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The development of a monoclonal antibody-based ELISA for the determination of histamine in food: application to fishery products and comparison with the HPLC assay

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Monoclonal antibodies (Mabs) to histamine were prepared by immunising mice with histamine–protein conjugates. Four Mabs were obtained which both exhibited high affinity for histamine after chemical derivatisation, and showed no cross-reaction with five other biogenic amines (cadaverine, putrescine, spermine, spermidine, tyramine) or with structurally related compounds such as histidine or serotonine. Using these antibodies, a competitive inhibition ELISA (CIELISA) was developed, available in the range 10–100 ng/ml. The assay has been used to quantify the histamine content of fish extracts previously treated for histamine derivatisation using 1,4-benzoquinone. Values for histamine content were obtained within 5 h. Results obtained either by CIELISA or by the currently used HPLC method were closely related.

INTRODUCTION

Histamine, 4-(2-aminoethyl)imidazole, is a primary amine arising from the decarboxylation of the amino acid L-histidine. Endogenous histamine plays important roles in a number of normal and abnormal biological processes including vasodilatation, anaphylaxis and gastric secretion (Beaven, 1978). Histamine also occurs exogenously in the food supply, and on occasion foodborne histamine does cross the intestinal barrier. If sufficient quantities of histamine enter the blood stream, an intoxication can occur. The histamine formed in foods is the result of the growth of bacteria that possess the enzyme histidine decarboxylase (Arnold & Brown, 1978). The concentration of other bioactive amines, such as cadaverine, putrescine, spermine, spermidine and tyramine, may also increase in food products following the microbial decarboxylation of other precursor amino acids such as phenylalanine, lysine, or arginine. Their presence in spoiled food may potentialise the histamine action. Food-related intoxications caused by histamine and/or other biogenic amines have been reported in several countries (Taylor, 1985; Straton *et al.*, 1991). Consequently, it is important to determine the level of these amines in food, especially for histamine. Indeed), the most frequent foodborne intoxications caused by biogenic amines involve histamine. Some national governments recommended a maximum histamine content of certain foods such as fishery products.

The main methods for histamine detection are fluorometric methods (Shore *et al.*, 1959) or chromatographic methods such as thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC) (Taylor, 1986). Their use is limited either by a lack of specificity (cross-reaction among bioactive amines) or by the requirement of expensive laboratory equipment and time-consuming manipulations.

There is a need to develop a simple and specific histamine assay which could be used routinely for a large number of samples. An immunoassay would be convenient and several tests using monoclonal antibodies (Mabs) have been developed for histamine evaluation in biological fluids (Guesdon *et al.*, 1986; Hammar *et al.*, 1990). However, their use for histamine evaluation in food products is rather difficult for several reasons, including inadequate sensitivity or lack of specificity. The aim of the research was to develop an immunoassay for the measurement of histamine in food products. To achieve this point, several anti-histamine Mabs were raised and characterised, which were subsequently used to realize a competitive inhibition ELISA.

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MATERIAL AND METHODS

Antigen preparation

Two kinds of antigens were prepared: (i) amine–protein conjugates, and (ii) amine derivatives.

Conjugates

Various histamine–protein conjugates were synthesised using two different methods, according to the nature of components involved in the coupling procedure.

One approach involved the modification, before the coupling reaction, of the carrier protein only. The carrier proteins used were as follows: casein (Merck, Darmstadt, Germany), keyhole limpet haemocyanin (KLH Calbiochem, La Jolla, USA), or bovine serum albumin (BSA, Behringwerke A.G. Marburg, Germany). The primary amines on these proteins were reacted with 1,4-benzoquinone (Sigma Chemical Co, Saint Louis, MO, USA) or with disuccinimidylsuberate (DSS, Pierce Chem. Co., Rockford, USA) as described by Avrameas *et al.* (1978) and Montesano *et al.* (1982), respectively. Then the activated proteins were reacted at pH 8·5 with the histamine primary amine (histamine dihydrochloride, Sigma). The following conjugates were obtained: histamine–benzoquinone–casein (HBC), histamine–benzoquinone–BSA (HBB), and histamine–DSS–KLH (HDK). An additional conjugate, a glycine–benzoquinon–casein (GBC) was synthesised as described for HBC, using glycine instead of histamine.

The second approach involved precoupling modifications of both histamine and protein. The histamine modification consisted of a reaction of the primary amine with 2-iminothiolane (Traut's Reagent, TrR, Pierce) as described by Blättler *et al.* (1985). The protein modification consisted in reacting the primary amines to *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP, Pierce) or to sulphosuccinimidyl-4-(*N*-maleidomethyl)-cyclohexane-1-carboxylate (sulphoSMCC, Pierce) as described by Carlsson *et al.* (1978) and Hashida (1984), respectively. Then the derivatised histamine and the activated protein (Thyroglobulin or Aldolase, Sigma) were reacted at pH 7 to obtain the following histamine–protein conjugates: histamine–TrR–SPDP–thyroglobulin (HTST), histamine–TrR–SPDP–adolase (HTSA) and histamine-TrR–sulphoSMCC–thyroglobulin (HTSST).

The linkage of a histaminyl group on the different proteins used was monitored by ELISA performed as described in the following section. The conjugates to be tested were coated to the polystyrene of the plate and the histaminyl group detected with an antihistamine Mab (D22) produced by Guesdon *et al.* (1986).

The two conjugates HBC and GBC were used as coating antigen for ELISA, GBC being a negative control. All the other conjugates (HBB, HDK, HTST, HTSA, HTSST) were only used as immunogens.

Derivatives

Histamine or related amino components (i.e. cadaverine, putrescine, spermine, spermidine, tyramine, serotonine, histidine, and glycine) were derivatised before to be used in the ELISA. The derivatisations were performed as follows:

- With benzoquinone: the amines or the amino acids were diluted to 10 μmol/ml in 0·2 M phosphate buffer pH 6, containing 3 mg of 1,4-benzoquinone per ml. The mixture was incubated for 1 h at 20°C and the reaction stopped by addition of 2 M glycine to give a pH of 8·5.
- With Traut's Reagent: the amines and amino acids were reacted as described above for histamine (see the section entitled 'Antigen preparation').
- With SPDP: amines and amino-acids were diluted in 0·1 M phosphate buffer pH 7 containing 4 mg of SPDP per ml, and incubated 1 h at 20°C.

Immunisation, fusion and Mab selection

Male BALC/C mice (Charles River, France) were injected subcutaneously with 100 μg of each the following conjugates in Freund's incomplete adjuvant: day 0 HBB, day 14 HDK, day 28 HTSST, day 42 HTST, day 56 HTSA, and day 70 HTSST. These mice were bled on day 0, 21, 32, 49, 62, and 77 for testing the antibody response by ELISA, performed as described below. On day 84 the mice showing antihistamine antibodies received an intravenous injection with 100 μg of HTSA. Hybridomas were generated by the fusion of their splenic lymphocytes with P3X.63-Ag.8.653 myeloma cells, essentially as described by Köhler and Milstein (1975).

The corresponding supernatants were tested by ELISA for histamine-specific antibody. Briefly polystyrene microtitre plates (Immulon Maxisorp, Nunc, Kamstrup Denmark) were coated either with GBC or HBC. These conjugates were diluted in 50 mM carbonate–bicarbonate buffer pH 9·6 and 100 μl of the corresponding solutions were dispended to obtain 300 ng per well. After 18 h at 4°C, unbound protein was washed off with 10 mM phosphate-buffered saline containing 0·1% Tween 20 (PBS-T). Then 100 μl aliquots of hybridoma supernatant were added to the conjugate-coated wells and incubated for 1 h at 37°C. The wells were washed with PBS-T, and antimouse goat IgG labelled with horseradish peroxidase (Jackson Immunoresearch, Baltimore Pike, USA), was added and incubated for 30 min at 37°C. After washing with PBS-T, 100 μl of a 0·005% solution of H_2O_2 in 0·1 M citrate buffer (pH 5) containing 1 mg of *O*-phenylnediamine/ml was added to each well. The enzymatic reaction was stopped after 30 min at room temperature by addition of 50 μl 4 M sulphuric acid. Absorbances were measured at 492 nm on a microtitre plate ELISA reader (Diagnostics Pasteur, Aulnay/Bois, France).

Isotypes determination and purification of antibodies

The isotypes were determined using an ELISA similar to the ELISA described in the above section. After the

addition of hybridoma supernatants to the HBC coated wells, peroxidase-labelled antibodies specific for mouse IgG1, IgG2a, IgG2b, IgG3 or IgM (Sigma), or specific for λ and κ chains were added (Nordic Immunological Laboratory, Tilburg, Netherlands). Then peroxidase substrate was added and the reaction terminated as above. Selected hybridoma cells were further grown in mice, following standard procedures. Mabs were precipitated from ascitic fluids by ammonium sulphate at 50% saturation. They were further purified by ion exchange chromatography and gel filtration according standard protocols (Tijssen, 1985). Purity was checked using polyacrylamide gel electrophoresis analysis.

Characterisation of the Mabs

Measurement of the dissociation constants (KD)
The KD were determined by measuring, by ELISA, the amount of free antibodies present at equilibrium in mixtures of the studied Mab and appropriate ligand (Friguet *et al.*, 1985). Concentrations from 10^{-1} M to 10^{-10} M of histamine or histamine–benzoquinone or HBC, were mixed with constant amounts of the purified Mab. Following incubation for 18 h at 4°C, the free antibodies remaining in the mixtures were assayed with the ELISA procedure described above. The data from these assays were used to calculate KD with a procedure based on Klotz analysis (Klotz & Hunton, 1971).

Evaluation of specificity in competitive inhibition ELISA (CIELISA)
The purified Mabs (100 μl) were mixed with 100 μl volume of serial dilutions (10^{-2} M to 10^{-8} M) of histamine or various related amino components, i.e. cadaverine, putrescine, spermine, spermidine, tyramine, glycine, histidine, or serotonin. They were used in their native form or derivatised with benzoquinone, Traut's Reagent, or SPDP. After incubation for 1 h at 20°C, the hapten–antibody mixtures were transferred into the well of a microtitration plate previously coated with HBC. After a 30 min incubation at 20°C, unbound proteins were removed with PBS-T and the immobilised Mab was detected with a second antibody (a peroxidase-labelled, anti-mouse IgG from Jackson Immunoresearch). Afterwards the assay was carried out as for ELISA.

Fish extract preparation

The fish extracts (tuna, herring or mackerel) were prepared from fresh, spoiled, or spiked, homogenised fish. Spiked samples were prepared according to Lüten *et al.* (1992) as follows: to an aliquot of homogenised fish were added appropriate amounts of the following amines: histamine, cadaverine, putrescine, tyramine, spermine and spermidine. These samples were involved in an international study of histamine evaluation in fish extracts and sent to several laboratories.

Samples (1 g) of the fresh, spiked, or spoiled homogenised fish were extracted with 10% trichloracetic acid (1 ml). The histamine content of the corresponding extracts was evaluated both by the HPLC method and the CIELISA performed with the four Mabs produced.

Histamine assay

HPLC method
The histamine content in the fish extracts was performed as previously described (Gouygou *et al.*, 1989). Briefly, histamine present in the extracts was derivatised with *o*-phthaldialdehyde (OPA) which forms fluorescent products. The OPA complexes obtained from TCA fish extracts were separated by reversed-phase HPLC under gradient conditions. Then histamine was detected by a fluorescence spectrophotometer at 450 nm.

CIELISA
The CIELISA was performed on HBC sensitised microplates blocked with fraction V (Sigma). Briefly, after the coating step, the wells were filled with a fraction V solution (1% w/v in PBS) and incubated 2 h at 37°C. Following three washings with PBS-T, the plates were used immediately or stored frozen (–20°C). The calibration curves were obtained as follows: serial dilutions of histamine–benzoquinone and a selected dilution of each of the four antibodies were added, incubated then transferred to each coated and blocked well. The total amount of antibodies bound to the wells was determined as described above. The extracts to be tested were treated with 1,4-benzoquinone to derivatise any histamine eventually present. So 100 μl of ethanol containing 3 mg 1,4-benzoquinone was added to 800 μl of serial dilution (1/10, 1/100, 1/1000) of the extract in phosphate buffer, pH 6. After 1 h incubation at 20°C, excess benzoquinone was inactivated and the pH adjusted to about 7·4 by adding 100 μl of 3·3 M triethanolamine solution containing 0·7 M glycine. Then the corresponding solutions were diluted 1/10 in PBS-T, and 100 μl were mixed with 100 μl of the purified Mab. After 1 h incubation at 20°C, 100 μl of the mixtures were transferred into each coated and blocked well. The subsequent steps in the ELISA were performed as described above.

RESULTS

Development of Mabs

Fusion of spleen cells, obtained from mice immunized with the five histamine–protein conjugates, with murine myeloma X63-Ag8 yielded about 100 rapidly growing hybridoma. Screening of the supernatants of the clones by the ELISA test revealed that 10 of them produced antibodies that bound to the conjugate HBC. To further investigate their antigenic specificity, the Mabs were retested against all the histamine–protein conjugates , the GBC conjugate, and the corresponding carriers.

Table 1. Some characteristics of the antihistamine antibody (Mab)[a]

Mab	Isotype	End-point titre (ng ml^{-1})	Dissociation constant mol		
			H	HB	HBC
4C9	IgG1 k	2	$3{\cdot}4 \times 10^{-4}$	$7{\cdot}3 \times 10^{-9}$	$7{\cdot}0 \times 10^{-9}$
9D9	IgG1 k	3	$4{\cdot}8 \times 10^{-4}$	$3{\cdot}4 \times 10^{-8}$	$2{\cdot}0 \times 10^{-9}$
7E10	IgG1 k	20	$5{\cdot}7 \times 10^{-4}$	$3{\cdot}5 \times 10^{-8}$	$3{\cdot}4 \times 10^{-9}$
4D9	IgG3 k	300	$1{\cdot}5 \times 10^{-4}$	$7{\cdot}0 \times 10^{-8}$	$1{\cdot}7 \times 10^{-8}$
8E7	IgM k	ND	ND	ND	ND

[a]Mabs end-point titre was calculated from the curves obtained in ELISA by plotting the absorbance at 492 nm versus the concentration of the purified Mabs (see Fig. 2). The dissociation constant was evaluated as described by Friguet *et al.* (1985). H, histamine; HB, histamine–benzoquinone; HBC, histamine–benzoquinone–casein.

Five Mabs bound to histamine–proteins only. No reaction was seen on the corresponding carriers, nor on other amine–protein conjugates, indicating that the histamine group was the most likely common feature recognised.

Table 1 lists the isotypes of these five Mabs, their dissociation constants (KD) and their end-point titre. As an example for the Mab 4C9, refer to Fig. 1 and Fig. 2. The IgM 8E7, showing poor purification yields, was not further studied.

Evaluation of Mabs specificity using CIELISA

The relative affinity of the four Mabs (4C9, 4D9, 9D9, and 7E10) was studied for eight compounds (cadaverine, putrescine, spermine, spermidine, tyramine, histidine, serotonine, and glycine) which may be present in spoiled food, or which show structural analogy with histamine. None of the four antibodies significantly bound to any of these components, either in their native form or in their derivatised form, as the inhibition obtained did not exceed 10%.

The study of the relative affinities of these same Mabs, for native or derivatised free histamine, or for protein bound histamine showed that all of them preferentially bound to the derivatised-form than to the native-form of the histamine molecule. This affinity enhancement was observed with each of the three coupling agent tested (i.e. 1,4-benzoquinone, SPDP, or Traut's Reagent), and varied from 10^3 to 10^5, depending of the antibodies and of the coupling agent (Table 2).

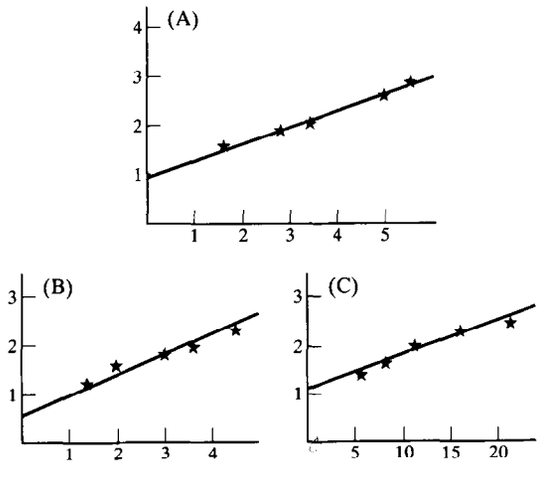

Fig. 1. Klotz plots of the binding of (a) histamine, (b) histamine–benzoquinone–casein conjugate, and (c) histamine–benzoquinone derivative, to 4C9 IgG measured by ELISA. Abscissa: (1/total antigen concentration) $\times 10^5$ mol^{-1} (a), or (1/total antigen concentration) $\times 10^{11}$ mol^{-1} (b and c). Ordinate: 1/fraction of bound antibody. Bound antibody was A_o/A_o -A, A being the absorbance measured for the antibody in the presence of antigen and A_o being the absorbance measured for the antibody in the absence of antigen. The slopes of the lines obtained, were taken as the dissociation constant (KD) as described by Friguet *et al.* (1985).

Histamine detection in food

Similar calibration curves were obtained using the four Mabs, and the detection limit of the assay was about 10–20 ng ml^{-1}, as shown Fig. 3.

The CIELISA and the HPLC method were compared for the determination of histamine present in seven extracts from fresh, spiked, or spoiled fish. The

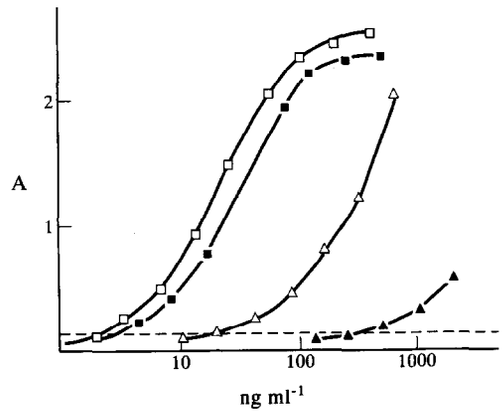

Fig. 2. Titration curves of Mabs (□) 4C9, (■) 9D9, (Δ) 7E10 and (▲) 4D9 obtained in ELISA using HBC sensitised plates. The absorbance (A) at 492 nm was plotted versus the concentration (ng ml^{-1}), of the purified Mab. Cut-off value (0·140) was three times the mean of the absorbances obtained in the absence of Mab. The end-point titres, respectively, 2, 3, 20, and 300 ng ml^{-1}, were evaluated using this titration curve and the cut-off value.

Table 2. Concentration (M) of various histamine form needed to obtain 50% inhibition binding on histamine–benzoquinone–casein (HBC)-coated plate of five Mabs (9D9, 4D9, 4C9, 7E10, and D22, and evaluated using CIELISA

Antigens	Concentration (M) allowing 50% inhibition			
	4C9	9D9	4D9	7E10
Histamine	5×10^{-3}	10^{-4}	10^{-3}	5×10^{-3}
Histamine–benzoquinone	10^{-7}	10^{-8}	10^{-6}	5×10^{-8}
Histamine–SPDP	10^{-6}	10^{-8}	10^{-5}	4×10^{-7}
Histamine–Traut's Reagent	7×10^{-7}	5×10^{-8}	7×10^{-7}	5×10^{-7}
HBB	2×10^{-7}	10^{-7}	2×10^{-7}	7×10^{-8}
HBC	10^{-6}	3×10^{-8}	3×10^{-7}	5×10^{-8}

[a]Briefly, the purified Mabs were incubated 1 h with 10^{-2}–10^{-8} M solutions of the various histamine forms. The mixtures were transferred to a HBC-sensitized plate and the total amount of antibody bound to the wells evaluated by ELISA. HBB, histamine–benzoquinine–BSA.

histamine concentration obtained with either the HPLC method or the CIELISA were closely related especially with the Mab 4C9. Further comparison was performed, using 4C9 or 4D9 in a study of 43 additional fish samples from various source: fresh or spoiled tuna or herring or mackerel. These samples encompassed a wide range of concentrations and positive correlations were found, using either 4C9 or 4D9, of $r = 0.9980$ or $r = 0.9939$, respectively (Fig. 4). In addition, within-assay and between-assay variability of both the 4C9-CIELISA and the 4D9-CIELISA were evaluated by assaying histamine for 10 fish samples in replicate ($n = 8$) as follows:

- Within-assay: each sample was extracted, then four aliquots of the corresponding extract were derivatised and tested in duplicate.
- Between-assay: each sample was extracted, derivatised, and tested on different days.

For the 4D9-CIELISA, the within-assay and between-assay CV's reached 10·0% and 12·2% respectively. For the 4C9-CIELISA, the CVs did not exceed 8·8% (within-assay) or 10·5% (between-assay), as shown in Table 3. So the assay precision was better, using 4C9.

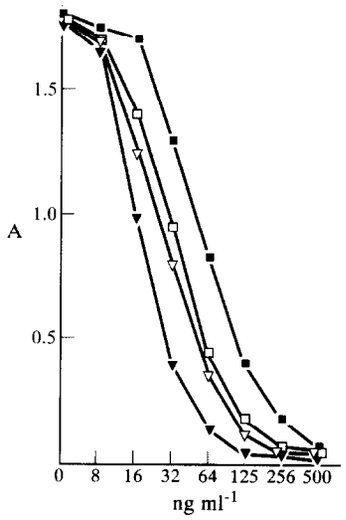

Fig. 3. Dose–response curve for histamine determined by the competitive inhibition ELISA (CIELISA). Ordinate: absorbance (A) at 492 nm; absissa: histamine concentration in ng ml^{-1}. Purified Mabs (9D9 ▲, 7E10 Δ, 4C90 □, 4D9 ■) were incubated for 1 h at room temperature with histamine–benzoquinone solutions of varying concentrations. The mixtures were then transferred to a HBC sensitised plate, and the total amount of antibody bound to the wells determined in ELISA. Each point represents the mean of four determinations.

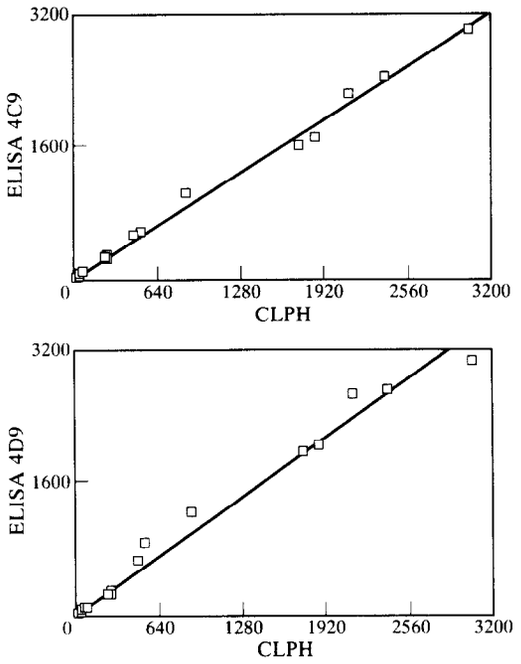

Fig. 4. Correlation between fish extracts histamine concentration (mg kg^{-1}) determined either by the HPLC method or by the competitive inhibition ELISA.

Table 3. Within-assay and between-assay variability of the 4C9-CIELISA evaluated by assaying histamine for ten fish samples in replicate ($n = 8$)

Samples	Variability within-assay		Variability between-assay	
	Mean (mg kg^{-1})	CV (%)	Mean (mg kg^{-1})	CV (%)
Tuna D0	24·4	7·9	24·7	9·5
Tuna D5	30·8	4·8	29·9	6·3
Tuna D15	1058	7·3	1052	10·5
Tuna D21	1644	5·0	1616	6·9
Tuna D15*	3038	3·0	3057	4·8
Tuna 53	5·0	7·0	5·1	8·1
Tuna 239	5·3	6·8	5·3	5·6
Tuna 271	269	5·5	262	6·2
Tuna mag	88	8·8	87	9·9
Mackerel D8	537	7·3	540	9·8

DISCUSSION

Several methods exist for the detection of histamine in food. The main ones are the fluorometric and the chromatographic methods.

The fluorometric methods consist of derivatisation of the histamine with *o*-phthaldialdehyde and measuring the fluorescence of the resulting compound (Shore *et al.*, 1959). This procedure is not entirely specific for histamine: interference by histidine, spermine and spermidine has been noted. In spite of an additional step before derivatisation (ion exchange chromatography or sequential extraction of the food extracts), specificity may be low. Chromatographic methods include TLC and HPLC (Taylor, 1986). TLC consist of migration of the food extract on silica gel and then detection of histamine with various spray reagents such as fluorescamine or ninhydrin. This method is simple but is only semiquantitative. HPLC is largely applied, but the histamine must be derivatised. Precolumn derivatisation (*o*-phthalate, dansylchloride) or postcolumn derivatisation (ninhydrin, *o*-phthaldialdehyde) are used. This method is highly specific but requires expensive laboratory equipment and is time-consuming.

So to date, a wide range of methods for the determination of histamine in foods has been described. However, only some laboratories are actually able to determine histamine in food products with an acceptable accuracy (Lüten *et al.*, 1992). As future regulation for the limitation of the histamine content in food products, especially fishery products, is to be expected (Lüten *et al.*, 1992), the number of histamine determination tests might increase. So there is a growing need for more rapid and specific methods for histamine analysis. As an alternative to the more conventional methods of analysis, biospecific procedures using antibodies are well-suited to meeting these demands. Immunoassay is soon widely used for detection of a variety of small molecules such as drugs or pesticides (Vanderlaan *et al.*, 1988), and offer a promising approach for food analysis (Fukal & Kas, 1989; Finglas & Morgan, 1994).

Histamine is a small molecule (mol. wt 111), and alone is not immunogenic, i.e. it does not stimulate antibody production in animals. In order to obtain antibodies, the histamine molecule must be coupled to a large immunogenic carrier molecule, usually a protein. The way in which the histamine–protein conjugate is produced, i.e. the use of different functional groups to couple to the protein, is crucially important in defining the specificity of the antisera produced.

Several methods to link the histamine molecule to a proteic carrier have been reported over the years, and may be classified in two major groups. The first group of methods consist of linking the histamine amino group to a coupling agent, which in a second step, will form a spacer between the histamine and protein molecules. In these cases the different Mabs produced (Guesdon *et al.*, 1986) showed low affinity for native histamine. However, an affinity enhancement was obtained after histamine derivatisation by the coupling agent used for the immunogen preparation. In the second approach, the histamine was coupled via its ring 1-nitrogen to the carrier protein. Hammar *et al.* (1990) obtained a Mab showing a KD of 100 nM for native histamine, but cross-reaction with histamine structurally related compounds was observed.

In order to produce new antihistamine Mabs, we produced several histamine–protein conjugates, by linking the histamine amino group to different coupling agents, which will form a spacer between histamine and the carrier protein. Mice were immunised with the conjugates obtained, following an original protocol. Each injection was performed with a different conjugate, i.e. the spacer between histamine and proteic carrier was different or the carrier was different, in order to raise high affinity antibodies to native histamine.

We obtained four Mabs which bound native histamine with KD in the range 15–57 mM. The histamine derivatisation allowed an affinity enhancement reaching 10^5. This enhancement is non-specific, as it could be obtained with at least three different coupling agents. Affinity enhancement by non-specific chemical derivatisation have been described for other haptens such as cyclic AMP (Delaage *et al.*, 1979) or the herbicide Benzaton (Qing Xiao Li *et al.*, 1991), but was not still described for histamine. Immunoenzymoassays were generally performed at pH around 7·5, and histamine occurs in the ionized state at this pH (Taylor 1986). An explanation may be that the antibody binding site cannot accept the charged hapten, i.e. histamine in our case, as proposed for other charged haptens as Benzaton (Qing Xiao Li *et al.*, 1991) and imidazole (Morel *et al.*, 1990).

We showed too that all the four Mabs appeared as highly selective for histamine since no cross-reaction is observed with structurally related compounds as histidine or serotonine, or with the other biogenic amines, which often occurred simultaneously in spoiled food. All these Mab properties indicate their potential usefulness in an immunoassay for the determination of histamine in food products, if a derivatisation step, before the assay is introduced. As a high-affinity enhancement was obtained with all the coupling agent tested, we choose the cheaper, the 1,4-benzoquinone.

The lower limit of detection in the present CIELISA was of the order of 10 ng ml^{-1}. The sensitivity of this test appeared as adequate, as upper limits of 100 mg histamine per kg in foods and 2 mg histamine $litre^{-1}$ in alcoholic beverages have been suggested (ten Brink *et al.*, 1990). A good correlation was observed when the commonly used HPLC method and the CIELISA performed with each the four Mabs, were used for the quantification of histamine in seven fish extracts. In this case, the best correlation was obtained with the Mab 4C9, as the histamine concentration obtained in CIELISA did not differ from those obtained by HPLC by more than 12·7%, instead of 24·6% to 50·6% when using the other Mabs. Additional results obtained in a study of 50 fish extracts, using either the 4C9 or the

4D9, confirmed this good correlation between the immunoassay and the chromatographic assay. The 4C9 appeared as the best Mab to be used in CIELISA, as the CV values obtained in this case, were lower than those obtained for 4D9. The CIELISA we described required several steps, i. e. derivatisation, histamine-Mab competition, and ELISA itself. These successive steps, were certainly responsible for the relatively high CVs values we observed. Work is now in progress to simplify this ELISA. However, the present CIELISA is soon sensitive and specific enough to assay histamine in fishery products, and may be completed within 5 h. This method does not require a sophisticated detection system and can be easily automated.

Recently, a Mab-based ELISA allowing the detection of spermine has been described (Garthwaite *et al.* , 1993). It can be expected that before long an antibody panel could be used to detect the different biogenic amines which may be present in spoiled food. So, immunoassay offers a very attractive alternative to the more conventional methods of biogenic amine analysis.

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