Successful Design and Synthesis of a Polarity-Triggered $\beta \rightarrow \alpha$ Conformational Switch Using the Side Chain Interaction Index (SCII) as a Measure of Local Stuctural Stability

Katja Gehenn, Rüdiger Pipkorn, and Jennifer Reed*

Department of Pathochemistry, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

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ABSTRACT: Certain sequences within proteins have the ability to undergo an abrupt cooperative conformational switch from β-strand to helix in response to decreasing polarity of the environment. This behavior was first observed at the CD4 binding site of the envelope glycoprotein gp120 of HIV-1, but evidence has accumulated that polarity-driven $\beta \rightarrow \alpha$ switches may be widespread, serving both to facilitate binding on protein/membrane or protein/protein contact and to signal that docking has occurred. The characteristics identified so far that distinguish switch sequences (a reverse turn at the N-terminus that acts as a helix initiation site, a conserved tryptophan residue downstream, and high potential for both the helix and β-fold) appear to be necessary but not sufficient, as some otherwise promising sequences found in data bank searches proved not to be capable of cooperative refolding. Analysis of existing switches has led to the development of the side chain interaction index (SCII) as a further parameter characterizing the $\beta \rightarrow \alpha$ polarity-driven switch. Data bank searches using this additional parameter have successfully identified a series of new potential switch sequences. All of them have in common the amino acid tetrad LPCR at the N-terminus and a tryptophan 5−20 residues C-terminal to it. Those with a high SCII as well, when synthesized and tested, exhibited strongly cooperative polarity-driven refolding. Control peptides, containing all other parameters but with a low SCII, did not. Using this new information, an artificial sequence was designed that had a high SCII as well as the initiation site, conserved tryptophan, and high P_α and P_β. When synthesized and tested, this sequence did in fact behave as a conformational switch, refolding cooperatively from β-fold to helix at a threshold value of 30% TFE. The successful design of a polarity-driven conformational switch opens the possibility of using this motif as a tool in protein engineering.

The "$\beta \rightarrow \alpha$ conformational switch" is a phenomenon in which some protein sequences are able to alter their conformation in response to changes in the polarity of the surrounding medium, reverting concertedly and abruptly from a β-type fold to a helix when the polarity is decreased below a critical level. This behavior was first seen in a 15-residue minidomain (LAV[1] peptide; sequence LPCRIKQFINMWQEV) of the envelope glycoprotein gp120 of HIV-1 (*1*), a domain responsible for the successful binding of the virus to the T-cell receptor CD4. The conformational flip plays a vital role in CD4 binding and is thus closely coupled to the biological activity of HIV-1. It was demonstrated that an N-terminal amino acid tetrad LPCR (*2*) with a high β-turn-forming potential plus a conserved tryptophan eight residues C-terminal to this tetrad was essential for the switch behavior of the LAV peptide and that both the switch and these amino acids were conserved throughout the various strains of HIV-1 (*3*). Data bank searches for sequences containing an LPCR tetrad and a tryptophan at a suitable position (5−15 residues) after the tetrad showed that this motif was not confined to HIV-1 and indeed might be widespread in proteins (*2*). Examinations of some of these other sequences showed that they exhibited switch behavior and led to the identification of another important characteristic: the residues between the LPCR tetrad and the tryptophan tended to have a fairly high potential for both β-sheet and helix structure (*3*). Certain discrepancies in the data suggested, however, that we did not yet have the complete story. Some promising sequences turned out not to be switches, although all the characteristics described above were present. Furthermore, initial attempts to build artificial peptides showing a switch behavior all failed (unpublished data).

Apparently, we were missing another necessary feature for the complete characterization of switch peptides. Closer examination of all functional switch motifs suggested a new common property, which we have dubbed the SCII (side chain interaction index; see Materials and Methods). This newly developed parameter is a measuring index for the probability of favorable side chain interactions within a sequence. As shown in Figure 1, the distribution of SCII values for the HIV 15mers and other naturally occurring peptides containing the LPCR tetrad and a W is significantly different

* To whom correspondence should be addressed. Telephone: (0049) 06221-423256. E-mail: j.reed@dkfz-heidelberg.de.

[1] Abbreviations: CD, circular dichroism; f-MOC, fluorenylmethoxycarbonyl; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethylgluronium hexafluorophosphate; HPLC, high-pressure liquid chromatography; LAV, lymphadenopathy-associated virus; MALDI, matrix-assisted laser desorption; *P*, probability; TFE, trifluoroethanol.

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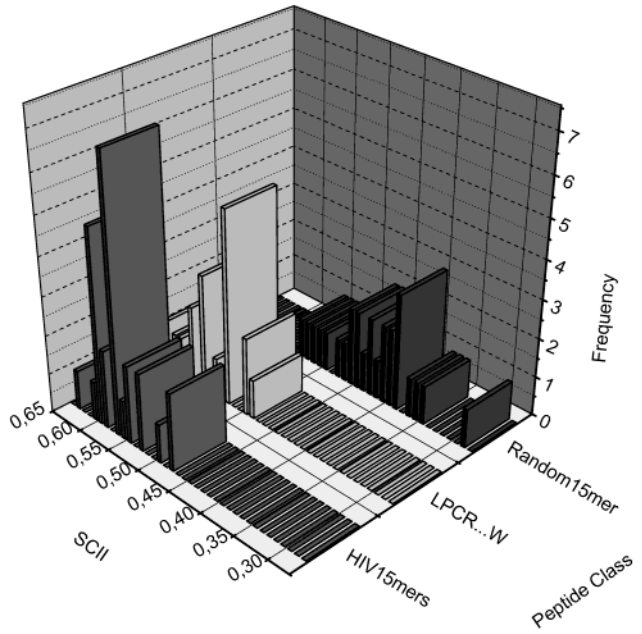

FIGURE 1: SCII values for the HIV 15mers, peptides containing LPCR...W, and random 15mers.

from that of random 15mers. It therefore appeared that an SCII value ≥ 0.5 could be important for characterizing switch peptides.

There have already been some indications from automated design of simple helical bundles or short β-sheet peptides that optimizing interactions leads to a more stable structure (*4*−*7*). The forces simulated have been restricted to van der Waals contacts, however. While these are easy to model, they are individually fairly weak and constitute only a fraction of the potential favorable interactions that can occur during folding. One can avoid the complexities of modeling all potential electrostatic and hydrophobic forces, at least at the initial stages of design, by relying on empirical data. The *Atlas of Protein Side Chain Interactions* (*8*) lists the natural propensities for any pair of side chains to interact. Drawn from actual structures in the protein data banks, this necessarily includes not only the classic forces (salt bridges, H-bonds, hydrophobic interactions) and more recently recognized cation−π and aromatic−amide interactions, etc., but has the advantage of including as well any aspects not yet discovered. These data were used to calculate the SCII.

Adding the SCII to the other known parameters allowed successful prediction of functional switch sequences in a data bank search. We have discovered two new peptides showing the typical switch behavior. Additionally, by adopting the newly developed SCII in addition to the other parameters, we have also succeeded for the first time in designing an artificial peptide that shows a strongly cooperative refolding from β-form to helix in response to altered polarity.

MATERIALS AND METHODS

Synthetic Peptides. The naturally occurring peptides LPCRAFGDVQLKWSKE (from pyruvate dehydrogenase phosphatase, rat), LPCRHVFCTACVVQRWRCP (adenylate cyclase regulatory protein, *Trypanosoma brucei brucei*), proposed from the sequence search as switch peptides, and LPCRSLSSHKLPWVTV (endothelial cell scavenger receptor precursor protein, human) as a control peptide were synthesized. The peptides were obtained from the Abteilung Zentrale Peptidsynthese, German Cancer Research Center, Heidelberg, Germany. All peptides were synthesized with the solid-phase peptide synthesis (SPPS) technique. The carboxyl group was activated with HBTU. The free α-amino group was blocked with f-MOC. Peptide purity was checked with HPLC and MALDI mass spectrometry and was >90% for all samples. In addition to the natural sequences, two designed peptides were constructed, one with the amino acid sequence LPCRQAQDVSMWIVG (called "KG") which fulfilled all criteria and should be a switch and one (LPCRQAHSTIRWSQL) which fulfilled all criteria except the SCII and serves as an additional negative control.

Sequence Search and Selection. The following parameters were calculated for all peptides and formed the basis of selection:

(1) Every peptide must contain the known helix initiation site LPCR at the N-terminus and a W at a suitable position (5−20 amino acids after the LPCR tetrad).

(2) The probabilities for forming secondary structure were calculated using folding algorithms (see below). Those for α-helix and β-sheet should be high and nearly equal. The probability for forming a reverse turn should be very small.

(3) The newly developed SCII (side chain interaction index; see below), proposed as an additional diagnostic tool in identifying switch peptides, should be ≥ 0.5 for all peptides except the control peptides.

Structure Probability Analysis. Analysis of the potential of primary sequences to form secondary structure elements was carried out using folding algorithms (*9*). P_α and P_β are the averaged probabilities of finding a particular residue in the α-helix or β-sheet, respectively. Turn probabilities were calculated as $P_t = f_i\, f_{i+1} f_{i+2} f_{i+3}$, where f_i, etc., is the statistical frequency of finding a given residue at position i to $i + 3$ of a reverse β-turn.

Side Chain Interaction Index. The SCII is a newly proposed measuring index for the probability of favorable side chain interactions within a sequence and is calculated as follows:

Using the *Atlas of Protein Side Chain Interactions* (*8*) for each residue within the peptide, the sum of favorable (i.e., 1.0 or greater) normalized contact propensities between it and each of the other 19 amino acids was calculated. Isoleucine, for example, has the following propensities equal to or greater than 1.0: Ile/Ala = 1.3, Ile/Cys = 1, Ile/Ile = 1.7, Ile/Leu = 1.5, Ile/Met = 1.4, Ile/Phe = 1.4, Ile/Trp = 1.3, Ile/Tyr = 1.0, and Ile/Val = 1.5, for a total sum of 10.8. The sum of favorable contact propensities between this residue and the other residues in the sequence was then calculated for each unique contact; i.e., if the residue concerned were an isoleucine and there were two alanines in the sequence between LPCR and W, the Ala/Ile propensity of 1.3 was only counted once rather than twice. The fraction of this sequence-specific sum divided by the first value was then calculated and assigned as the index number for that residue. Thus an index number of 0.75 would mean that of all the potential favorable contacts theoretically available to that residue, 75% were present in the sequence under investigation. This number was calculated for every residue within the peptide, no matter how many times it appeared. Thus for the example above, each Ala would have the same fraction since the proportion of potential favorable partners

Table 1: Overview for the Positive Peptides (Showing Switch Behavior) and for the Negative Controls

protein	sequence	P_α	P_β	P_t	SCII
(a) Positive Peptides					
pyruvate dehydrogenase phosphatase (PDP2_RAT)	**LPCR**AFGDVQLK**W**SKE	1.08	1.04		0.58
adenylate cyclase regulatory protein (ESA8_TRYBB)	**LPCR**HVFCTACVVQR**W**RCP	1.11	1.25	0.78	0.58
envelope gp120 (HIV-1)	**LPCR**IKQFINM**W**QEV	1.1	1.19	0.83	0.58
polygalacturonase (tomato)	**LPCR**DAPTALTF**W**NK	1.04	0.92	1.6	0.53
KG (artificial peptide)	**LPCR**QAQDVSM**W**IVG	1.08	1.07		0.6
(b) Negative Controls					
endothelial cell scavenger receptor precursor protein (human)	**LPCR**SLSSHKLP**W**VTV	0.93	1.01	1.38, 1.47	0.3
KG control (artificial)	**LPCR**QAHSTIR**W**SQL	1.03	1.04		0.43
artificial peptide	**LPCR**QLRSQLR**W**SQL	1.05	1.06	0.79	0.32
artificial peptide	**LPCR**LFKQLFK**W**LFQ	1.16	1.13		0.36

would be the same for both but each would be counted. The average of these fractional propensities was then calculated for the entire sequence. This number is the side chain interaction index, or SCII, and constitutes a measure of the degree to which favorable interactions are present in the sequence.

Circular Dichroism (CD) Spectroscopy. Circular dichroism spectra were measured using a Jasco J-710 automatic recording spectral polarimeter. The instrument was calibrated by use of 0.05% β-andosterone in dioxane. CD spectra were taken from 190 to 240 nm at a sensitivity of 100 mdeg/cm and a scanning speed of 5.0 nm/min with a 4.0 s time constant. Samples were routinely measured in a 1.0 mm quartz cuvette at 20 °C at a concentration of 100 μg/mL in TFE, respectively, MeOH increasing in 20% steps from 0% to 100% and with 10 mM Tris-HCl, pH 7.5, as the aqueous buffer. Where necessary for better focus, smaller steps were taken. Curves were signal-averaged with a minimum of four repeats and presented with a similarly signal-averaged buffer baseline subtracted. Spectra digitally recorded in millidegrees were converted to θ_{MR} (mean residue ellipticity) for curve fitting and secondary structure analysis.

Secondary Structure Analysis. Curves were fitted by using the computer program PEPFIT (*10*). This program is based on known CD spectra of model peptides, taking the mean distribution of amino acid side chains occurring in proteins into account.

Sequence Similarity Search. A search for the sequence LPCR and a W occurring 5−20 amino acids after the tetrad was carried out using the HUSAR module of the Biological Sequence Analysis program at the German Cancer Research Center to scan the Swiss Prot Data Bank.

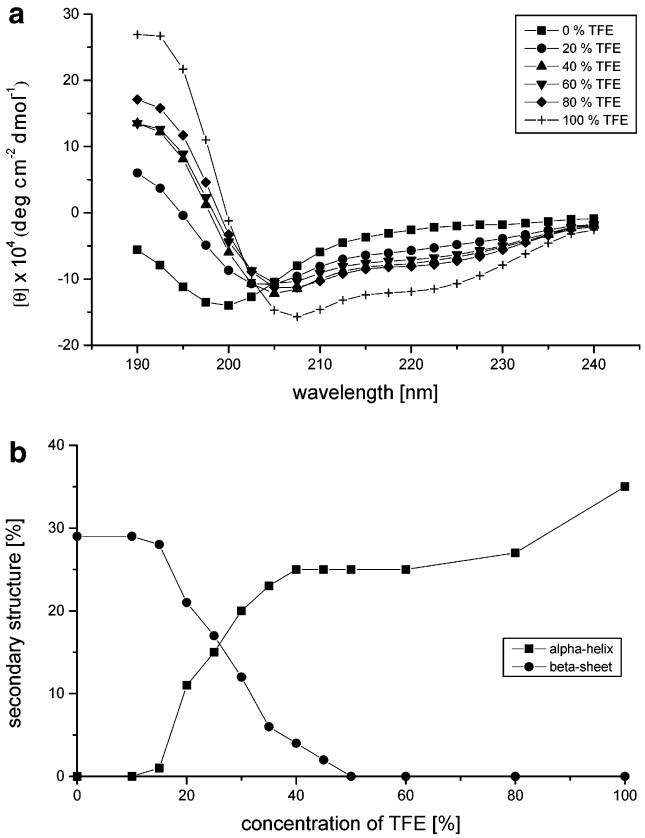

FIGURE 2: (a) Change in the far-UV CD spectrum of the PDP2_RAT peptide as a function of TFE concentration. TFE concentrations from 0% to 100% were measured. Shown are the results at 20% intervals. (b) Secondary structure content of the PDP2_RAT peptide (focus: titration at 10% intervals).

RESULTS

A search of protein data banks for sequences containing the N-terminal tetrad LPCR and a tryptophan residue 5−15 residues downstream resulted in 66 hits. Three of these sequences were selected for synthesis as peptides. Two of them (PDP2_RAT, ESA8_TRYBB) meet all of the parameters for switch peptides, including the new SCII; the third peptide (SREC_HUMAN, control) conforms to parameters 1 and 2 but has a much lower SCII (see Table 1).

Figure 2a shows the far-UV spectra of the first peptide (PDP2_RAT) under increasing concentrations of trifluoroethanol (TFE). The content of secondary structure is shown in Figure 2b. TFE is known to favor regular secondary structure by promoting the formation of intrachain hydrogen bonds. For most peptides the gain of regular secondary structure is more or less linear with increasing TFE concentration. Instead of a linear increase in secondary structure, the PDP2_RAT peptide starts out with a moderate level of β-sheet (~30%) and no helical structure. This peptide conformation remains unaltered until the TFE concentration reaches 20%. At this point an abrupt structural change takes place from primarily β-sheet to 25% helix. By 50% TFE this cooperative transition is complete, and there is no noticeable alteration in secondary structure content between 40% and 80% TFE. This concerted transition from one secondary structure to another, triggered at a particular cusp of polarity, is what we call the conformational switch.

Figure 3a shows the curves of CD measurement of our second predicted switch peptide (ESA8_TRYBB). The secondary structure content as a function of the TFE concentration derived from these is shown in Figure 3b. As

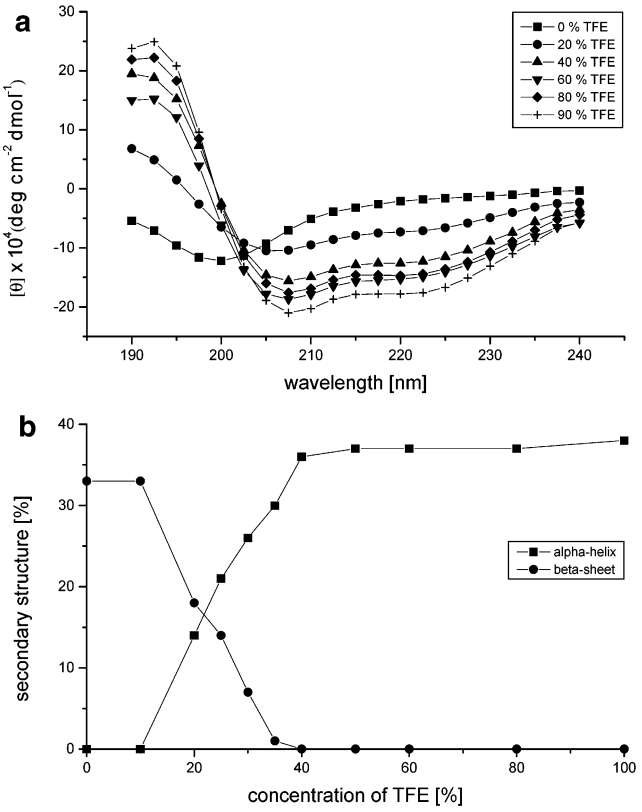

FIGURE 3: (a) Change in the far-UV CD spectrum of the ESA8_TRYBB peptide as a function of TFE concentration. TFE concentrations from 0% to 100% were measured. Shown are the results at 20% intervals. (b) Secondary structure content of the PDP2_RAT peptide (focus).

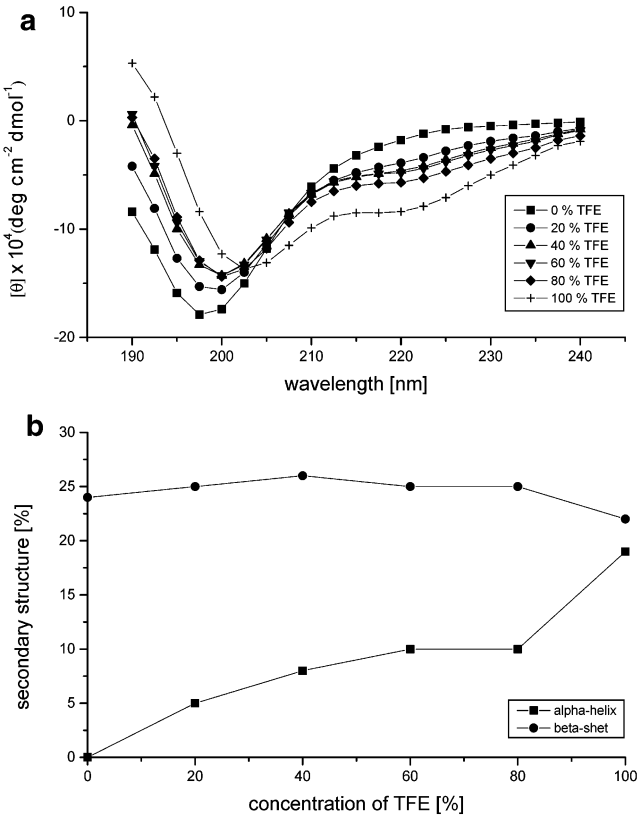

FIGURE 4: (a) Far-UV CD spectrum of the SREC_HUMAN peptide as a function of TFE concentration. TFE concentrations from 0% to 100% were measured. Shown are the results at 20% intervals. (b) Secondary structure content of the SREC_HUMAN peptide.

seen here the peptide contains 33% β-sheet and 0% helix at the beginning of the TFE titration. This structure remains until 10% TFE, at which point there is again a cooperative transition from β-sheet to helix at ~25% TFE. This process is complete at 40% TFE, and further increases in polarity up to 100% TFE do not change the content of regular secondary structure elements. Thus the second peptide with all three predictive characteristics also exhibits a strong switch behavior.

To test the assumption that the SCII is an indispensable parameter for characterizing switch peptides, it was necessary to measure control peptides with all other characteristics but having a lower SCII (prediction: "no switch"). The results of testing one of these peptides (SREC_HUMAN) are presented in Figure 4. Figure 4b shows clearly that there is no cooperative change in secondary structure. The content of β-sheet remains at 24% without noticeable alterations up to 100% TFE. The amount of α-helix increases over the whole range of TFE concentration. These results, two peptides with strong switch behavior having a high SCII and the control peptide with a low SCII showing no switch behavior, suggested that we might have identified all necessary parameters for characterizing a switch peptide. Furthermore, retroactive calculation of the SCII for the two failed attempts at designing synthetic switch sequences, LPCRLFKQLFKWLFQ and LPCRQLRSQLRWSQL, found values of 0.36 and 0.32, respectively. The SCII appears to have an excellent predictive value.

The next step was to confirm the method by designing an artificial switch peptide using these parameters. An additional refinement was to ensure that there was no other proline besides the one in the tetrad, because proline is a known helix breaker and is thus undesirable in an artificial switch peptide, and that charged amino acids (arginine and aspartate) were arranged in such a way that formation of a salt bridge should not occur either in the helical form or in the β-form of the peptide. The length of the peptide was modeled on the LAV peptide, with the tryptophan set at position 8 after the LPCR tetrad and the total length extended to 15 residues. The remaining amino acids (67% of the sequence) were arranged without reference to the LAV peptide, using only the established switch criteria as a basis for choice.

As seen in the CD spectra of our constructed peptide KG, there is a conspicuous jump between the 20% TFE curve and the 40% TFE curve (Figure 5a). The 20% curve does not show the two negative bands between 200 and 240 nm indicative of significant helix content, but these appear in the spectrum at 40%. The content of helix and β-sheet in the polar → apolar gradient is shown in Figure 5b. A cooperative transition is clearly visible at ~30% TFE. From a TFE concentration of 0% to 20% there is 33% β-sheet and no helical structure within the peptide. Then a rapid decrease of β-sheet to 0% takes place. Simultaneously, the content of the helix rises to ~34%. This process stops after 40% TFE, and there are no further alterations in secondary structure with decreasing polarity.

As TFE is quite apolar, the TFE/buffer gradient tends to be steep. A robust test of the cooperative nature of the switch transition is to retitrate the peptide with a "flatter" gradient such as methanol/buffer. The results of such a titration for

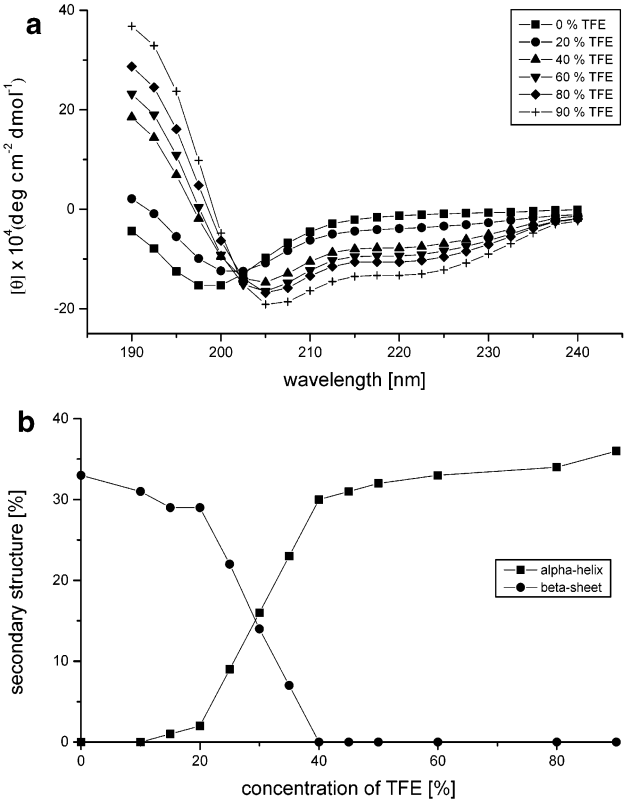

FIGURE 5: (a) Change in the far-UV CD spectrum of the KG peptide as a function of TFE concentration. TFE concentrations from 0% to 90% were measured. Shown are the results at 20% with respect to 10% intervals. (b) Secondary structure content of the KG peptide (focus: TFE).

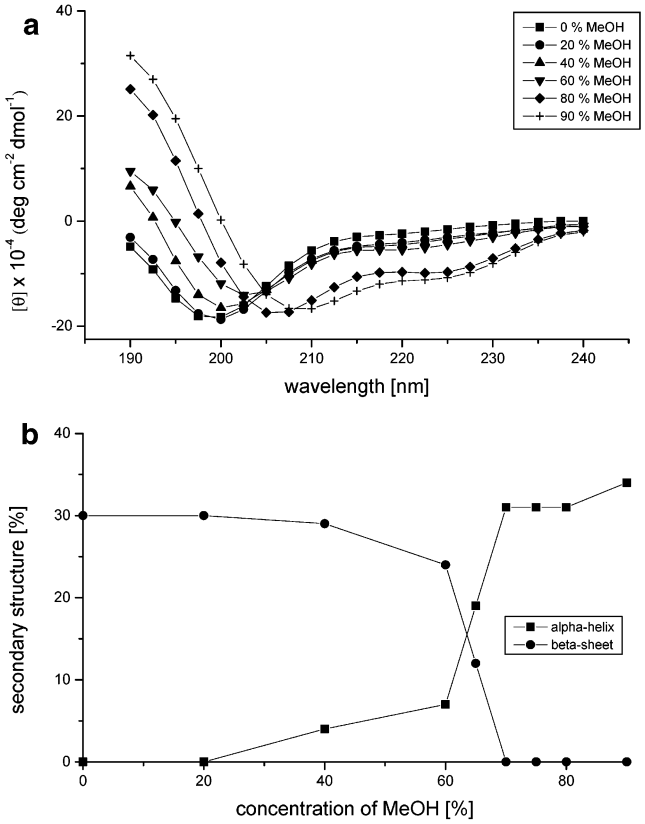

FIGURE 6: (a) Change in the far-UV CD spectrum of the KG peptide as a function of MeOH concentration. MeOH concentrations from 0% to 90% were measured. Shown are the results at 20% with respect to 10% intervals. (b) Secondary structure content of the KG peptide (focus: MeOH).

the designed peptide are presented in Figure 6. The change in structure from β-type to helical again occurs in a cooperative manner; the percentage of the apolar component at the critical point, however, is now higher, as might be expected given the higher polarity of methanol over TFE.

The results of the artificial control peptide (KG control) are shown in Figure 7. The transition between helix and β-sheet is quite linear. As predicted, the artificial control peptide shows no switch behavior.

DISCUSSION

Two main points have been established in the research presented here. The first concerns the SCII and its usefulness as a means of assessing the potential of a sequence to exhibit polarity-driven conformational switch behavior. The second concerns our understanding of the molecular mechanisms driving switch behavior as evidenced by the design of an artificial switch.

The concept of the SCII arose out of an apparent paradox that occurred during experiments with the original LAV switch peptide. It was discovered that the Trp residue was essential to cooperativity. Some form of interaction with the Trp residue allowed the β-fold to remain stable over a limited range of increasing TFE concentration, rather than converting to helix as a linear function of polarity. An overtly conservative substitution to Val had the effect of abolishing cooperative folding completely. Interestingly, substitution with Phe had an intermediate effect, suggesting that it was some form of aromatic interaction that was responsible. Charged to Ala substitutions were carried out on all residues capable of interacting with an aromatic side chain in an attempt to identify the partner, but the results indicated that multiple interactions were occurring (*3*). This seemed impossible, given the relatively weak interaction energies assumed at that time to be associated with aromatic rings. The work of Dougherty (*11*) resolved this paradox in that it showed that the Trp quadrupole was considerably stronger than predicted and might well be capable of establishing a network of interactions with several side chains at once. The hypothesis was formed that such a network acted to stabilize the β-fold until a critical polarity was reached. All of this suggested that a high level of side chain interaction might distinguish switch sequences, and the SCII was developed as an index of interaction potential. Application of the SCII to the switch sequences from all known HIV strains and to the non-HIV sequences discovered to date revealed that there was a statistically significant difference between the values for these and for an equal number of 15-residue sequences selected at random (Figure 1). It looked very much as though we had found a third identifying characteristic for switch peptides. The first test was to employ it to predict switch behavior in freshly selected natural sequences. The information from the new data bank search and CD tests for switch activity can be summarized as follows:

(1) The occurrence of switch peptides containing the helix initiation site LPCR at the N-terminus and a tryptophan residue at a convenient position after the tetrad is apparently a general phenomenon. Of the 66 hits for LPCR(5−20)W, 36 or 55% had a high P_α and P_β plus an SCII $\geq$0.5 and

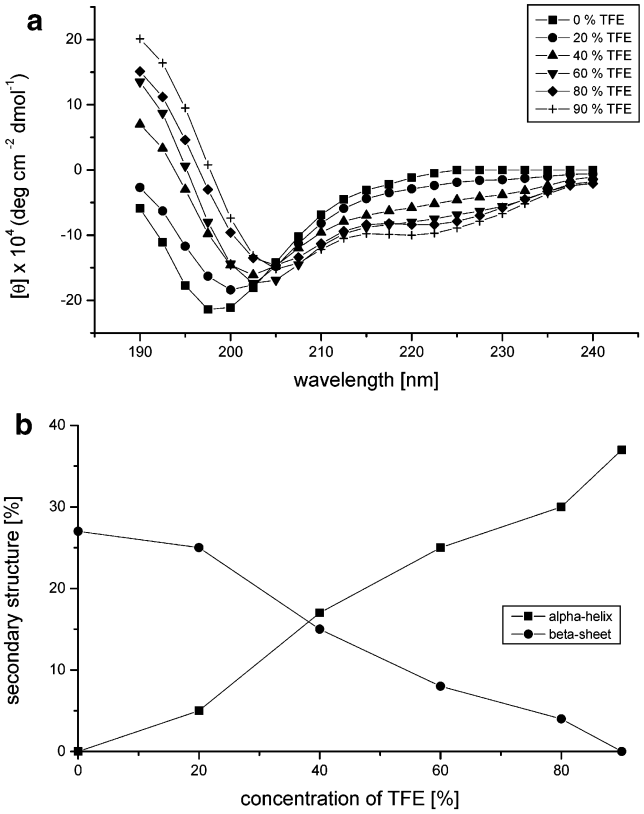

FIGURE 7: (a) Far-UV CD spectrum of the designed negative control peptide, KG control, as a function of TFE concentration. TFE concentrations from 0% to 90% were measured. Shown are the results at 20% with respect to 10% intervals. (b) Secondary structure content of the KG control.

would be predicted to show switch behavior. Two of them fulfilled all described parameters and were thus synthesized and tested and found to be $\beta \rightarrow \alpha$ conformational switches, thus adding pyruvate dehydrogenase phosphatase from *Rattus norvegicus* and the adenylate cyclase regulatory protein of *T. bruceii bruceii* to those already found in the envelope glycoprotein gp120 of HIV-1 and polygalacturonase from tomato (LPCRDAPTALTFWNK).

(2) The newly developed SCII is an essential parameter for characterizing and identifying switch peptides, and it has an excellent predictive value. The control peptide (found in human endothelial cell scavenger receptor precursor protein) does not show any switch behavior despite conforming to all known parameters except the high SCII. The same is true for the two earlier designed peptides that failed to show switch behavior and a newly designed artificial peptide with a low SCII. In total, five peptides with all parameters plus high SCII have been shown to exhibit switch behavior, and four peptides with all parameters except a high SCII do not (Table 1). Thus a high SCII is a further important criterion for the specification of switch peptides.

(3) Taking into account this third parameter of a high potential for interaction among the sequence side chains has resulted in the first successful design of an artificial peptide showing polarity-driven conformational switching.

The findings presented here open the possibility of designing proteins that will respond to a specific contact by changing their conformation in a predicted manner. Before the full potential can be realized, however, the following points have to be clarified:

(1) Does the switch behavior of the newly identified peptides play a physiological role, as it does in the env glycoprotein from HIV-1? Is it necessary for protein−protein or protein−membrane binding?

(2) The switch peptides found so far undergo the folding transition at different triggering polarities (i.e., LAV, ∼60% TFE; PDP2_RAT, ∼25% TFE; our own peptide, ∼30%). Which attributes are responsible for the different transition points? That is, what would allow us to design, for example, a conformational switch sensitive to protein−membrane rather than protein−protein contact?

(3) Do $\beta \rightarrow \alpha$ conformational switches exist with helix initiation sites differing from the LPCR tetrad? What "families" of conformational switches exist and do different families display different specificities?

Even at our present level of understanding, though, the $\beta \rightarrow \alpha$ conformational switch adds a new and, literally, flexible tool to protein engineering. In addition, the SCII offers a rapid method of scanning sequences that, having a high propensity for favorable interactions at a local level, may be capable of independent folding and achieve stability rapidly during the folding process.

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