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Sensitivity and specificity enhanced enzyme-linked immunosorbent assay by rational hapten modification and heterogeneous antibody/coating antigen combinations for the detection of melamine in milk, milk powder and feed samples

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ABSTRACT

The adulteration of food products with melamine has led to an urgent requirement for sensitive, specific, rapid and reliable quantitative/screening methods. To enhance the sensitivity and specificity of the enzyme-linked immunosorbent assay (ELISA) for the detection of melamine in milk, milk powder and feed samples, rational hapten modification and heterogeneous antibody/coating antigen combinations were adopted. Three melamine derivatives with different length of carboxylic spacer at the end were synthesized and linked to carrier proteins for the production of immunogens and coating antigens. Monoclonal antibody against melamine was produced by hybridoma technology. Under optimal experimental conditions, the standard curves of the ELISAs for melamine were constructed in range of 0.1–100 ng mL^{-1}. The sensitivity was 10–300 times enhanced compared to those in the published literatures. The cross-reactivity values of the ELISAs also demonstrated the assays exhibited high specificity. Five samples were spiked with melamine at different concentrations and detected by the ELISA. The recovery rates of 72.8–123.0% and intra-assay coefficients of variation of 0.8–18.9% ($n=3$) were obtained. The ELISA for milk sample was confirmed by high-performance liquid chromatography with a high correlation coefficient of 0.9902 ($n=6$). The proposed ELISA was proven to be a feasible quantitative/screening method for melamine analysis.

1. Introduction

Melamine (2,4,6-triamino-1,3,5-triazine, $C_3H_6N_6$, Fig. 1) was commonly used as an industrial chemical in the production of melamine–formaldehyde polymer resins for laminates, coatings, commercial filters, glues or adhesives, plastics and flame-retardants [1]. Melamine can be found in food and beverages due to migration from melamine-containing resins [2], or as a metabolite product of cyromazine, an insecticide used on animals and crops [3]. The high nitrogen content of melamine has led to its being used as adulterants in feed and food, presumably to inflate the amount of measurable nitrogen, as determined by Kjeldahl method, producing products that appear to have more protein at a reduced cost. Melamine was originally viewed as nontoxic when administered in the purified form as a supplement [4]; however, it can form lethal kidney stones [5], especially when combined with cyanuric acid [6], due to precipitation of insoluble melamine cyanurate. High and prolonged dietary exposure to melamine results in the formation of bladder stones and increase incidence of urinary bladder tumors. Large outbreaks of nephrotoxic renal failure occurred in dogs and cats attributed to ingestion of melamine-containing pet food in 2004 and 2007 [7,8]. In September 2008, infant formulas that were illegally adulterated with melamine led to health problems for thousands of infants in China [9]. In many countries, the tolerance level for melamine is regulated to be 1 mg kg^{-1} for baby formula and 2.5 mg kg^{-1} for food containing $>15\%$ milk [10]. Therefore, determination of melamine is of biological, clinical, and food industry importance, and sensitive, specific and high throughout analytical methods are needed to determine melamine residues in food and feed, and particularly in dairy products for children.

Many methods including capillary electrophoresis [11], HPLC [12], LC-MS/MS [13,14], GC–MS [15], nuclear magnetic resonance spectroscopy [16], MALDI-MS [17] have been developed for the detection of melamine. These methods are accurate, but they are expensive and time-consuming and often require complicated

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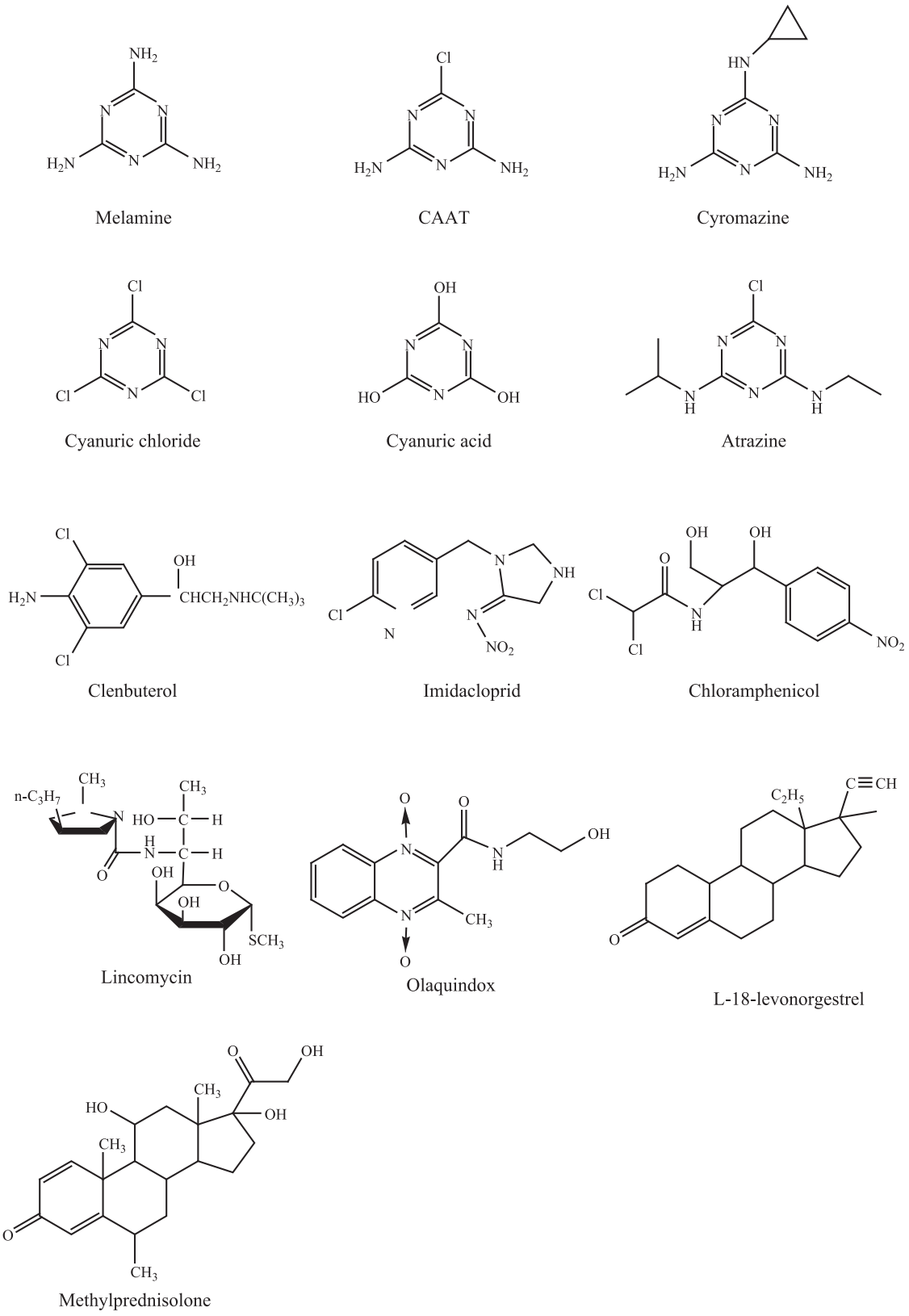

Fig. 1. Molecular structures of melamine and other compounds used for cross-reactivity testing.

sample preparation before analysis. Recently various other techniques such as visual detection by use of gold nanoparticles [18,19] and surface-enhanced Raman spectroscopy [20,21] were proposed for melamine detection. However, these methods suffer from low sensitivity or specificity.

Immunoassays, especially enzyme-linked immunosorbent assays (ELISAs), are analytical methods which are based on the specific interaction between an antibody and corresponding antigen. Immunoassays are generally rapid, high sensitivity and specificity, simple sample preparation, high throughput, and therefore, low cost per