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PROTEIN ANTIGEN CONFORMATION: FOLDING PATTERNS AND PREDICTIVE ALGORITHMS; SELECTION OF ANTIGENIC AND IMMUNOGENIC PEPTIDES

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ABSTRACT

Efforts to understand the nature of protein antigenicity have taken two major approaches: clarification of the precise amino acids responsible for a natural immune response against a protein, or attempts to induce antibodies using chemically synthesized peptide fragments. A considerable body of experimental results are now available on both approaches, allowing some conclusions to be made. In general, the conventional view that major antigenic sites contain concentrations of charged and polar residues is upheld. Furthermore the results show a striking agreement with the method for predicting antigenic determinants developed several years ago (HOPP and WOODS, 1981). Many groups have successfully used the prediction method either as a guide for testing the location of antigenic sites in whole proteins or in order to choose sequences suitable for the production of chemically synthesized peptide immunogens. Even when experimenters did not rely upon predictions, their results often identified antigenic sites that would have been predictable by the method. In fact, most of the findings for all well characterized major disease organisms could have been predicted by this procedure. These results, many of them obtained without reference to the method, serve as a strong indicator in favor of use of the method in future investigations, of antigenic determinants of proteins.

Our most recent experiments have demonstrated that a distinction can be drawn between the amino acids most important for antigenic determinant predictions (Asp, Glu, Lys and Arg) and those most likely to occur in highly exposed portions of a peptide chain (Gly, Pro, Asn and Ser). We are currently using a newly developed hydrophilicity scale (acrophilicity) for predicting surface portions of proteins, with better success than any other available procedure (see e.g. Fig. 1).

The new method is also superior for locating signal and transmembrane hydrophobic segments. We also use the older antigenic determinant prediction method to identify the subset of surface sites that are likely to be most antigenic. The sites predicted to be antigenic are also the most likely sites for other types of protein interactions. Examples identified so far include sites of proteolytic processing of precursor forms of proteins and hormones, sites of phosphorylation and glycosylation, as well as other sites such as nucleic acid interaction sites, a nuclear transport site, complement binding sites and cell binding sites.

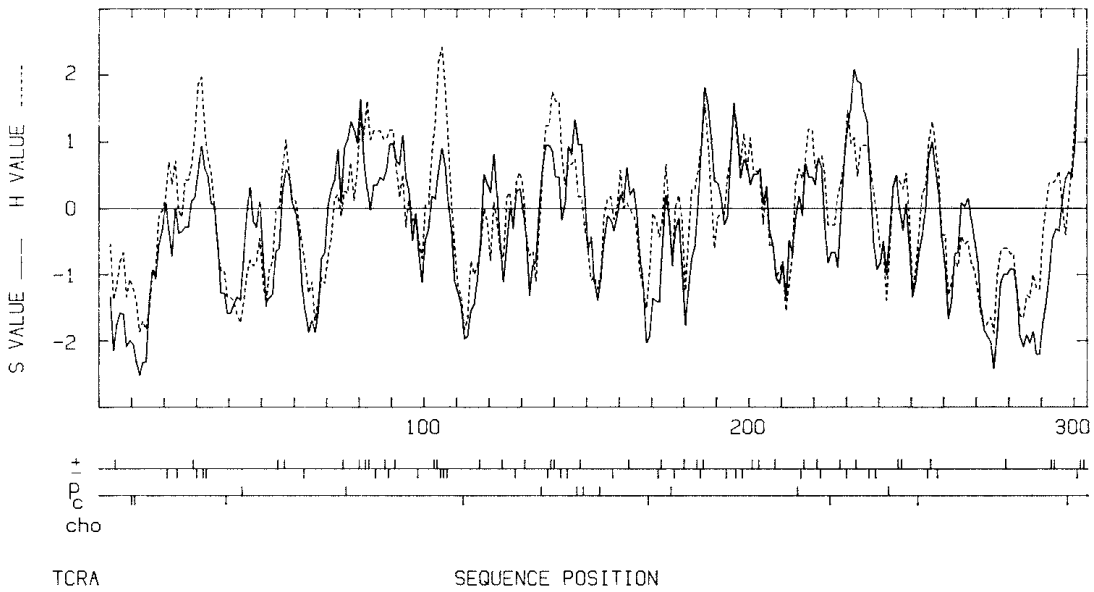

Figure 1 - Topological analysis of the T cell receptor A chain (TCRA). The solid line profile represents acrophilicity averages and the dotted profile represents hydrophilicity averages. The tick marks on the two lines below the plot indicate the following residues: (+) Arg, Lys, (–) Asp, Glu, (P) Pro, (C) Cys. The space below these lines (cho) is used to indicate Asn-X-Ser/Thr glycosylation sites, none of which occur in this molecule. The lowest acrophilicity values occur at the N terminus, and near the C terminus, and indicate the presence of a signal peptide and a transmembrane segment, respectively.

We are now using both methods simultaneously in order to identify the probable structural features of a protein and to locate the most likely functional regions as well.

HUMORAL IMMUNITY

Table I lists a number of proteins, including many viral and bacterial antigens, for which substantial investigations have been reported that describe the locations of antibody binding sites that occur in natural immune responses or in more artificial systems, such as hybridoma production. All of these results have identified antigenic sites that were predictable among the top hydrophilicity peaks for the particular protein. They represent the majority of all findings for these proteins and organisms, which serves to underscore the almost universal agreement of experimental findings with the hydrophilicity predictions. In cases where a second or third peak has been verified as antigenic but the highest peak has not, it is often the case that the investigators have not yet tested the most hydrophilic sequence; rarely is any data presented that disagrees with the postulation of antigenic activity at hydrophilic high points.

CELLULAR IMMUNITY

Although the hydrophilicity method was originally developed for use in locating antigenic determinants recognized by antibodies, it has become apparent that it is useful for considerably more than its original intent. As seen in Table II, there is a growing list of findings, for various cell mediated immunity phenomena that are specific for hydrophilic regions on protein antigens. These include reactions that probably involve recognition by the newly described T cell antigen receptor, and, in keeping with the antibody-like nature of this receptor it appears at this early stage that the same correlation of hydrophilicity and antigenicity will be found as is the case for antibody binding sites.

Table I - Humoral immune responses.

Hydrophilicity peak number	Antigen	Synthetic Peptide Used	Type of Response	Reference
1	Influenza hemagglutinin	+	Protective	MULLER *et al.* (1982)
1, 3	» »	+	»	SHAPIRA *et al.* (1984)
2	» »	+	Antisera, hybridomas	WABUKE-BUNOTI *et al.* (1984)
1, 2, 3	» »	–	Antigenic variation	WILEY *et al.* (1981)
1, 5	» »	+	Hybridomas	WILSON *et al.* (1984), HOPP and WOODS (1983)
1	Streptococcal M protein	+	Protective	BEACHEY *et al.* (1984)
1, 3, 5	Poliovirus VP1	+	Neutralizing	EMINI *et al.* (1983)
3	» »	+	»	WYCHOWSKI *et al.* (1983)
3	» »	–	»	EVANS *et al.* (1983)
1, 3	Foot-and-mouth disease VP1	+	»	BITTLE *et al.* (1982), PFAFF *et al.* (1982)
1	Hepatitis B surface antigen	+	Antisera	HOPP and WOODS (1981), PRINCE *et al.* (1982), BHATNAGAR *et al.* (1982)
3	» » »	+	»	DREESMAN *et al.* (1982)
3	» » »	+	Protective	GERIN *et al.* (1983)
2, 4, 5	» » »	+	Antisera	LERNER *et al.* (1981)
2, 3	Gonococcal pilin	+	»	ROTHBARD *et al.* (1984)
3	Herpes virus gpD	+	Neutralizing	COHEN *et al.* (1984)
1, 3, 5	Influenza neuraminidase	–	Antigenic variation	COLMAN *et al.* (1983)
1, 2	Yes and src gene products	+	Antisera	GENTRY *et al.* (1983)
1	Polyoma virus middle T antigen	+	»	ITO *et al.* (1983)
2	Histocompatibility antigen HLAB7	–	Alloantisera	LOPEZ DE CASTRO *et al.* (1983)
2	» » HLADR	+	Hybridomas	NIMAN *et al.* (1983)
2	Beta-2 microglobulin	–	Hybridoma	PARHAM *et al.* (1983)
1, 2	Scorpion toxin II	+	Antisera	GRANIER *et al.* (1984)
3, 5	Acetylcholine receptor	+	»	BARKAS *et al.* (1984)
1	Growth hormone	+	»	NERI *et al.* (1984)
1	IgG CH3 domain	–	Allotypic marker G1m	KEHOE and SEIDE-KEHOE (1979)
1	Interferon alpha	+	Antisera	ARNHEITER *et al.* (1983)
1	Interleukin 2	+	»	ALTMAN *et al.* (1984)
1	Myoglobin	+	Hybridoma	SCHMITZ *et al.* (1983)
1, 3	Metallothionein	–	Autoimmune antisera	WINGE and GARVEY (1983)
1	Tubulin	–	Hybridoma	WEHLAND *et al.* (1984)

Table II - Cellular immune response

Hydrophilicity peak number	Antigen	Synthetic Peptide Used	Type of Response	Reference
1	Influenza hemagglutinin	+	T cell proliferation	LAMB *et al.* (1982)
2	» »	+	Cytolytic T cells	WABUKE-BUNOTI *et al.* (1984)
1	Insulin A Chain	–	T cell proliferation	SHAPIRO *et al.* (1984)
1	Ragweed allergen RA5	–	Allergenic determinant	ROEBBER *et al.* (1983)
1	Myelin basic protein	+	Encephalitis	HASHIM (1978)
1	Cytochrome c	+	Delayed type hypersensitivity	WANG and REICHLIN (1979)
1	Myoglobin	+	MIF secretion	STAVITSKY *et al.* (1975)
1	Histocompatibility antigen H2 K^{bm1}	–	Gene conversion antigenic site	SCHULZE *et al.* (1983)

One further implication of this observation is that it should not be too difficult to adapt our findings for synthetic vaccine production of humoral responses to the question of synthetic vaccine stimulation of cell-mediated immunity, if these two phenomena are both related to antigenic sites that are predictable by hydrophilicity analysis.

OTHER TYPES OF SITES

The expression of antigenicity by the product of a viral or bacterial gene is not only a result of the folded chain conformation, but may also be influenced by post-translational modifications. Hence, it is important to know the existence of sites of proteolytic removal of portions of an antigen that might come into play during maturation of an antigen, or of the existence of possible antigenic modifications due to derivatization of the peptide chain, for example by phosphorylation, glycosylation, acetylation or other post-translational processing events. In this light, Table III and IV are of interest because, even though they represent a review of only a small portion of the current literature, they demonstrate that a surprisingly diverse array of post-translational events are indeed associated with the most hydrophilic regions of protein sequences.

The location of the proteolytic cleavage that activates the membrane penetrating ability of influenza virus turns out to be the highest hydrophilic peak for several strains of influenza, and a similar processing site occurs in the envelope glycoprotein of the acquired immunodeficiency syndrome (AIDS) associated virus HTLV type II.

Table III - Proteolysis sites.

Hydrophilicity peak number	Protein	Type of Site	Reference
1	Influenza hemagglutinin	Fusion activation processing	HOPP and WOODS (1983)
1	HTLV envelope glycoprotein	Proteolytic processing	SODROSKI *et al.* (1984)
1, 2	Proinsulin	C peptide removal	NBRF database *
1	Cholecystokinin	Processing and amidation	GUBLER *et al.* (1984)
2	Neuropeptide tyrosine	» »	MINTH *et al.* (1984)
1, 2, 3, 4	Alpha factor	Processing	KURJAN and HERSKOWITZ (1982)
2	Bovine growth hormone	Single thrombin site	LIBERTI and DURHAM (1983)
3	Rat growth hormone	Spontaneous proteolysis	MACIAG *et al.* (1980)
4	Human growth hormone	Single plasmin site	RUSSEL *et al.* (1981)
4	Atrial natriuretic factor	Processing	NAKAYAMA *et al.* (1984)
1	Human factor X	»	LEYTUS *et al.* (1984)
1	Trypsinogen	Activation	NBRF database
2	Bovine protein C	Processing	» »
1	Complement C4	»	OGATA and SEPICH (1984)
1	Sex limited protein (Slp)	»	» » »
5	Urokinase	Activation	NBRF database
1	Kappa casein	Single chymosin site	» »
1	Phosphorylase a	Subtilisin limited proteolysis	FLETTERICK and MADSEN (1980)
2, 4	Staphylococcal nuclease	Tryptic limited proteolysis	COTTON *et al.* (1972)
2	Citrate synthase	Chymotryptic and subtilisin limited proteolysis	LILL *et al.* (1984)

* Protein sequence database, National Biomedical Research Foundation, Georgetown University Medical Center, 3900 Reservoir Road, Washington D.C. 20007.

The other examples listed in Table IV indicate that other types of protein modifications may be locatable by hydrophilicity analysis as well. It appears that hydrophilicity may be one factor that is involved in the preferential glycosylation of one Asn-X-Thr/Ser site over another in cases where not all of the potential glycosylation sites of a protein are glycosylated.

Table IV - Post-translational modification sites.

Hydrophilicity peak number	Protein	Type of site	Reference
1	Phosphorylase a	Single phosphorylatable serine	FLETTERICK and MADSEN (1980)
1	Polyoma virus middle T antigen	Phosphorylatable tyrosine	HUNTER *et al.* (1984)
1	Troponin I	Phosphorylatable threonine	NBRF database
3	Histone H2A	Phosphorylated serine	» »
1	Major histocompatibility antigens (cytoplasmic)	» »	TYKOCINSKI *et al.* (1984)
3, 4	Alpha-S1 casein	Phosphorylation	NBRF database
2, 3	Human factor IX	Gamma-carboxyglutamic acid	» »
3	Bovine factor X	» »	» »
4	Bovine protein C	» »	» »
1	Hepatitis B surface antigen	Preferred CHO site	PETERSON (1981)
1	Tubulin	Tyrosine ligase	WEHLAND *et al.* (1984)
1	Troponin I	Acetyl N terminus	NBRF database
2	Myelin basic protein	» » »	» »
3	Histones H2A and H4	» » »	» »
3	Cytochrome c	» » »	» »
1, 3	Histone H4	Acetyl lysine	» »
3	Histone H2A	» »	» »
1	Histone H4	Methyl lysine	» »
1	Calmodulin	Trimethyl lysine	WATTERSON *et al.* (1980)

Other types of derivatizations also appear to be predictable, including sites of phosphorylation at Ser, Thr and Tyr residues, and identification of methylatable or acetylatable lysine residues.

In addition to the above-mentioned hydrophilic sites, there are other types of protein interaction sites that are correlated with hydrophilicity. These may have varying degrees of relevance to immune responses, but will be mentioned here for the sake of completeness.

Table V demonstrates that hydrophilic sites seem to be involved in a great number of different types of interactions of proteins with other molecules around them. This implies that the antigen-antibody interaction represents a special case of the more general phenomena relating protein interaction sites to the highly exposed, hydrophilic sequences that make them up.

Table V - Other protein interaction sites.

Hydrophilicity peak number	Protein	Type of Site	Reference
1	IgG CH2 domain	C1q binding site	PRYSTOWSKY *et al.* (1981)
3	Fibronectin cell binding domain	Cell binding site	PIERSCHBACHER and RUOSLAHTI (1984)
1	Tubulin	MAP binding	SERVANO *et al.* (1984)
1	Band 3 protein N domain	Hemoglobin binding	WALDER *et al.* (1984)
2	Large T antigen of SV40	Nuclear transport	LANFORD and BUTEL (1984), KALDERON *et al.* (1984)
1, 2	Tobacco mosaic virus	RNA binding	BLOOMER *et al.* (1978)
2	DNAbp II (*B. stearothermoph*.)	DNA binding	TANAKA *et al.* (1984)
2	ssDNAbp *(E. coli)*	DNA binding	WILLIAMS *et al.* (1983)
4	T4 gene 32 protein	» »	WILLIAMS and KONIGSBERG (1978)
1, 3	Troponin C	Calcium binding	NBRF database
3, 4	Calmodulin	» »	WATTERSON *et al.* (1980)
2	Concanavalin A	Manganese binding	REEKE *et al.* (1975)
4	» »	Calcium binding	» » »

IMPROVING THE METHOD

Because hydrophilicity analysis, as it was originally published, was incapable of predicting the locations of all known antigenic sites, considerable effort has been made by our group to find ways to increase the prediction success rate. Only one of the many variations has yielded an improved success rate; this is an upward adjustment of the hydrophilicity values of the N and C termini in recognition of the fact that these are typically highly exposed and are often involved in antigenic determinants. This improved method still correctly predicts antigenic determinants with a 100% success rate at the highest hydrophilic peak and now is substantially more successful in lower peaks, so that it is worthwhile to consider antigenicity to be likely at all of the top three peak hydrophilicity regions of a protein sequence.

By way of comparison, the only other published method that has been used to predict the locations of antigenic sites, that of KYTE and DOOLITTLE (1982), has only a 60% success rate among the top three peaks while our method now has an 80% success rate.

A DISTINCTION BETWEEN SURFACE EXPOSURE AND ANTIGENICITY

Our attempts to improve hydrophilicity analysis made it clear that antigenic sites are a subset of surface sites, usually characterized by the presence of one or more charged amino acids. This led to the development of a new scale of amino acid values based on the frequency of occurrence of each amino acid in regions of proteins that are highly exposed on protruding portions of the peptide chain. We call these the acrophilicity values to reflect the fact that they express the "height-loving" nature of the amino acids.

As seen in Table VI, the two scales are similar in their lower halves; that is, the hydrophobic amino acids are also acrophobic. However, at the tops, the two scales are very different. While the charged residues dominate the top of the hydrophilicity scale, it is the small amino acids that dominate the acrophilicity scale. In fact, the acrophilicity scale is essentially a size scale, with only minor exceptions. Because this scale was obtained by direct observations of 49 crystallographically determined protein structures (HOPP and MERRIAM, in preparation) it is clear that protein folding is strongly influenced by the size of local groups of amino acids, and that this effect is at least as important as hydrophilic/hydrophobic considerations.

A comparison of our new scale to the hydropathy scale of KYTE and DOOLITTLE (1982) and the turn prediction values of CHOU and FASMAN (1978) demonstrated that neither of these methods are very useful for finding highly exposed sites, having 70 and 76% success among their top five peaks respectively, while our scale yielded a 91% success rate.

We also found that it is possible to compensate for the ambiguous nature of glycine and serine (which occasionally occur in buried positions) by varying their values according to the nature of neighboring residues. This makes it possible to identify signal peptides and transmembrane segments without compromising the surface predictions.

Table VI - Comparison of hydrophilicity and acrophilicity values.

Hydrophilicity		Acrophilicity	
Asp	3.0	Gly	3.0
Glu	3.0	Pro	2.6
Lys	3.0	Asn	2.3
Arg	3.0	Asp	2.1
Ser	0.3	Ser	1.8
Asn	0.2	Lys	1.4
Gln	0.2	Glu	0.5
Gly	0.0	Arg	0.3
Pro	0.0	Thr	–0.1
Thr	–0.4	Gln	–0.2
His	–0.5	His	–0.4
Ala	–0.5	Ala	–0.5
Cys	–1.0	Val	–1.7
Met	–1.3	Met	–1.8
Val	–1.5	Tyr	–2.0
Leu	–1.8	Leu	–2.5
Ile	–1.8	Ile	–2.5
Tyr	–2.3	Cys	–2.6
Phe	–2.5	Phe	–2.7
Trp	–3.4	Trp	–3.0

We are currently using acrophilicity analysis to determine the topological features of proteins, and then using hydrophilicity analysis to select the subset of surface sites that are likely to be antigenic, or involved in other types of protein interactions. A copy of our computerized version of this analysis is available on request.

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