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Heterologous screening of hybridomas for the development of broad-specific monoclonal antibodies against deoxynivalenol and its analogues

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Abstract

Hapten heterology was introduced into the steps of hybridoma selection for the development of monoclonal antibodies (MAbs) against deoxynivalenol (DON). Firstly, a novel heterologous DON hapten was synthesized and covalently coupled to proteins (i.e., bovine serum albumin (BSA), ovalbumin and horseradish peroxidase) using the linkage of cyanuric chloride (CC). After immunization, antisera from different DON immunogens were checked for the presence of useful antibodies. Next, both homologous and heterologous enzyme-linked immunosorbent assays (ELISAs) were conducted to screen for hybridomas. It was found that heterologous screening could significantly reduce the proportion of false positives and appeared to be an efficient approach for selecting hybridomas of interest. This strategy resulted in two kinds of broad-selective MAbs against DON and its analogues. They were quite distinct from other reported DON-antibodies in their cross-reactivity profiles. A unique MAb 13H1 derived from DON-CC-BSA immunogen could recognize DON and its analogues in the order of HT-2 toxin > 15-acetyl-DON > DON > nivalenol, with IC_{50} ranging from 1.14 to 7.69 µg/mL. Another preferable MAb 10H10 generated from DON-BSA immunogen manifested relatively similar affinity to DON, 3-acetyl-DON and 15-acetyl-DON, with IC_{50} values of 22, 15 and 34 ng/mL,

respectively. This is the first broad-specific MAb against DON and its two acetylated forms and thus it can be used for simultaneous detection of the three mycotoxins.

Keywords: hapten heterology; competitive ELISA; cross-reactivity

Abbreviations: MAb, monoclonal antibody; DON, deoxynivalenol; BSA, bovine serum albumin; CC, cyanuric chloride; ELISA, enzyme-linked immunosorbent assay; 3-ADON, 3-acetyl-DON; 15-ADON, 15-acetyl-DON; DON-3-G, DON-3-glucoside; NIV, nivalenol; FUS-X, fusarenon-X; DOM, the de-epoxy metabolite of DON; TMB, 3,3',5,5'-tetramethylbenzidine; OVA, ovalbumin; HRP, horseradish peroxidase; SecAb-HRP, rabbit anti-mouse IgG secondary antibody labeled with HRP; PBS, phosphate buffered saline; CBS, carbonate buffered saline; CR, cross-reactivity.

1. Introduction

Monoclonal antibodies (MAbs) are powerful biological tools for the modern analytical sciences. Until now, hybridoma technology is still the most common method for MAb development, in which hybridoma screening is a key step to select high-affinity MAbs but rather a labor-intensive and time-consuming process. An ideal screening method should be fast, reliable and easy to accomplish. For generating MAbs against low-molecular-weight analytes (haptens, e.g., pesticides, antibiotics and mycotoxins), one of the traditional methods for hybridoma screening is homologous indirect enzyme-linked immunosorbent assay (ELISA), i.e. the coating hapten is the same as the immunizing hapten. However, through this way, a high proportion of false positives are frequently encountered (Cervino et al., 2008). The problem can be associated with the fact that many of the initially selected positive clones could have strong affinity to the spacer arms or the whole hapten-arm regions, which led to poor or even no inhibition by the free target compounds (Kim et al., 2003). Since hapten heterology is often employed to modify the performance of competitive immunoassay for small molecules (Kim et al., 2003; Wang et al., 2011), it can also be introduced into the steps of hybridoma selection. To date, using heterologous ELISAs for hybridoma screening is yet limited to very few kinds of MAb generation (Liu et al., 2010; Muldoon et al., 2000; Shim et al., 2010; Wang et al., 2010). Hence, in the present study, we attempted to explore the applicability of heterologous screening for hybridoma selection.

Deoxynivalenol (DON; vomitoxin) was the molecule of interest, given that it is a prevalent trichothecene mycotoxin that contaminates cereal-based food and feed. So far, all the previously reported DON-MAbs were selected by homologous screening and most of them displayed much higher affinity either to 3-acetyl-DON (3-ADON) (Baumgartner et al., 2010; Casale et al., 1988; Maragos and McCormick, 2000; Tangni et al., 2010) or to 15-acetyl-DON (15-ADON) (Dos Santos et al., 2011; Maragos et al., 2011; Nicol et al., 1993; Sinha et al., 1995; Xu et al., 2010), the two derivatives that often co-occur with DON. Thus, the relevant immunoassays could only be used to screen for DON/3-ADON or DON/15-ADON, not three of them simultaneously. In 2010 health authorities considered the toxicity of the acetylated derivatives equal to that of DON (JECFA, 2010), but a recent study suggested the higher toxicity of 15-ADON should be taken into account (Pinton et al., 2012). Moreover, DON-3-glucoside (DON-3-G), another DON masked form detected in cereals and beers, also has non-

negligible contribution to the overall DON contamination (Berthiller et al., 2009), though the toxicology data are insufficient. Therefore, it is necessary to develop generic or broad-specific (broad-selective) MAbs with similar affinity to DON and its analogues of concern, so as to rapidly determine the sum of these mycotoxins in a single test and assess the health risk from "total" DON exposure.

In the current work, a new heterologous DON hapten was introduced for immunization and hybridoma screening. Results indicated that MAbs selected by the heterologous ELISAs were broad-selective to DON and its analogues, which were different from other previous DON-antibodies. To the best of our knowledge, it is the first report to employ hapten heterology for hybridoma screening to produce broad-specific MAbs against DON and its analogues.

2. Materials and methods

2.1. Reagents and materials

DON, 3-ADON and 15-ADON standards were obtained from Fermentek (Jerusalem, Israel). Other mycotoxin reference standards namely nivalenol (NIV), HT-2 toxin, T-2 toxin, fusarenon-X (FUS-X) and the de-epoxy metabolite of DON (DOM) were purchased from Sigma-Aldrich (Bornem, Belgium), except that DON-3-G was from Biopure Referenzsubstanzen GmbH (Tulln, Austria). **Figure 1** lists the chemical structures of related compounds used in this work.

Figure 1 Chemical structures of DON and its related analogues

Colorburst™ blue 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution containing hydrogen peroxide was supplied by Alerchek (Springvale, Maine, USA). Rabbit anti-mouse immunoglobulins (anti-mouse IgG secondary antibody; protein concentration of 2.5 g/L) were purchased from DakoCytomation (Glostrup, Denmark). Bovine serum albumin (BSA), ovalbumin (OVA), horseradish peroxidase (HRP), 1,1-carbonyldiimidazole, cyanuric chloride, N,N'-diisopropylethylamine, rabbit anti-mouse IgG secondary antibody labeled with HRP (SecAb-HRP), phosphate buffered saline (PBS, 0.01 M, pH 7.4) tablet, carbonate buffered saline (CBS, 0.05 M, pH 9.6) capsule, complete and incomplete Freund's adjuvants, Tween 20 and skim milk powder were obtained from Sigma-Aldrich (Bornem, Belgium). Deionized water was purified by a Millipore Milli-Q system (Molsheim, France). Other chemicals and solvents were of analytical grade and bought from VWR International (Leuven, Belgium).

Nunc-Immuno™ F96-well microplates and Nunclon™ cell culture plates were from Nalge Nunc International (Roskilde, Denmark). Protein concentrators (9K MWCO, 20 mL) were purchased from Thermo Scientific (Rockford, USA).

2.2. Preparation of DON-protein conjugates

A highly reactive agent, cyanuric chloride (CC), was used to make a new heterologous hapten derivative for DON. The DON-CC-BSA conjugate was prepared in a two-step procedure based on previous publications (Abuchowski et al., 1977; Abuknesha and Griffith, 2005). Briefly, a solution of DON (7 mg) in cold acetonitrile (3.5 mL) was added to a vigorously stirred solution of cyanuric chloride (4.4 mg) in acetonitrile (9 mL) cooled to -20 °C for 1h. Afterwards, a solution of N, N'-diisopropylethylamine (6.1 mg) in ice-cold acetone (1.8 mL) was added. The mixture was kept at 55-60 °C for 5 h and then it was allowed to mix for another 16 h at room temperature. The product was evaporated to dryness at 40 °C under a stream of nitrogen, followed by adding a solution of 3.6 mg of BSA in 2 mL of 0.1 M sodium tetraborate (pH 9.2) and stirring for 1 h at 4 °C. The resultant DON-CC-BSA conjugate was purified by dialyzing extensively with PBS and concentrated using a protein concentrator (9K MWCO, 20 mL). Likewise, the same method was used for preparing DON-CC-OVA and DON-CC-HRP conjugates. Additionally, a common DON-BSA conjugate was synthesized by the 1, 1-carbonyldiimidazole coupling reaction, adopted from published literatures (Maragos and McCormick, 2000; Xiao et al., 1995), as well as DON-OVA and DON-HRP probes. Both DON-CC-protein and DON-protein could be the mixtures of conjugates linked either at the C-3, the C-15, or even both. **Figure 2** presents the structures of the two types of DON-protein conjugates. The protein concentrations of conjugates were determined with bicinchoninic acid using a commercial test kit (Pierce, Rockford, IL, USA).

Figure 2 Structures of DON-protein conjugates

2.3. Immunization and cell fusion

BALB/c female mice (6-8 weeks old) were immunized with DON-CC-BSA or DON-BSA conjugate. For each mouse, the volume of injection was 200 μL every time. The first dose consisted of 100 μg of the conjugate per mouse, which was subcutaneously injected as an emulsion of PBS and complete Freund's adjuvant. Two subsequent boosts were given intraperitoneally at 3-week intervals, with the same amount of immunogen emulsified by incomplete Freund's adjuvant. Afterwards, identical injections were given at 1-month

intervals and one week after each boost, mice were tail-bled and titers of antisera were measured by indirect ELISA to determine whether additional immunizations would be necessary prior to cell fusion. After a resting period of at least 3 weeks from the last injection in adjuvant, mice selected to be spleen donors for hybridoma production received a final intraperitoneal injection of 100 μg of the conjugate dissolved in PBS (without adjuvant). Three days after the final injection, cell fusion was performed according to standard procedures and some details were previously described (Burmistrova et al., 2009).

2.4. Hybridoma selection and subcloning

Ten to twelve days after cell fusion, plates of hybridoma culture supernatants were initially screened by non-competitive indirect or direct ELISAs, based on homologous or heterologous DON antigens. The positive wells were picked out and screened again by competitive ELISAs. Only those wells that gave substantial inhibition (more than 20%) by DON and had no cross-reactivity with OVA were selected. The interesting hybridomas were expanded, rechecked and subcloned by limiting dilution for 2-3 cycles to ensure the monoclonality. The resulting stable clones were expanded and stored in liquid nitrogen. For large-scale production of MAbs, each cell-line was cultured in a two-compartment bioreactor CELLine 350 (INTEGRA Biosciences AG, Zizers, Switzerland) for more than three months. The collected supernatant was further used for MAb characterization.

2.5. Indirect ELISA

All incubations except for the first coating step were carried out at 37 °C, and after each incubation, the plates were washed three times (300 μL/well) with PBST (PBS containing 0.05 % Tween 20) using an automatic microplate washer "96PW" (TECAN, Salzburg, Austria). High-binding polystyrene 96-well microplates were coated with DON-CC-OVA or DON-OVA diluted in CBS (100 μL/well). After incubation at 4 °C overnight, the plates were blocked with 2% skim milk in PBS (300 μL/well) for 30 min. For competitive indirect ELISA analysis, standard solutions of DON and PBS control were added (50 μL/well), followed by adding 50 μL/well of diluted antibodies (antisera or culture supernatants) in PBS. After shaking and incubation for 1h, 100 μL/well of SecAb-HRP was added and incubated for another 1 h. Then, 100 μL/well of TMB substrate solution was added. The reaction was stopped after 15 min with 2 M sulphuric acid (50 μL/well), and the absorbance at 450 nm was measured by a Bio-Rad model 550 microplate reader (Richmond, CA, USA).

2.6. Direct ELISA

The microplates were coated with 100 μL/well of anti-mouse IgG secondary antibody diluted in CBS (5 μg/mL) by an overnight incubation at 4 °C. Then, the plates were blocked in the same above-mentioned way. For testing, antibodies diluted in PBS were added (100 μL/well). After 1 h incubation and the washing step, standard solutions of DON and PBS control were added (50 μL/well), followed by adding another 50 μL/well of DON-CC-HRP or DON-HRP diluted in PBS. The plates were shaken and incubated for another 1 h, and color development was carried out as described in the procedure of indirect ELISA.

2.7. Assay sensitivity test

Checkerboard assays, in which antibodies were titrated against various amounts of coating antigens or enzyme tracers, were conducted to select appropriate working concentrations for evaluation of assay sensitivities to DON. Standard competitive curves were obtained by plotting relative absorbance (ratio of absorbance measured at the standard concentration and zero concentration: $B/B_0 \times 100\%$) against the logarithm of analyte concentration. Sigmoid curves were simulated by means of Origin 8.0 software. From the equations, IC_{50} values (i.e., analyte concentrations at which the maximum absorbances were inhibited by 50%) were determined to assess the assay sensitivity.

2.8. Cross-reactivity study

To evaluate MAb specificity or assay selectivity, a set of DON analogues were utilized to perform cross-reactivity studies. IC_{50} of each tested compound was based on its corresponding competitive curve. Cross-reactivity (CR) values were calculated according to the following equation: $CR(\%) = [IC_{50}(DON) / IC_{50}(analogue)] \times 100\%$，where CR values were calculated using IC_{50} values with units of μg/mL. Likewise, CR_{molar} values were calculated using $IC_{50\ molar}$ values with units of nmol/mL (Xu et al., 2009).

3. Results and discussion

3.1. Immune response to DON immunogens

As mentioned above, most of existing DON-MAbs have much higher affinity either to 3-ADON or to 15-ADON, because the reported DON immunogens often contain the ester-based linkages at the C-3 or the C-15 of DON, which resemble the acetyl groups of 3-ADON or 15-

ADON. Therefore, in our initial study, we attempted to employ a totally different linker for making DON-protein conjugates. Using cyanuric chloride (CC) to form a spacer arm, DON was successfully coupled to proteins. Mice injected with DON-CC-BSA developed very high immune responses. After the third boost, the antiserum titers were higher than 10,000 with DON-CC-OVA as coating antigen. **Table 1** lists the polyclonal antibody characteristics from the best DON-CC-BSA mouse. Judged from the working concentration of antigen and antiserum, it is clear that the polyclonal antibody had much higher affinity to the homologous hapten DON-CC, suggesting the strong recognition of both DON and the CC bridge region. But concerning the inhibition results, the DON-OVA coated ELISA was more sensitive to DON, which proved that the heterologous format did improve the assay sensitivity for DON.

Table 1 Characteristics of antisera from the best DON-CC-BSA and DON-BSA mice

Immunogen	ELISA format	Serum dilution	Coating antigen	Maxium absorbance	Inhibition by
DON-CC-BSA	Homologous	1/16,000	DON-CC-OVA (1)	0.96	11.2%
DON-CC-BSA	Heterologous	1/4,000	DON-OVA (4)	0.92	34.8%
DON-BSA	Homologous	1/16,000	DON-OVA (0.5)	0.88	57.9%
DON-BSA	Heterologous	1/16,000	DON-CC-OVA (4)	0.81	54.5%

Meanwhile, immunization with the usual DON-BSA was implemented for comparison. The produced antisera could recognize both DON-OVA and DON-CC-OVA very well, despite that the dose of DON-CC-OVA was higher than that of DON-OVA (see **Table 1**). However, using the heterologous coating antigen did not promote the assay sensitivity for DON. By contrast, the polyclonal antibody derived from DON-BSA displayed stronger affinity to the free DON than that from DON-CC-BSA. The phenomena must be associated with the fact that DON-BSA contained a smaller linkage compared to DON-CC-BSA, leading to the reduced influence from the spacer arm. Given that the molecular weight of DON is less than 300 Da, the influence of the linkage arm is indeed crucial (Chappey et al., 1994).

3.2. Hybridoma screening by indirect ELISA

The best DON-CC-BSA mouse was first sacrificed for hybridoma production. Due to a great amount of spleen cells, seventeen 96-well plates were used to distribute the fused cell suspension. Non-competitive indirect ELISAs based on both DON-CC-OVA and DON-OVA

were used for primary hybridoma screening. When using DON-CC-OVA (4 μg/mL) for homologous screening, almost all the wells were visually positive. This could be envisaged from the very high titer of antiserum and the satisfying fusion rate (every well contained at least one cluster of hybridomas). Whereas, using DON-OVA (8 μg/mL) for heterologous screening, only a few wells in each plate manifested strong signals. These wells showed even deeper color in the corresponding DON-CC-OVA-coated plate. In other words, these double-checked positive wells exhibited higher affinity to the DON part than other wells did. By contrast, it seemed that a lot of hybridomas could secrete antibodies to recognize the whole DON-CC structure or the CC linker, which led to the high background of DON-CC-OVA-coated ELISA, as the CC ring structure is also an important epitope for eliciting the immune response.

In total, 47 positive wells were quickly selected based on the results of DON-OVA-coated heterologous ELISA. After adding the fresh medium overnight, they were rechecked by the competitive indirect ELISA. Top 6 wells with both strong signal and substantial inhibition by DON were expanded and re-selected. Eventually, the best clone 13H1 was subjected to subcloning for three cycles to get a stable single-cell line.

3.3. Hybridoma screening by direct ELISA

Since the antiserum titer of the DON-BSA mice was considerably high, it could be foreseen that a great number of positive clones would be obtained when using indirect ELISA with DON-OVA for hybridoma screening. Moreover, the standard indirect ELISA screening can result in a large number of false positives, which give rise to a lot of unnecessary workload (Cervino et al., 2008) and exorbitant waste of the precious experimental materials in the following procedures. With the aim to efficiently select the target clones that can secrete high-affinity MAbs, direct ELISA was applied to screen hybridomas from the DON-BSA mice, although the sensitivity of direct ELISA was not as high as that of indirect ELISA (Cervino et al., 2008). However, from another point of view, only clones with high affinity to the target antigen should be detected as the positives by direct ELISA, reducing the number of clones with fairly low affinity.

For initial screening of DON-BSA hybridomas, non-competitive direct ELISAs based on both DON-HRP and DON-CC-HRP were carried out. Likewise, much more positive wells were found when using the homologous tracer DON-HRP, compared to the case of using the heterologous tracer DON-CC-HRP. The wells, which were positive from DON-CC-HRP-

based ELISA, gave even stronger signals in the corresponding DON-HRP-based ELISA. Thus, the top 8 positive wells were easily chosen for further inhibition tests by competitive direct ELISA. Among them, two clones 10H10 and 14D4 could recognize free DON very well and exhibited cross-reactivity to both 3-ADON and 15-ADON; another clone 4B10 had much higher affinity to 3-ADON but very poor reactivity to DON. Subsequently, hybridomas 10H10 and 14D4 were subcloned to obtain the final stable single-cell lines. Given that these two MAbs possessed very similar characteristics, only clone10H10 was used for large-scale culturing.

To be emphasized, the advantage of heterologous screening is that it can notably reduce the proportion of false positives, and hence one may efficiently get the interesting hybridomas with high affinity to the free target analyte. Consequently, it generates time-saving and is labor-saving as well as being cost-effective.

3.4. Assessment of assay sensitivity to DON

The two representative clones 13H1 and 10H10 were individually cultured in CELLine 350 bioreactors for large-scale production of MAbs. In view of practical applications, the competitive direct ELISA format, a popular format in commercial ELISA kits, was used for MAb characterization. After checkerboard titration, the standard curves were set up with the optimal working dilutions of MAbs and tracers. As shown in **Figure 3**, for clone 13H1, the heterologous format based on DON-HRP did improve the assay sensitivity to DON, with IC_{50} value decreasing from 4.34 µg/mL (DON-CC-HRP-traced ELISA) to 2.13 µg/mL (DON-HRP-traced ELISA). Whereas, for clone 10H10, the heterologous format based on DON-CC-HRP was rather less sensitive ($IC_{50} = 0.064$ µg/mL) than the homologous format based on DON-HRP ($IC_{50} = 0.022$ µg/mL). These results confirmed that the degree of hapten heterology could influence the assay sensitivity of direct competitive ELISA, but whether improving or not, it depends on the specific case. The assay sensitivity may not be in parallel with the degree of hapten heterology, and this finding is similar to that of Wang et al. (Wang et al., 2011). Additionally, ELISAs based on clone 10H10 showed much higher sensitivity to DON than those from clone 13H1 (differences of two orders of magnitude), which could be foreseen from the primary results of antisera above-mentioned.

Figure 3 Standard curves of direct competitive ELISAs based on different MAbs and tracers (n=6). B/B_0: ratio of absorbance measured at the standard concentration and zero concentration.

3.5. Assay selectivity

The selectivity of immunoassay is mainly based on the specificity of the involved antibody. In this work, DON-HRP-traced competitive direct ELISA was also used for cross-reactivity measurement, including eight common DON analogues (see **Figure 1**) for tests. **Table 2** lists the cross-reaction results of MAbs 13H1 and 10H10.

Table 2 Cross-reaction results of MAbs 13H1 and 10H10

Compound	MW	Clone 13H1				Clone 10H10			
		IC_{50}, μg/mL	CR, %	$IC_{50\ molar}$, nmol/L	CR_{molar}, %	IC_{50}, μg/mL	CR, %	$IC_{50\ molar}$, nmol/L	CR_{molar}, %
DON	296	2.13	100	7.20	100	0.022	100	0.074	100
3-ADON	338	34.12	6	100.95	7	0.015	147	0.044	167
15-ADON	338	1.62	131	4.79	150	0.034	65	0.101	74
NIV	312	7.69	28	24.65	29	> 10	< 0.2	> 32	< 0.2
T-2 toxin	466	> 100	< 2	> 214.6	< 3	> 10	< 0.2	> 21	< 0.3
HT-2 toxin	424	1.14	187	2.69	268	> 10	< 0.2	> 24	< 0.3
FUS-X	354	> 100	< 2	> 282.5	< 3	> 10	< 0.2	> 28	< 0.3
DON-3-G	458	15.67	14	34.21	21	0.072	31	0.157	47
DOM	280	5.47	39	19.54	37	0.079	28	0.282	26

It seemed that the clone 13H1 could be considered as a unique broad-selective antibody, with even higher sensitivity to HT-2 toxin and 15-ADON than to DON, minor affinity toward NIV, DOM and DON-3-G, and very low cross-reactivity against 3-ADON, while no obvious cross-reaction with T-2 toxin and FUS-X was found. Interestingly, this MAb could recognize HT-2 toxin but not T-2 toxin, as both of them belong to type A trichothecene (absence of a carbonyl at the C-8) and just differ from the C-4. The observations were mainly ascribed to the immunogen DON-CC-BSA, in which the CC linker must be located more at the C-15 than at the C-3 and it significantly affected the electronic configuration of the DON molecule. Probably, the molecular property of the immunizing hapten DON-CC was more close to HT-2 toxin than to other tested compounds, which led to the fact that the generated antibody had the greatest affinity to HT-2 toxin. These results were in agreement with Muldoon et al.'s

perspective that both steric and electronic features governed antibody recognition (Muldoon et al., 2000).

MAb of clone 10H10 gave better sensitivity to 3-ADON than to DON, moderate affinity to 15-ADON, and minor cross-reactions with DON-3-G and DOM, but no cross-reactivity toward the other trichothecenes. It was interesting to observe that this anti-DON MAb could recognize both 3-ADON ($IC_{50} = 0.015$ µg/mL) and 15-ADON ($IC_{50} = 0.034$ µg/mL) very well, and the differences among the three CR values were much smaller than those from other reported anti-DON MAb-based ELISAs (Li et al., 2012; Maragos et al., 2006; Maragos and McCormick, 2000; Tangni et al., 2010; Xu et al., 2010). In other words, the discrepancies of the assay sensitivities to DON, 3-ADON and 15-ADON are so narrow that it is allowed to utilize the novel MAb for simultaneous detection of the three mycotoxins. As a result, the "total" DON content can be rapidly estimated in one assay and an overall health risk of DON exposure for the consumers can be assessed. The broad-selectivity of this MAb was probably due to the involved strategy of heterologous screening for hybridomas instead of the homologous one. Previous researches (Liu et al., 2010; Muldoon et al., 2000; Wang et al., 2010) also indicated that application of this screening strategy increased the likelihood of isolating hybridomas secreting very broad-specific MAbs.

Note that when comparing target analytes to analogues with different molecular weight (MW), cross-reactivity values calculated from units of mole per volume should be more accurate than those from units of weight per volume (though the latters are often used)(Xu et al., 2009), since cross-reactivity is a structure-related phenomenon in antibody-antigen binding. Generally, the gaps between CR and CR_{molar} values would not be too big, except that the antibody is broad-specific and shows high affinity to the analogue whose MW has a very significant deviation from that of the target analyte. Herein, as for the cross-reactivity of MAb 13H1 to HT-2 toxin, the usual CR was 187%, while the CR_{molar} increased up to 268%. Also as described in a few references (Ruprich and Ostrý, 2008; Tangni et al., 2010), some DON-ELISA tests displayed very high cross-reactivity to the masked form DON-3-G and the corresponding CR_{molar} values must be much larger than the normal CR values. Hence, we suggest that evaluation of the cross-reactivity of a broad-specific antibody should be based on the CR_{molar} values as far as possible. Certainly, CR data obtained in the current work can be somewhat different from the values declared later in the future application studies (because of various assay formats, sample matrixes and so on), but the general tendency should be similar.

4. Conclusion

In this study, two new types of anti-DON MAbs have been developed by heterologous screening of hybridomas, and this screening strategy appears to be an efficient approach for selecting hybridomas of interest. The superior MAb 10H10 has relatively similar affinity to DON, 3-ADON and 15-ADON, with IC_{50} values of 22, 15 and 34 ng/mL, respectively. This is, so far, the first broad-specific MAb against DON and its two acetylated forms, which can be used for simultaneous monitoring of the three mycotoxins. Thus, it would be further applied to different kinds of DON-related immunoassays, or immunoaffinity columns for sample clean-up before chromatographic analysis.

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