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Short communication

Plant antigens cross-react with rat polyclonal antibodies against KLH-conjugated peptides

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Abstract

Keyhole limpet hemocyanin (KLH)-conjugated peptides are routinely used to raise polyclonal antibodies for biochemical or immunolocalization studies. Rats are suitable for producing antisera against plant antigens as they often lack non-specific response towards plant materials. We attempted to obtain rat antisera against peptides derived from several plant proteins. However, most antisera recognized the same background KLH-related plant antigen (KRAP) in *Arabidopsis* and tobacco. We characterized KRAP with respect to size and cellular localization and examined possible antigen-specific reasons for the failure of most immunizations. We also found no reports of successful use of rat anti-KLH-peptide antibodies in plant studies. We thus believe that the rat-KLH:peptide system is poorly suited for production of antibodies, especially against plant antigens, and should be used with caution, if at all.

Keywords: Keyhole limpet hemocyanin; Rat antisera; Synthetic peptides; Cross-reactivity; Plant antigens

1. Introduction

Polyclonal antibodies against synthetic oligopeptides are routinely used in biochemistry and molecular biology. Since molecules <12 kD do not efficiently elicit vertebrate immune responses, peptide antigens have to be conjugated prior to immunization to a suitable carrier, such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). KLH is a large copper-containing glycoprotein from the marine mollusk, *Megatura crenulata*, that carries a number of lysine residues suitable for chemical coupling of peptides, and triggers a strong immune response producing antibodies with very low or no non-specific cross-reactivity (Dixon et al., 1966; reviewed in Harlow and Lane, 1988). Immunogenic peptide design is guided by several criteria, such as accessibility on the surface of the molecule (especially if the antisera are to be used for in situ immunolocalization), and good solubility, which often represents the limiting factor.

Polyclonal antisera from herbivorous animals, such as rabbits and chicken, often display non-specific background reaction towards plant antigens, while rat antisera usually exhibit minimal such cross-reactivity. This makes rats the animals of choice for production of antibodies in plant biology. Moreover, the amount of antigen needed for immunization is relatively small, the yield of sera is sufficient for most applications, and commercially available anti-rat secondary antibodies do not cross-react with anti-mouse primary antibodies, making double labeling experiments possible.

We have obtained rat antisera against a series of oligopeptides derived from the sequences of several plant proteins. Unexpectedly, we found that most of our antisera recognize the same conserved plant antigen unrelated to the peptides used for immunization, while no peptide-specific antibodies

Abbreviations: BSA, bovine serum albumin; FH2, formin homology 2; KLH, keyhole limpet hemocyanin; KRAP, KLH-related antigen of plants; PLD, phospholipase D; SEC, size exclusion chromatography.

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were detected. We present the results of the preliminary characterization of this background KLH-related antigen, and examine the possible peptide sequence-related causes of the systematic failure of specific antibody production.

2. Materials and methods

2.1. Peptide design and antibody production

Antigens and peptide sequences are summarized in Table 1.

For formin-derived antigens, sequence conservation and predicted 3D-structure were used as primary criteria. 3D models of the AtFH3 and AtFH16 FH2 domains were generated by threading the antigen sequence onto a known FH2 domain structure (PDB: 1UX5) as described elsewhere (Cvrčková et al., 2004; Grunt et al., 2008); for the PTEN domain, published models were used (Cvrčková et al., 2004). Hydrophilicity determined according to Hopp and Woods (1981) was considered as a secondary criterion. For the remaining antigens and for comparison purposes, predictions of hydropathy (Kyte and Doolittle, 1982) and antigenicity (Welling et al., 1985) were performed at http://www.expasy.org/tools/pscale, antigenicity predictions using Kolaskar and Tongaonkar's (1990) method at http://bio.dfci.harvard.edu/Tools/antigenic.pl. Secondary structure and exposed residues predictions, performed at the Predict Protein server (Rost et al., 2003) were taken into account as secondary criteria.

Peptides were synthesized and polyclonal rat antibodies produced commercially by Moravian Biotechnology (Brno, Czech Republic). Peptides were prepared on a PE Biosystems Pioneer synthesiser using the Fmoc/tBu method (Chan and White, 2004). Acylation reactions were carried out using amino acids activated with HBTU in the presence of HOBt and DIPEA. Amino acids, resins and solvents were purchased from Novabiochem, Applied Biosystems and Biosolve, respectively. Cleavage of peptide from the resin and side chain deprotection were carried out by treatment of peptidyl resin with TFA/TIPS/water (95:2, 5:2,5). Synthetic peptides were coupled to KLH (for immunization) and BSA (for screening of animal response) using a single step coupling protocol (Harlow and Lane, 1988). The peptide CLN was conjugated to BSA via an added cysteine to improve solubility, as well as to KLH, for immunization. Peptide conjugation to KLH/BSA via cystein was carried out using MBS (*m*-maleimidobenzoic acid *N*-hydroxysuccinimide ester) activated KLH/BSA (Pierce). For each peptide, 3 rats were immunized using standard procedures (Harlow and Lane, 1988). Animal response was screened using a dot blot assay as follows; peptides coupled to either BSA or KLH, as well as carrier-only controls (all at 20 μg/ml in PBS), were bound to nitrocellulose membrane strips for 2 h at 37 °C; the following steps were performed at room temperature. After blocking in 10% fetal calf serum (FCS) in D-MEM for 2 h, 2 μl aliquots of serially diluted sera were spotted on antigen-coated membranes, incubated for 2 h, washed 3× in PBS, incubated with horseradish peroxidase-conjugated goat anti-rat antibodies (Sigma A9037) diluted 1:100 in D-MEM plus 10% FCS, washed 3 × 5 min in PBS and developed using 1, 4-chloronaphtol.

To remove antibodies against the KLH carrier, selected antisera were commercially affinity-purified on a KLH-containing column (Hena s.r.o., Czech Republic).

2.2. Plant materials

Pollen of *Nicotiana tabacum* cv. Samsun was harvested, stored and germinated in a medium containing 10% sucrose and 0.1% H_3BO_3 for 1–2 h as described previously (Potocký et al., 2003). *Arabidopsis thaliana* suspension cultures, *N. tabacum* BY2 cells and *A. thaliana* Col-0 plants were maintained and harvested as described (Hála et al., 2005; Synek et al., 2006).

Table 1
Peptides used in this study, corresponding target antigens (with Uniprot or Genbank accessions in brackets), immunization outcomes (see Section 3.2) and relevant peptide parameters (see Section 3.4). N.A. – not available (tobacco has at least 2 isoforms; *Arabidopsis* homologue is 90 kDa); KT – method of Kolaskar and Tongaonkar (1990); * – possible specific reaction detected by dot blot, but only KRAP seen on Western blots (further characterization is in progress). For each peptide/recognized antigen combination, the number indicates the number of positive antisera (out of 3 rats). No major differences in probability of surface exposure or secondary structure were predicted

Peptide name	Peptide sequence	Target			Recognized antigens			Peptide parameters		
		Antigen	Predicted size (kDa)	Domain	KRAP75	KRAP90	Specific	Antigenicity		Hydropathy
								Welling	KT	
NVT	NVTTEEVVDAIKEGNELPVELL	AtFH3 (O23373)	65–70+	FH2 (specific)	3	1	1?*	−0.2	1.06	0.04
GRS	GRSSLTWPAERFLKIL				3	1	0	−0.18	1.07	0.04
DEL	DELQIQYGESQTAE	AtFH16 (Q9FF15)	79	FH2 (specific)	1	1	0	−0.49	1.01	−0.61
TED	TEDVFGGPDHNIDD				2	0	0	−0.22	0.97	−0.85
CLN	CLNRDEVDTLWHIKE	AtFH13 (Q9LVN1)	140	PTEN (conserved)	2	1	0	−0.13	1.02	−0.6
GEG	GEGGCRPIFRIYGQD	AtFH18 (Q9SK28)	123	PTEN (conserved)	1	0	0	−1	1.01	−0.42
LFL	LFLEFGNGDDSNSQLASVT	AtExo70A1 (NP_195974)	72	Internal	3	1	0	−0.38	1.00	−0.4
LER	LERLLGELFEGKSMNEPR	AtExo70A1 (NP_195974)		C-terminal	3	3	0	−0.36	0.98	−0.57
GDL	GDLELHIVHARHLPN	NtPLDδ	N.A.	N-terminal	1 (weak)	0	2	0.49	1.08	−0.06
QEL	QELKSSQLKDVHPSD	NtPLDδ	N.A.	Internal	0	0	1 (weak)	0.39	1.06	−0.83