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Development of a surface display ELISA to detect anti-IgG antibodies against bovine α S1-casein in human sera

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Abstract

The aim of the present study was to develop a surface display ELISA (SD-ELISA) for IgG-serum reaction against bovine casein α S1 (CSN1S1). In a SD-ELISA, the antigen is displayed on the surface of *Escherichia coli* using the autodisplay technology and whole cells of *E. coli* are used to coat the microplates for serum testing.

After establishing the setup of the SD-ELISA with polyclonal rabbit antiserum against bovine CSN1S1, the SD-ELISA was validated with 20 human sera, of which 10 sera were proven to have an IgG-mediated reaction against bovine CSN1S1 and 10 sera were shown to be negative for this reaction. Receiver operating characteristics (ROC) analysis revealed sensitivity of 100% and a specificity of 100% at a cut-off value of 0.133. Furthermore, human serum of 48 patients with known reactivity against human CSN1S1 (31 positive and 17 negative) was examined by the newly developed SD-ELISA to exclude cross-reactivity. Twenty human sera showed an IgG-mediated reaction against bovine CSN1S1. Eleven of these sera were positive for the reactivity against human CSN1S1, and nine were negative.

In conclusion it was demonstrated that the performance of SD-ELISA is comparable to established ELISA without loss in sensitivity or specificity. Based on the advantages of this method – in particular no need for time-consuming and expensive antigen production and purification – the SD-ELISA is a potent alternative to convenient methods for identification and especially high-throughput screening of new antigens in the field of food allergies.

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Keywords

Autodisplay; SD-ELISA; IgG-mediated cross-reactivity; Bovine casein; Human α s1-casein

1. Introduction

Caseins are one of the major milk proteins of most mammalian species [1]. Eighty percent of bovine milk proteins are caseins [1,2] with α S1-casein (CSN1S1) being the most abundant protein. In contrast, the casein fraction constitutes only approximately 40% in human milk [3]. Most caseins are phosphoproteins and ameliorate the intestinal uptake of calcium-phosphates and other minerals by incorporating them into clusters of diverse casein family members, termed micelles [4]. Besides its role for the suckling infant, bovine milk proteins are abundant ingredients in a typical western diet [3]. It is known, that bovine CSN1S1 is a major food allergen, stimulating allergic reactions [5–8]. Symptoms include digestive problems, skin allergies, and anaphylactic shocks [9]. The detection of an immunoglobulin G (IgG) reaction against CSN1S1 could not unequivocally be associated to cow's milk allergy (CMA). In contrast to IgG-reaction 89% of CMA patients show an immunoglobulin E (IgE) reaction against bovine CSN1S1 [10]. For bovine CSN1S1, there are six IgG epitopes identified [11,12]. The most recognized binding domain for the IgG antibodies in cow's milk allergy was found between amino acids 15–36 of bovine CSN1S1 [11]. Phosphorylation did not display any influence on the antibody reaction against bovine CSN1S1 in this study, because epitopes were not phosphorylated [13].

The concentration of serum IgG antibodies to bovine milk proteins is dependent upon the duration of exposure to cow's milk during early infancy [14]. In a prior study, an IgG-mediated antibody reaction against human CSN1S1 in sera of healthy breast-fed adults by a surface display (SD) ELISA was detected [15]. The SD-ELISA is based on the autodisplay technology [16,17] and allows the testing of human sera on antibody reactions against antigens without further purification steps of the recombinant antigen.

The aim of this study was to establish a SD-ELISA for the detection of IgG antibody reactions against bovine CSN1S1, and evaluate its sensitivity, specificity and precision in comparison to an established indirect ELISA. Subsequently, it was intended to investigate its applicability by analyzing a possible cross-reactivity of the IgG mediated reaction in human sera against the bovine and the human CSN1S1. The SD-ELISA technique may subsequently be used for the detection of various potential antigens and modifications thereof in patients with cow's milk allergy.

2. Materials and methods

2.1. Human sera

Twenty human sera which were tested for their IgG-mediated antibody reaction with an indirect ELISA previously (10 sera with an IgG-mediated reaction against bovine CSN1S1 and 10 sera without a reaction) [10], were used to establish the SD-ELISA against bovine CSN1S1. In addition, a total amount of 48 human sera of healthy adult individuals with

known reactivity against human CSN1S1 (31 with and 17 without antibodies as previously determined [15]) were analyzed for an IgG-mediated antibody reaction against bovine CSN1S1 by the newly established SD-ELISA. Sera were obtained, treated and stored as described before [10,15].

2.2. Materials

Goat anti-human IgG conjugated with horseradish peroxidase was obtained from Beckman Coulter (Krefeld, Germany), rabbit anti-bovine casein was obtained from GeneTex Inc. (Irvine, CA, USA), goat anti-rabbit IgG conjugated with horseradish peroxidase was obtained from Sigma-Aldrich (product number A0545 Munich, Germany), goat anti-rabbit IgG conjugated with fluoresceine isothiocyanate (FITC) was obtained from Bethly (Montgomery, TX, USA), restriction endonucleases were purchased from New England Biolabs (Ipswich, MA, USA), 3,3',5,5'-tetramethylbenzidine (TMB) was obtained from Sigma-Aldrich (Munich, Germany). Maxisorp[®] microplates were purchased from Nunc (Langenselbold, Germany).

2.3. Bacterial strains and plasmids for bovine CSN1S1 surface display

Escherichia coli strain UT5600(DE3) (F⁻ ara 14 leuB6 azi-6 lacY1 proC14 tsx-67 entA403 trp E38 rfbD1 rpsL109 xyl-5 mtl-1 thi1, ompT-fepC266) was used to express the proteins [18]. The corresponding gene encoding for bovine CSN1S1 without signal peptide (UniProt database: P02662), optimized for codon usage for *E. coli* K12 strains was obtained from Eurofins MWG Operon (Ebersberg, Germany) in the plasmid backbone pCR2.1. The plasmid was transformed into *E. coli* UT5600(DE3). Plasmid pKP10 encoding for human CSN1S1 [15], was used for construction of the gene encoding the precursor protein for the surface display of bovine CSN1S1. Both plasmids were cleaved with XhoI and Acc65I restriction sites to insert the DNA fragment encoding for bovine CSN1S1 into the plasmid backbone of pKP10. The plasmid was transformed into the strain *E. coli* DH5a for cloning. Strain UT5600(DE3) was used for protein expression.

2.4. SDS-PAGE and flow cytometer analysis

As a negative control *E. coli* UT5600(DE3) presenting a small peptide (PEYFK-epitope) instead of the bovine CSN1S1 was used [18]. For both strains lysogeny-broth (LB) containing 50 mg/L of carbenicillin, 10 µmol/L ethylenediaminetetraacetate (EDTA) and 10 mM β-mercaptoethanol were inoculated and cultured at 37 °C. Protein expression was induced by addition of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), outer membrane protein preparation, and surface detection was performed as described before [16]. To test surface accessibility a proteinase K digestion was performed analog to Schumacher et al. [19] with a final concentration of 0.125 mg/L proteinase K. Flow cytometer analysis was performed [16], with the primary antibody rabbit anti-bovine CSN1S1 (1:25 in phosphate buffered saline [PBS, pH 7.4] and 3% bovine serum albumin) [19].

2.5. SD-ELISA protocol

The general method of the SD-ELISA was illustrated before [15]. For each serum to be tested, three wells of a 96-well plate were coated with *E. coli* cells presenting bovine CSN1S1 and three wells were coated with control *E. coli* cells. The difference in the mean of the absorption value measured for *E. coli* cells presenting bovine CSN1S1 and control cells was calculated (presented as bars). The standard deviation of each triplet was determined (presented as error-bars). Wells of a microplate were coated with *E. coli* cells. Afterwards unspecific binding sites were blocked and cells were incubated – firstly with human sera and secondly with a horse radish peroxidase (HRP) labeled antibody – to detect a color reaction by addition of 3,3',5,5'-tetramethylbenzidine (TMB). LB-Medium was inoculated with a starter culture (1:100) [16]. Protein expression was induced at OD₅₇₈ of 0.5 by addition of IPTG (1 mM final concentration) for 16 h at 4 °C in PBS (pH 7.4). Subsequently, cells were washed twice with PBS (pH 7.4) and suspended in PBS volume to a final OD₅₇₈ of 0.5. 96-wells microplate (Maxisorp®; Nunc, Langenselbold, Germany) were coated with 100 µL cell suspension overnight at 37 °C. Unspecific binding sites were blocked with 120 µL PBS (pH 7.4) and 10% fetal calf serum for 3 h at 30 °C. Afterwards the cells were incubated with a rabbit anti-bovine casein serum or human sera (100 µL, diluted 1:200) for 1 h at 30 °C and washed three times with PBS (pH 7.4) with 0.1% Tween 20. The microplate was incubated with 100 µL of goat anti-human IgG conjugated with HRP for 45 min at 30 °C and washed three times with PBS (pH 7.4) with 0.1% Tween 20. 100 µL of TMB, an HRP substrate, was added for 25 min at 30 °C and the reaction was stopped with 100 µL of 2 M H₂SO₄. Subsequently, the IgG reaction against bovine CSN1S1 was quantified by measuring the absorption at 450 nm as well as the reference wavelength of 620 nm with a microplate reader (Berthold Technologies, Bad Wildbad, Germany).

2.6. Stripping procedure for the preparation of human sera before being applied to the SD-ELISA

To reduce IgG antibodies against *E. coli* in human sera, all samples were pre-incubated with control cells (stripping procedure, Supplementary Fig. I). Antibodies against *E. coli* were eliminated by centrifugation. The supernatant was used for the SD-ELISA. A similar approach has previously been used to successfully establish a Ro/SS-A SD-ELISA [15,16]. In detail, 200 mL LB-medium containing 50 mg/L of carbenicillin was inoculated with negative control cells and incubated overnight (37 °C, 200 rpm). The protein expression was induced by 1 mM IPTG for 60 min (30 °C, 200 rpm). Cells were harvested, washed twice with PBS (pH 7.4) and resuspended to a final OD₅₇₈ of 20 in PBS (pH 7.4). 1 mL of this solution was centrifuged (2 min, 13 200 rpm). The supernatant was rinsed out, the cell pellet was suspended with 145 µL PBS and 5 µL human sera and incubated for 5 h at 37 °C and 800 rpm in a thermo shaker (Labnet Vortemp 56, Oakham, UK). After centrifugation (2 min, 13 200 rpm) the supernatant was transferred to a reaction tube with a cell pellet as described above. The solution was incubated for 15 h (37 °C, 800 rpm). The supernatant was diluted with 850 µL PBS (pH 7.4) and 10% fetal calf serum to a final dilution of 1:200 of the human sera and applied to the SD-ELISA.

Supplementary Fig. I related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jpba.2014.03.032>.

2.7. Data analysis

Calculations of means, standard deviations, cut-off values, ROC analysis and spearman correlations were performed with Graph-Pad Prism[®] 5.0. The Scheffé test was performed with OriginLab[®] 8.6. The amino acid sequence alignment was carried out with the online open software tool EMBOSS of the European Bioinformatics Institute.

3. Results and discussion

3.1. Autodisplay of bovine CSN1S1

In order to detect the antibody reaction against bovine CSN1S1 *via* SD-ELISA, the protein had to be displayed on the surface of *E. coli* by autodisplay. Autodisplay has been described as an efficient and convenient system for surface expression of diverse recombinant proteins in *E. coli* [17]. The corresponding gene encoding for bovine CSN1S1 was inserted into the plasmid encoding for human CSN1S1 and transformed into *E. coli* as previously described [15]. The fusion protein consisted of four domains: first, the signal peptide (SP); second the so-called passenger bovine CSN1S1; third, a linker protein; and fourth, a β -barrel from the autotransporter (Supplementary Fig. II) [20].

Supplementary Fig. II related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jpba.2014.03.032>.

The protein expression was analyzed by flow cytometry. For each strain LB were inoculated and cultured to an OD₅₇₈ of 0.5. The protein expression was induced by addition of IPTG (1 mM final concentration). The *E. coli* cells were incubated with a polyclonal rabbit anti-bovine casein serum and afterwards with a goat anti-rabbit IgG-antibody conjugated to FITC. As shown in Fig. 1a, mean fluorescence (mF) of cells expressing bovine CSN1S1 (54.1) was 15 times higher than the mean fluorescence of the control cells (3.42). Obviously not all of the cells, however, were displaying bovine CSN1S1. The efficiency of surface display was determined by integrating the areas under the curve for the positive and the negative population in the flow cytometer analyses as indicated in Fig. 1a. A ratio of about 80% of *E. coli* cells with surface displayed bovine CSN1S1 could be estimated among the whole *E. coli* whereas about 20% appeared to be negative.

To support these findings suggesting protein expression on the surface of *E. coli*, outer membrane isolations of control cells and *E. coli* UT5600(DE3) presenting bovine CSN1S1 were prepared. As a control, the same *E. coli* strain without the induction of bovine CSN1S1 expression was used. One sample of the outer membrane isolate was obtained after digesting whole cells with proteinase K in order to demonstrate that bovine CSN1S1 was on the surface of *E. coli*. Outer membrane isolates were separated by SDS-PAGE. A band of the approximated size of the fusion protein (70 kDa) was only observed for the *E. coli* cells presenting bovine CSN1S1 induced with IPTG (Fig. 1b, lane 3). This band disappeared after digestion with proteinase K (lane 4). The natural outer membrane proteins Omp F/C and OmpA remained unaffected. These results indicate that bovine CSN1S1 was expressed on the outer membrane of *E. coli* UT5600(DE3)pET AB002.

3.2. Establishment of SD-ELISA and comparison to indirect ELISA

The results in Section 3.1 indicate that bovine CSN1S1 was displayed on the surface of *E. coli* cells. Based on the SD-ELISA for the detection of antibodies against human CSN1S1 in human sera [15], a modified protocol for the detection of an antibody reaction against bovine CSN1S1 was performed as described in Section 2.5. A temperature for the SD-ELISA to detect bovine CSN1S1 of 30 °C turned out to improve detection sensitivity in comparison to room temperature as described before [21].

Temperature and time of induction with IPTG were varied for full expression of bovine CSN1S1 on the cell surface. The low temperature of 4 °C led to a reduced synthesis pace [22]. The probability for binding to chaperones increased and promoted a more efficient transport through the inner membrane of the unfolded autotransporter fusion protein [22,23]. Because of the slower synthesis rate the induction time was increased from one hour to overnight in PBS (pH 7.4). The mechanism of the secretion is not completely identified [17].

For each tested serum three wells of a 96-well plate were coated with *E. coli* cells presenting bovine CSN1S1 and three wells were coated with negative control cells. The difference in the mean of the absorption value measured for *E. coli* cells presenting bovine CSN1S1 and control cells was calculated (presented as bars), to normalize the effects resulting of an antibody reaction of the sera to *E. coli*. The standard deviation of each triplet was calculated (presented as error-bars).

The efficiency of immobilization of *E. coli* cells in the wells of 96-well Maxisorp[®] microplates was determined before by Petermann et al. [16]. It turned out that maximal absorption values were obtained after reaction with primary and secondary antibodies in a corresponding SD-ELISA, when 100 µL of cells at an OD₅₇₈ = 0.5 were applied. Under these conditions, Lee et al. [24] observed a coverage value of 200–300 *E. coli* cells per mm². As in the experiments presented here these conditions were identical, an identical amount of *E. coli* cells being adsorbed in the wells of the microplate can be deduced.

To prove the functionality of the SD-ELISA to bovine CSN1S1 four different primary antibody concentrations were tested and plotted. As shown in Supplementary Fig. III there is a linear correlation of the *E. coli* presenting bovine CSN1S1 and the antibody concentration with a linear regression of 0.993.

Supplementary Fig. III related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jpba.2014.03.032>.

The matrix of human sera is more complex, than the one of a commercially available antibody and *a priori* is supposed to contain antibodies against *E. coli* cells. To eliminate these antibodies against *E. coli* and prevent false positive results, human sera were stripped with *E. coli* control cells in two cycles for a total of 20 h (Supplementary Fig. I). This step is crucial for a SD-ELISA and is a disadvantage in comparison to standard ELISA protocols, which do not require such procedure.

Subsequently, the performance of the SD-ELISA was compared to an indirect ELISA for the detection of IgG subclass antibody reaction against bovine CSN1S1 by Hochwallner et al.

[10]. The recombinant bovine CSN1S1 of the indirect ELISA was overexpressed in *E. coli* BL21(DE3) and afterwards purified by column chromatography [7]. The amino acid sequence of the recombinant purified bovine CSN1S1 was identical with that of the bovine CSN1S1 displayed on the cell surface of *E. coli* in the SD-ELISA. Each of the 20 human sera (divided into two populations: 10 with and 10 without an observed IgG-mediated reaction against bovine CSN1S1 by Hochwallner et al. [10]) was tested four times with SD-ELISA against bovine CSN1S1 and control cells (Supplementary Fig. IV). Mean and standard deviation were calculated. It clearly indicated, that the serum reaction against control cells laid between 0.05 and 0.32 for both, positive and negative sera. In contrast, the antibody reaction against cells displaying bovine CSN1S1 for sera tested before as IgG positive was significantly higher and appeared to be in the range of 0.36–2.81. For easier understanding, however, the difference of values obtained with cells presenting bovine CSN1S1 and control cells was calculated for each serum and the data obtained thereby were presented in the following figures as bars (Fig. 2a).

Supplementary Fig. IV related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jpba.2014.03.032>.

The same sera that were identified to exhibit an IgG-mediated reaction against CSN1S1 in the indirect ELISA appeared to be positive in the SD-ELISA (Fig. 2b). The positive and negative populations were different in a Scheffé test ($p \leq 0.01$). The accuracy of the SD-ELISA was determined with the receiver-operating-characteristics (ROC) analysis, a method often used in diagnostic tests [25,26]. The area under the curve (AUC) of 1 in the ROC analysis is perfect. A cut-off value of 0.133 with 100% specificity and 100% sensitivity was determined.

The data of a prior study by Hochwallner et al. [10] for IgG subclasses 1–4 were compared with the results of the total IgG reaction by SD-ELISA with the spearman correlation coefficients (ρ) [27,28]. The ρ confirmed that the absorption values of the SD-ELISA for the detection of IgG correlated strongly to the prior results for IgG1, IgG2 and IgG4 ($\rho = 0.817$; 0.935; 0.851, respectively). For IgG3, which is less than 10% of total IgG, a moderate correlation to the total IgG as assessed by SD-ELISA was observed ($\rho = 0.646$).

Hochwallner et al. identified the highest levels for IgG1 and IgG4 against CSN1S1 and weakest for the IgG3 antibody reaction [10]. This presumes that IgG3 antibody reaction has only a weak influence on total IgG reaction. In order to test whether the SD-ELISA can lead to the same assay results as the conventional ELISA, the absorption values obtained with both methods were compared (Fig. 2a). For the SD-ELISA, the values for the complete IgG repertoire were determined, and compared to the values obtained for IgG1 reaction, known to represent around 90% of the entire IgG repertoire in the standard ELISA. Although the absolute values varied between the two methods, with cut-off value as determined for the SD-ELISA of 0.133 (Fig. 2b) and the cut-off for the standard ELISA which was set as twofold the highest buffer control [10], absolutely identical assay results were obtained. The obtained results indicated that whole cell SD-ELISA are an alternative to an established ELISA without any loss in preciseness or sensitivity, but the advantage of no further purification steps of recombinant obtained protein. Thus, the SD-ELISA technique opens up

new possibilities for convenient testing of human sera against diverse dairy proteins and potential modifications. For instance, instead of whole proteins, peptides derived thereof could be presented using the autotransporter to more accurately determine the antigenic region. This introduces new possibilities for targeted *in vivo* research in cow's milk allergy and food intolerance disorders. Because an immune reaction against bovine milk proteins, in particular CSNs, appears to be frequent in western civilization, the SD-ELISA could be a rapid and convenient tool to identify such reaction and the bovine milk protein responsible for.

3.3. No cross-reactivity between bovine and human CSN1S1

There is no data available to confirm or exclude a cross-reactivity of bovine CSN1S1 to human CSN1S1. Human CSN1S1 has originally been identified by its sequence homology to caseins of other mammalian species, especially by identity of the signal peptide (SP) [4,29,30]. There are no known immunological cross-reactivities between bovine and breast-milk unequivocally identified today [31]. Nevertheless, often such cross-reactivities are assumed, e.g. for α -lactalbumin or β -Casein [32].

In a former study, we tested 48 human sera of healthy volunteers for an antibody reaction against human CSN1S1 [15]. Breast-fed persons exhibited an IgG-mediated reaction against human CSN1S1 and could be distinguished of formula-fed persons, statistically confirmed by a ROC analysis with an AUC of 0.844. These sera with known reactivity against human CSN1S1 were checked for their reactivity against bovine CSN1S1 in order to exclude cross-reactivity. There was an IgG-mediated antibody reaction against bovine CSN1S1 detected in 20 sera of the 48 healthy donors (Fig. 3). One sample showed a negative absorption difference (-0.146 for no. 12).

These samples were divided into two populations: one with an IgG-reaction against human CSN1S1 (IgG positive) and one without an IgG-reaction against human CSN1S1 (IgG negative) (Fig. 4a). 11 out of 29 samples with an IgG reaction against human CSN1S1 showed an IgG-reaction against bovine CSN1S1. 9 of the 19 sera without an IgG-reaction against human CSN1S1 showed an IgG reaction against bovine CSN1S1. The populations were not statistically different in Scheffé test ($p \geq 0.05$). Next, a ROC analysis was performed, calculating sensitivity and specificity for each possible cut-off value (Fig. 4b). The AUC (0.632) for this ROC curve substantiated that there was no cross-reactivity between bovine and human CSN1S1. The comparison of the results by spearman correlation ($\rho = -0.029$) for the ELISA between bovine and human CSN1S1 strengthened the results. Comparison of the amino acid sequences of bovine and human CSN1S1 (using the Needleman-Wunsch Algorithm, Fig. 5) showed that there is only marginal homology between both proteins, and especially no homology in the known binding domains of bovine anti-CSN1S1 antibodies in cow's milk allergy. Both CSN1S1s had an identity of 32.8% and a homology of 44.7% when the entire amino acid sequence with signal peptide was compared (Fig. 5). The identity of both signal peptides was 93.3%, but because they were cleaved off during cellular production of CSN1S1 [17], the identity between both proteins without signal peptide appears to be more relevant for a possible cross-reactivity of antibodies. The identity of the bovine with the human CSN1S1 was 28.6%. This is in good

agreement with the results of the present study, which exclude a cross-reactivity of bovine and human CSN1S1. In consequence this may contribute to a change of view, namely that the bovine CSN1S1 and the human CSN1S1 are completely different in sequence, immunogenicity and hence most probably in function as well.

4. Conclusion

In this study, a whole cell SD-ELISA to detect IgG antibodies against bovine CSN1S1 in human sera was established. The performance of the SD-ELISA is essentially comparable to other ELISA tests without any loss in sensitivity or specificity.

Furthermore, it was clearly demonstrated that bovine CSN1S1 antibodies are not cross-reactive with human CSN1S1 using SD-ELISA.

This technology provides researchers in the field of food allergies and especially cow's allergy with a potent and convenient tool for the identification and high-throughput screening of new antigens. Furthermore, selected sequences of known allergens can be expressed using the Autodisplay technology to identify antigenic sequences within the proteins *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Expression and surface detection of bovine CSN1S1. (a) Flow cytometry of *E. coli* control cells (clear, left peak) and cells presenting bovine CSN1S1 (gray filled) with two populations (left: cells without bovine CSN1S1 on the surface; right: cells with bovine CSN1S1). Cells were incubated with rabbit antiserum against bovine CSN1S1 and labeled with a secondary antibody conjugated to FITC. Mean fluorescence of cells presenting bovine CSN1S1 (54.1) was 15 times higher than the one of control cells (3.42). (b) SDS PAGE of outer membrane proteins of *E. coli* presenting bovine CSN1S1, without induction with IPTG (lane 2), with induction with IPTG (lanes 3 and 4) and digestion with proteinase K (lane 4) and of control cells induced with IPTG (lane 1). Only lane 3 contains the band of the approximated size of fusion protein at 70 kDa.

Fig. 2.

Validation of the SD-ELISA against bovine CSN1S1. (a) The antibody reaction against bovine CSN1S1 of 20 human sera for validation of the SD-ELISA to IgG against bovine CSN1S1 (white bars) and compared to the values obtained with a standard indirect ELISA for IgG1 reaction against bovine CSN1S1 [7] (black bars). The cut-off value of 0.133 determined for the SD-ELISA is illustrated as a dotted line. (b) The antibody reaction against bovine CSN1S1 divided into the populations tested to possess an IgG reaction (IgG positive) and not possess an IgG reaction (IgG negative); with the cut-off value (dotted line).

Fig. 3.

Antibody reaction against bovine CSN1S1 in human sera. Forty-eight human sera of healthy volunteers were tested for their antibody reaction against bovine CSN1S1 with SD-ELISA. Every serum was measured three times independently and the mean was calculated (shown as bars). Standard deviations are given as lines.

Fig. 4.

Analyzing the serum reaction. (a) Comparison of the antibody reaction against bovine CSN1S1 of sera with a positive antibody reaction against human CSN1S1 and without an antibody reaction against human CSN1S1 (negative IgG) (dotted line = cut-off value IgG reaction; dashed). (b) Comparison of the ROC curve for the antibody reaction against bovine CSN1S1 (filled dots) with an AUC of 0.622, the antibody reaction against human CSN1S1 (empty dots) with an AUC of 0.844 and a coin flip (black line) with an AUC of 0.5.

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1  MKLLILTCLVAVALARPKHPIKH-----QGLP----QEVLN---      32
   |:|||||||||||||||.:::      :.:|      :.:|
1  MRLILITCLVAVALARPKLPLRYPERLQNPSESSEPIPLESREEYMNGMN      50

33  --ENLLRFFVAPFPEVFGKEKVNELSKDIGSESTEDQAMEDIKQMEAESI      80
   .:|:|      ||...||...:||||:.....:|:|...
51  RQRNILR-----EKQTDEIKDTRNESTQNCVVAEPEKMESIS          88

81  SSSEEIVPNSVEQKHIQKEDVPSEYLGYLEQLRLKKYKVPQLEIVPNS      130
   |||:|:.....      .||..||:|.  ||:.....
89  SSSEEMSLSKC-----AEQFCRLNEYN--QLQLQAAH              118

131 AEERLHSMKEGIHAQQKEPMIGVNQELAYFYPELFRQFYQLDAYPSGAWY      180
   |:|:..|.|.|.|.      |:|.|||.|||.||
119 AQEQIRRMNENSHVQVP-----FQQLNQLAAYPYAVWY            151

181 YVPLGTQYTDAPSFSDIPNPIGSENSEKTTMPL-W                214
   | |...|...|.||||.||||.||||.||||.:.| |
152 Y-PQIMQYVPFPFSDISNPTAHENYEKNNVMLQW                185

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Fig. 5.

Sequence alignment of bovine and human CSN1S1. Comparison of the amino acid sequence of bovine CSN1S1 (top row) and human CSN1S1 (bottom row) with the Needleman-Wunsch algorithm (with the symbols '|' for identity, ':' for homology and '.' for no similarity between amino acid sequence).