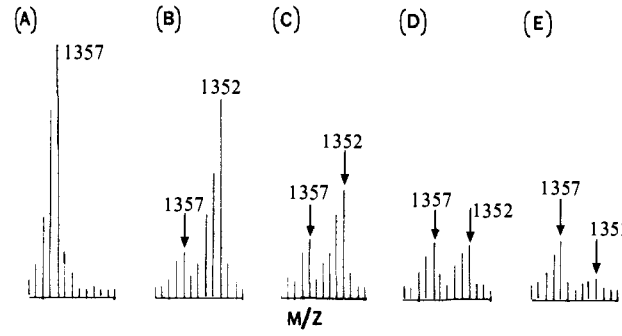

Figure 1. Positive-ion FAB mass spectra in the mass range m/z 1350–1360 of ~0.5 nm of peptide fragments from trypsin digest of glucagon (in 3 μL of 1 M trichloroacetic acid in glycerol). The sample was loaded on a steel probe tip, and spectra were obtained with 8-keV Xe atoms (40-μA current): (A) peptide fragment MH^+, m/z 1357, alone; (B) initial scan after addition of an approximately equimolar amount of peptide fragment MH^+, m/z 1352; (C) spectrum in (B) after 1.5 min in Xe beam; (D) spectrum in (B) after 2.5 min in Xe beam; (E) spectrum in (B) after 4.0 min in Xe beam.

Table II. Bull and Breese Indices and FAB Analysis of Peptides Obtained from Tryptic Digestion of Horse Heart Cytochrome *c* (**2**)

frag	B and B indices[a]	MH^+, m/z	pres (+) or abs (-) in FAB MS[b,c]
Ac-1–5	(+328)[d]	589	+
6–7	+635	204	-
9–13	-458	634	+
14–22	*e*	1634	+
23–25	+693	261	-
26–27	+575	284	-
28–38	0	1168	+
40–53	+251	1270	-
54–55	+675	261	-
56–60	-218	604	+
61–72	-157	1495	+
74–79	-248	678	+
80–86	-457	779	+
89–91	+497	405	-
92–99	-499	964	+
101–104	+575	434	-

[a,b,c] As for Table I. [d] Does not allow for acetylation of the N-terminus. [e] The heme group will make this fragment more hydrophobic than the peptide portion (B and B index +389).

MH^+ due to the hydrophobic peptide (see Figure 1B–E). This observation is, of course, consistent with the more hydrophobic peptide initially occupying the surface of the matrix, being sputtered away by the early bombardment, and the more hydrophilic peptide gaining later access to the surface; it emphasizes the dynamic nature of the FAB process.

Support for the above conclusions has been obtained by FAB analysis of a tryptic digest of horse heart cytochrome *c* (**2**). The

Ac-^{1}Gly-Asp-Val-Glu-^{5}Lys-^{6}Gly-^{7}Lys-^{8}Lys-^{9}Ile-Phe-
Val-Gln-^{13}Lys-^{14}Cys(heme)-Ala-Gln-Cys-His-Thr-Val-Glu-
^{22}Lys-^{23}Gly-Gly-^{25}Lys-^{26}His-^{27}Lys-^{28}Thr-Gly-Pro-
Asn-Leu-His-Gly-Leu-Phe-Gly-^{38}Arg-^{39}Lys-^{40}Thr-Gly-
Gln-Ala-Pro-Gly-Phe-Thr-Tyr-Thr-Asp-Ala-Asn-^{53}Lys-
^{54}Asn-^{55}Lys-^{56}Gly-Ile-Thr-Trp-^{60}Lys-^{61}Glu-Glu-Thr-
Leu-Met-Glu-Tyr-Leu-Glu-Asn-Pro-^{72}Lys-^{73}Lys-^{74}Tyr-
Ile-Pro-Gly-Thr-^{79}Lys-^{80}Met-Ile-Phe-Ala-Gly-Ile-
^{88}Lys-^{87}Lys-^{88}Lys-^{89}Thr-Glu-^{91}Arg-^{92}Glu-Asp-Leu-Ile-
Ala-Tyr-Leu-^{99}Lys-^{100}Lys-^{101}Ala-Thr-Asn-^{104}Glu

2

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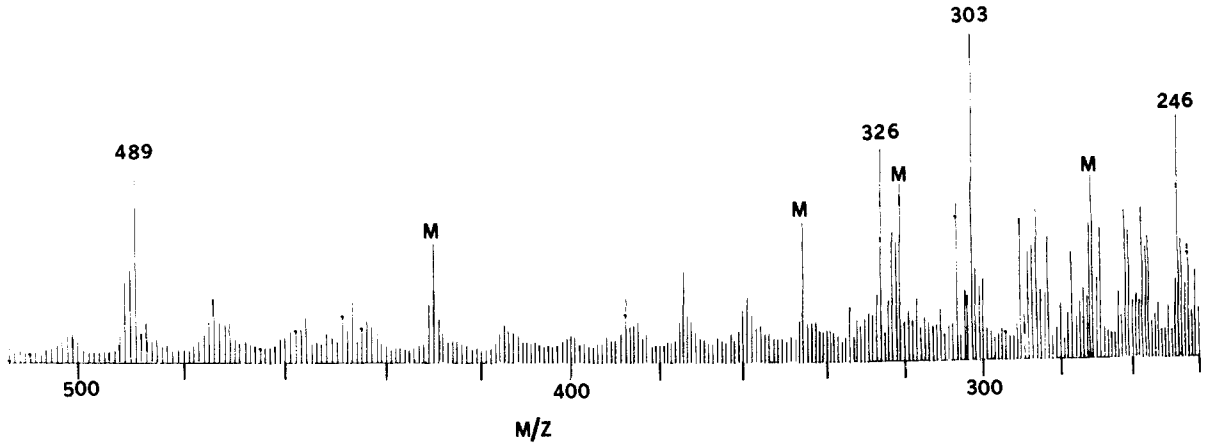

Figure 2. Partial positive-ion FAB mass spectrum (m/z 240–520) of isopropyl ester derivatives of peptides obtained by tryptic digestion of horse heart cytochrome *c*. The matrix is 1 M trichloroacetic acid in glycerol, and the scan was obtained 7 min after introduction of the sample into the ion beam. Peaks marked "M" are due to the matrix, since they were also observed when the matrix was run alone.

fragments anticipated to occur in this digest are indicated by broken lines in **2**. All those fragments that were not observed in an initial FAB scan of this complex mixture have positive values of an index based on the Bull and Breese scale (Table II). Conversely, all those that were observed are relatively hydrophobic and have a zero or negative value of the index (Table II) (with the exception of the N-terminal fragment, which is not typical insofar as the hydrophilicity associated with the normal N-terminal N^+H_3 group is absent due to acetylation of the protein).

In general in the analysis of complex mixtures, it is desirable that the more polar peptides appear after the minimum number of scans. We planned, therefore, to modify the peptide mixture chemically so that all the peptides present in the mixture would have greater hydrophobicities and hence compete more effectively for the surface of the matrix. Ideally, the chemical method should be essentially quantitative in yield and achievable without any tube-to-tube transfers (to be applicable at the submicrogram level if necessary). Additionally, it must remove a common cause of hydrophilicity in peptides. We have used esterification with 1 M HCl in 2-propanol (at 37 °C for ca. 24 h).[13] When the above digest of glucagon was treated in this manner, all the peptide fragments were observed in the initial scans of the FAB mass spectrum. When the digest of horse heart cytochrome *c* was similarly treated and then analyzed by FAB, the longest peptide (40–53, Table I), which had not been seen prior to derivatization, was seen in the initial scans. Moreover, later scans over the mass range 500–200 resulted in the appearance of MH^+ signals (m/z) associated with isopropyl esters of the remaining six most hydrophilic peptides: 6–7, 246; 23–25, 303; 26–27, 326; 54–55, 303; 89–91, 489; 101–104, 518; Figure 2.

To explore further the phenomenon of suppression, we have studied the sensitivity of FAB in the analysis of commercially available peptides of varying hydrophobicity, run as pure components. δ-Sleep-inducing peptide (DSIP, Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu) was selected as a relatively hydrophilic peptide (B and B index +556) for comparison with the relatively hydrophobic peptides bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, B and B index −104) and oxytocin (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2, B and B index −145). The MH^+ signal due to each pure peptide, dissolved in 2 μL of glycerol containing 5% water, was monitored. The experiments were carried out successively, without change of the instrument parameters. The signal response due to MH^+ of the hydrophilic peptide DSIP was so poor that the sample sizes of DSIP, bradykinin, and oxytocin used were respectively 20, 2, and 2 nM; for comparison of the data, the signal response for DSIP was then divided by a factor of 10. The average ratio of the MH^+ signal heights in scans taken over a period of 0–2 min after the initiation of bombardment was 1:8:20 (DSIP/bradykinin/oxytocin), i.e., a correlation of increasing signal response with increasing hydrophobicity. This conclusion was then reinforced by taking the hydrophilic DSIP peptide and comparing the MH^+ abundance with the MH^+ abundances obtained from DSIP dimethyl ester and DSIP diisopropyl ester (each run separately with 3.6 nM of material in 2 μL of glycerol containing 5% water). The average ratios of the MH^+ abundances, in scans obtained within 10 s of the initiation of bombardment, were 1:20:100 (DSIP/DSIP-Me_2/DSIP-Ip_2).

In the above comparison, DSIP was chosen not only because it is hydrophilic but also because its net charge at physiological pH is −2 [contrast bradykinin (+2) and oxytocin (0)]. It might therefore be argued that the poor MH^+ abundance from DSIP is associated with the inappropriateness of its normal charge for positive-ion FAB. We have established that this is not the case. In negative-ion FAB experiments, the relative $(M - H)^-$ abundances obtained for equimolar amounts of DSIP, bradykinin, and oxytocin (each run separately) were 1:18:20 1 min after the initiation of bombardment and 1:7:10 after 2 min. This experiment, carried out in the same matrix as that of the corresponding positive-ion experiment, shows that the signal intensity of $[M - H]^-$ increases markedly with increasing hydrophobicity as in the positive-ion experiment. This is despite the fact that an $(M - H)^-$ signal response determined largely by the net charge in a polar medium would lead to the counter expectation $[M - H^-]$ (DSIP) > $[M - H^-]$ (bradykinin). In these experiments, the signals due to the two hydrophobic peptides are rapidly depleted to the chemical "noise" level within 4 min of the commencement of xenon bombardment. In contrast, the much smaller signal due to $[M - H]^-$ of the hydrophilic DSIP remains almost constant for the first 4 min and then increases by a factor of 1.5–2 for a further 10 min. These results are comparable to those found in the analysis of a mixture containing both hydrophobic and hydrophilic components (Figure 1). The rapid depletion of signal is expected for peptide initially occupying the surface (hydrophobic), and a poor initial signal, followed by signal improvement and prolonged response, is expected for peptides initially below the surface (hydrophilic) of the matrix. A final check of the consistency of the model for DSIP analysis was carried out. DSIP, N-terminally acetylated DSIP (prepared with 1:1 Ac_2O/H_2O), and di-N-acetylated DSIP (containing *N*-Ac-Trp, when derivatization with 1:1 Ac_2O/pyridine was used) were run separately in equimolar ratios as before. The $[M - H]^-$ ratios, averaged over the first 2 min of scanning, were 1:4:8, i.e., increasing signal respose with increasing hydrophobicity as before.

The results for the two enzymic digests, considered in conjunction with the data for the FAB responses associated with isolated hydrophobic and hydrophilic peptides, have led us to study

(13) The use of higher concentrations of HCl causes the conversion of some amide side chains to the corresponding isopropyl esters.

two possibly important practical consequences.

First, the possibility that sufficiently hydrophilic peptides, even in the absence of hydrophobic components, might not give a detectable signal in FAB in early scans but give useful signals at later times. We have found this situation in studying a mixture of Ser-Pro-Gln-Pro-Ser-Arg-Arg-Gly-Ser*-Glu-Ser-Ser-Glu-Glu (B and B index +461) with the same peptide phosphorylated at the serine residue indicated by an asterisk.[14] With a sample containing ca. 1 nm of each of these peptides, no signals (MH^+) are detected above the noise during the first 2 min of bombardment. However, in the period 8–12 min, signals due both components are observed with a S/N ratio = 4:1.

Second, since pure hydrophilic peptides (positive B and B index) can appear in initial FAB scans (albeit with a poor signal response), it seemed clear that in principle they might not be completely suppresed in an enzymic digest. In a mixture, the degree of suppression of a hydrophilic peptide should depend on the actual positive magnitude of the index and the number of hydrophobic peptides and their degree of hydrophobicity. This being so, it appeared likely to be fortuitous in the two enzymic digests (Tables I and II) that observation or suppression of a peptide correlated so precisely with a negative or positive value of the Bull and Breese index. This doubt is also indicated by the data for the diisopropyl ester of residues 40–53 (**2**) from horse heart cytochrome *c* (seen in initial scans of the esterified mixture). Based on the reasonable assumption that the change $-CO_2^- \rightarrow -CO_2CH(CH_3)_2$ will produce a maximum index change of ~−1000 (the difference in the Bull and Breese values for aspartic acid and leucine), our index value for this derivatized peptide is +110. Hence, a peptide with a small positive value of the index is seen in initial scans of a mixture containing numerous hydrophobic components. To obtain more deta regarding this point, we carried out an experiment with a mixture of hydrophilic and hydrophobic synthetic peptides and carried out a third tryptic digestion (and subsequent isopropyl ester derivatization) on myoglobin.

An approximately equimolar mixture of the hydrophilic peptide Arg-Arg-Leu-Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-Arg-Gly (abbreviated to R--G; B and B index +136) and the hydrophobic peptide Met-Glu-His-Phe-Arg-Trp-Gly [adrenocorticotrophic hormone fragment 4–10, abbreviated ACTH(4–10); B and B index −97] was examined by positive-ion FAB using an aqueous glycerol matrix as before. The ratio of the MH^+ abundances was ~1:3 [R--G/ACTH(4–10)] in scans taken in times up to 3 min after the initial bombardment. In this mixture analysis, the component with a positive Bull and Breese index is seen, but as expected its signal is suppressed relative to that from the more hydrophobic component. The signal responses can be manipulated in the expected manner. The more hydrophilic peptide was converted to its tetraisopropyl ester derivative and this derivative then added to ACTH(4–10) to give an equimolar mixture; the MH^+ signal from the ester was approximately 10 times as abundant as that from ACTH(4–10).

The data for the tryptic digest of myoglobin (horse) are given in Table III. The data accord beautifully with the physical requirements of the model and emphasize points that should be borne in mind when analyzing an enzymic digest using this approach. First, di- and tripeptides with negative indices may not be observed in the analysis, especially in early scans; this is evidenced by the data for the four italicized peptides in Table III. The physical basis for this effect is clear; for very short peptides, the influences of the polar N^+H_3- and $-CO_2^-$ termini should be weighted heavily and yet are ignored in the index calculation. Second, the dividing line between "presence" and "absence" for all other peptides in this digest is not zero, as found in Tables I and II, but $\leq +51$ and $\geq +160$. Given this proviso, the correlation is perfect for 15 peptides ranging from tetrapeptides to hexadecapeptides in the mixture.

Table III. Bull and Breese Indices and FAB Analysis of Peptides Obtained from Tryptic Digestion of Myoglobin (Horse)

frag[d]	B and B indices[a]	MH^+	pres (+) or abs (−) in FAB MS[b,c]
GLSDGEWQQVLNVWGK	+3.8	1815	+
VEADIAGHGQEVLIR	+51	1606	+
LFTGHPQTLEK	−130	1271	+
FDK	*−150*	*409*	−
FK	*−530*	*294*	−
HLK	*−166*	*397*	−
TEAEMK	+286	708	−
ASEDLK	+160	662	−
HGVTVLTALGGILK*	−224	1378	+
GHHEAELK }	+329	920	−
PLAQSHATK }	+248	952	−
HK	+575	284	−
IPIK	−652	471	+
TLEFISDAIIHVLHSK	−434	1884	+
HPGNFGADAQGAMTK	+388	1501	−
ALQLFK	−502	748	+
NDIAAK	+288	631	−
YK	*−485*	*310*	−
ELGFQG	−12	650	+

[a] Defined as in Table I. [b] Spectra obtained in glycerol containing 1 M trichloroacetic acid. [c] As defined in Table I. [d] Peptide fragments are presented in order from the N-terminus, using the one-letter code for amino acids.

Other points of interest arise from the analysis of the digest. Lysine-71 (indicated by an asterisk in Table III) is followed by an additional lysine in the protein. Incomplete cleavage at lysine-71 is indicated by an MH^+ ion in the digest at 1506, appearing in accord with its negative index value. Additionally, when isopropyl ester derivatives of the peptide digest were analyzed, signals due to all peptides except the HK derivative were seen. This experiment also indicates that the Lys–Pro bond in the two contiguous peptides that are bracketed together in Table III is not in fact cleaved to a significant extent. The evidence for this is as follows; (i) MH^+ ions corresponding to isopropyl esters of peptides arising from cleavage at Lys–Pro are not observed; (ii) an MH^+ ion is observed at m/z 1979 corresponding to the triisopropyl ester derivative commencing GHH... and terminating in ...ATK.

Conclusion

The analysis by FAB mass spectrometry of peptides in a protein digest is greatly facilitated by (i) preparation of a derivative that will increase the hydrophobicity of the most polar peptides and (ii) repetitive scanning of the resultant mixture. Alternatively, in some cases it may help to separate the most hydrophilic peptides from the most hydrophobic by HPLC fractionation and separately assay the resulting fractions by FAB. Our data give convincing support to the concept that the most hydrophilic components of mixtures may be suppressed in FAB analysis because of their low concentrations in the surface of the matrix. Such low concentrations appear to be influenced by at least two factors. One is the intrinsic tendency of even a pure hydrophilic peptide to avoid the matrix/"vacuum" interface. The second is the preference, in a mixture of hydrophilic and hydrophobic peptides, of the hydrophobic component to occupy this interface and so to tend to suppress any signal that might otherwise arise from the low concentrations of the hydrophilic component at or near the interface.[15]

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(15) It should be noted that good FAB sensitivity is not found with *extremely* high hydrophobicity, e.g., as found in sterols and terpenes. The sample should not be so hydrophobic that the polar matrix is unable to break intermolecular bonds in the sample, since in such circumstances the dynamic behavior associated with theexpression of surface properties would not exist. Additionally, the polarity of the sample must be sufficient to support the internal solvation of charge in MH^+ or $(M - H)^-$.

Experimental Section

The FAB mass spectra were taken on a Kratos MS 50 mass spectrometer equipped with a magnet having a mass range of 10 000 Da at full (8-kV) accelerating voltage. The atom beam was provided with an Ion Tech FAB gun operating with xenon at 8 kV with a current of 30–40 μA. The magnet was typically scanned a rate of 100 s/decade.

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Stereochemically Constrained Peptides. Theoretical and Experimental Studies on the Conformations of Peptides Containing 1-Aminocyclohexanecarboxylic Acid

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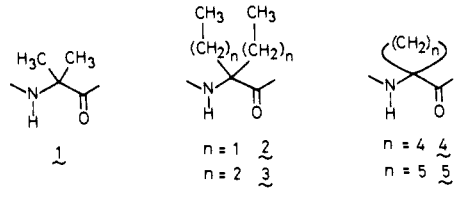
Abstract: Conformational energy calculations on the model system *N*-acetyl-1-aminocyclohexanecarboxylic acid *N'*-methylamide (Ac-Acc^6-NHMe), using an average geometry derived from 13 crystallographic observations, establish that the Acc^6 residue is constrained to adopt conformations in the $3_{10}/\alpha$-helical regions of ϕ, ψ space ($\phi = \pm 50 \pm 20°$, $\psi = \pm 50 \pm 20°$). In contrast, the α,α-dialkylated residue with linear hydrocarbon side chains, α,α-di-*n*-propylglycine favors fully extended backbone structures ($\phi \approx \psi \approx 180°$). The crystal structures of two model peptides, Boc-(Acc^6)$_3$-OMe (type III β-turn at -Acc^6(1)-Acc^6(2)-) and Boc-Pro-Acc^6-Ala-OMe (type II β-turn at -Pro-Acc^6-), establish that Acc^6 residues can occupy either position of type III β-turns and the $i + 2$ position of type II β-turns. The stereochemical rigidity of these peptides is demonstrated in solution by NMR studies, which establish the presence of one intramolecular hydrogen bond in each peptide in $CDCl_3$ and $(CD_3)_2SO$. Nuclear Overhauser effects permit characterization of the β-turn conformations in solution and establish their similarity to the solid-state structures. The implications for the use of Acc^6 residues in conformational design are considered.

The introduction of α,α-dialkylated amino acids into peptide chains provides a means of restricting the available range of backbone conformations.[1] The best studied member of this class of amino acids is α-aminoisobutyric acid (Aib).[2] The Aib residue (**1**) has been shown to strongly stabilize conformations in the $3_{10}/\alpha$-helical regions of the conformational map ($\phi \sim \pm 60 \pm 20°$, $\psi \sim \pm 30 \pm 20°$).[3] Peptides containing the residues α,α-diethylglycine (Deg; **2**) and α,α-di-*n*-propylglycine (Dpg; **3**) have been shown to occur in fully extended conformations ($\phi \sim 180°$, $\psi \sim 180°$).[4] Preliminary studies on fully protected peptides containing 1-aminocycloalkanecarboxylic acids (Acc^n, where n is the number of carbon atoms in the cycloalkane ring) suggest that both 1-aminocyclopentanecarboxylic acid (Acc^5, **4**)[5] and 1-aminocyclohexanecarboxylic acid (Acc^6, **5**) residues[6] stabilize folded conformations similar to those observed in Aib peptides. A synthetic chemotactic peptide analogue formyl-Met-Acc^6-Phe-OMe has been shown to possess significantly higher biological activity than the parent peptide, formyl-Met-Leu-Phe-OMe[7], stimulating interest in the conformational characteristics of Acc^6 residues. In this report we present conformational energy calculations on Ac-Acc^6-NHMe and compare the results obtained with similar computations for Aib[1a,8] and Dpg[4a,c] residues. An



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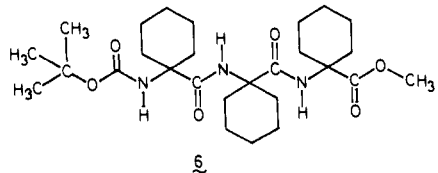
n = 1 **2**
n = 2 **3**

n = 4 **4**
n = 5 **5**



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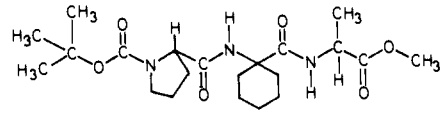

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(2) (a) Abbreviations used: Aib, α-aminoisobutyric acid; Acc^n, 1-aminocycloalkanecarboxylic acid with n atoms in the cycloalkane ring; Deg, α,α-diethylglycine; Dpg, α,α-di-*n*-propylglycine; Boc, *tert*-butyloxycarbonyl; Z, benzyloxycarbonyl; *p*BrBz, *p*-bromobenzoyl; *m*ClAc, monochloroacetyl; (b) All chiral amino acids used are of the L configuration. (c) Backbone torsion angles are defined according to the IUPAC–IUB Commission on Biochemical Nomenclature: *Biochemistry* **1970**, *9*, 3471–3479.

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