Immobilized Metal Ion Chromatographic (IMAC) Determination of Ovomucoid in Hen's Egg White

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Abstract

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An immobilized metal ion high-performance liquid chromatographic technique was developed for the determination of one of the egg allergens – ovomucoid – in egg white and food products containing egg white. The method was tested using standard samples as well as complete egg white and its reproducibility was determined. The possible application of this method is discussed. The method is based on the HPLC chromatography on the chelating column saturated with copper ions. The sample is applied to the column at pH 7.5 and eluted isocraticaly with a buffer containing 1.75 mM imidazole in the optimum case.

Keywords: ovomucoid; determination; HPLC; egg white; analytical method; IMAC; HPLC

Egg white ovomucoid is one of the most important food allergens (COOKE & SAMPSON 1997; URISU *et al.* 1997). It is the "true" allergen producing IgE in sensitive patients (ZHANG & MINE 1998). According to the act of the European Commission, it is recommended (see e.g. the recommendation of the year 1997 of OECD code SG/ICGB(97)1) to declare the concentration of all the allergens present in foods; as a consequence, a method for the quantification of the allergens has to be developed.

Ovomucoid can be determined presently only by immunochemical methods (KAMINOGAWA *et al.* 1985; TAKAHASHI *et al*. 1999). The cross-reactivity of the appropriate immunoglobulin with some other proteins (not only that of egg white) (ZHANG & MINE 1998) may cause errors in the determination of ovomucoid in a mixture.

This paper reports the development of a simple IMAC method for the determination of ovomucoid based on the absence of an accessible histidine moiety (or other groups interacting with copper ions) on the molecule of this protein whereas all other proteins present in egg white bind to these ions. Moreover, the lack of histidine on the surface of other proteins used in food products is rare. Then the method presented here could replace the immunochemical method for the determination of this allergen.

MATERIAL AND METHODS

IMAC (Immobilized Metal Ion Affinity Chromatography) experiments were carried out with the use of HPLC system (equipment ECOM, Prague, CR) utilizing the column Separon HEMA 1000 Budge IDA (8 × 250 mm) (from Tessec, Prague, CR) saturated with copper ions.

Proteins in the effluent were evaluated on the ground of their absorption at 280 nm.

The sample (hen's egg white) was diluted with distilled water to the final concentration of 10% (w/v), the insoluble part was separated by centrifugation, and the clear supernatant was directly used for the separation.

Standard proteins used in this work, i.e. ovalbumine, ovomucoid, and avidin, were the products of SIGMA Fine Chemicals and were of the reagent grade. Lysozyme, the food grade, was purchased from PROMED factory, Prague, CR.

The following protocol was used for IMAC of the diluted egg white: the diluted egg white samples were adjusted to pH 7.5 and the concentration of NaCl in the samples was adjusted to 0.5 M. The samples were then injected into the column equilibrated with 50 mM acetate, pH 7.5, containing 0.5 M NaCl (buffer A) and imidazole in the concentration range of 1.25 to 2.75 mM.

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The proteins were eluted isocraticaly with the starting buffer.

RESULTS AND DISCUSSION

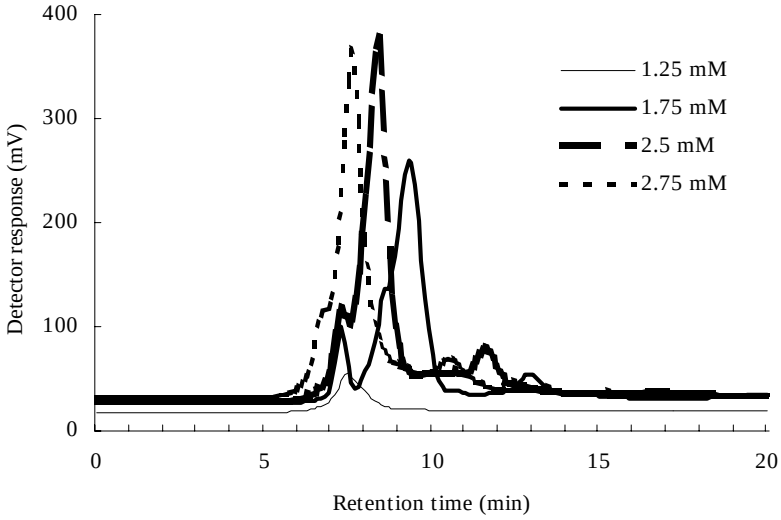
In the first instance, the column was run with egg white samples in buffer A and the separation was studied as a function of the imidazole concentration. The results shown in Fig. 1 indicate a strong dependence of the separation of the individual proteins on the concentration of imidazole. Both the highest and the lowest concentrations gave a poor resolution. The proteins were not eluted at all (with the exception of ovomucoid) from the column at the lowest concentration of imidazole and were eluted as a single peak (with the exception of lysozyme) in the case of the highest concentration. The medium concentrations (1.25 and 1.75 mM) gave relatively similar resolutions, however, 1.75 mM imidazole was supposed to be better. The individual egg white proteins were identified at the optimum concentration of imidazole using standard egg white protein samples (Fig. 2). The separation of egg white ovomucoid at this imidazole concentration is very good and its analytical application is possible.

The retention time of ovomucoid is equal to 7.33 both in the egg white and in the standard samples.

In the following, the calibration curve was determined with the method of internal standard which seemed to be the best way of calibration in this and similar cases.

The egg white samples were run with the addition of different amounts of pure ovomucoid under standard conditions (buffer A containing 1.75 mM imidazole) using the isocratic elution; the peak areas were plotted against the added ovomucoid concentration.

As shown in Fig. 2, the egg white proteins other than ovomucoid cannot be separated for analytical purposes. Ovalbumine has nearly the same retention time as avidin and, therefore, neither of these proteins can be determined



The chromatography was run on the IMAC sorbent saturated with Cu^{++} ions in 50 mM acetate pH 7.5 containing 0.5 M NaCl and different concentrations (1.25 to 2.75 mM) of imidazole

Fig. 1. The dependence of the resolution of egg white proteins in IMAC chromatography on the concentration of imidazole in the buffer

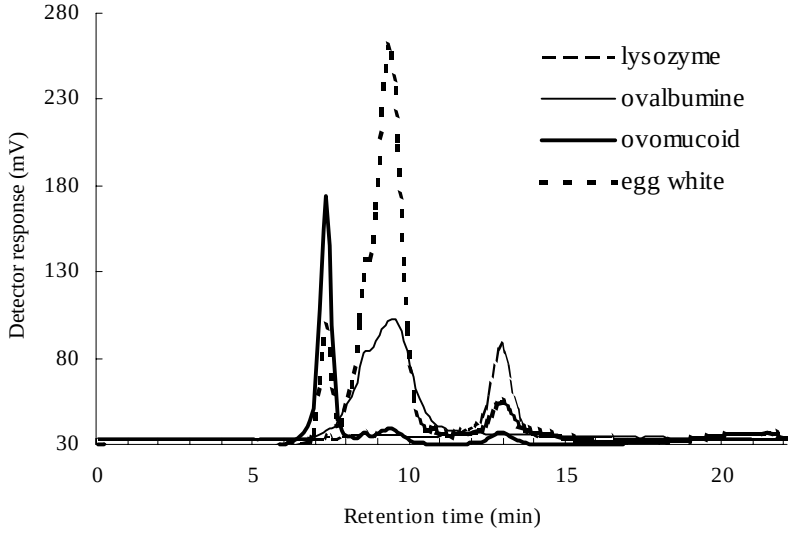


Fig. 2. The IMAC chromatography of egg white and the standards for the identification of individual protein peaks (buffer A with 1.75 mM of imidazole)

quantitatively by this IMAC method. Nevertheless, the ovalbumine concentration can be roughly estimated from the peak area because the relative concentration of avidin is low compared to the concentration of ovalbumine. The systematic error caused by this fact is not too great for the approximate estimation.

Conclusion

HPLC on immobilized metal ion described in this paper can serve as a new simple procedure for the quantitative determination of ovomucoid and could also be used as a method for the approximate estimation of the content of ovalbumine in egg white and food products prepared from eggs. Ovomucoid is well separated at the retention time equal to 7.33 min but the peak of ovalbumine, which appears at the retention volume of approx. 9.39 min, represents the sum of the peak of ovalbumine itself and that of avidine. The ovalbumine standard presents two peaks which are not fully separated at the retention volumes of 8.59 and 9.53 min whereas the avidine standard presents a sharp peak at 8.63 min. As ovalbumine forms the major protein in egg white (approx. 54% of all proteins) and avidin amounts to 0.05% only, the area of the peak with the retention time 9.39 min could be used as a rough estimate of the ovalbumine concentration, namely in the food products prepared from egg white where other methods are less reliable.

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Souhrn

PROŠKOVÁ A., KUČERA J. (2002): **Stanovení ovomukoidů ve vaječných bílcích chromatografií na imobilizovaných iontech kovů (IMAC)**. Czech J. Food Sci., **20**: 95–97.

HPLC na imobilizovaných iontech kovů byla použita pro stanovení jednoho z hlavních alergenů vaječných bílků – ovomukoidu. Tato metoda je vhodná pro stanovení ovomukoidu ve vaječném bílku a potravinářských výrobcích obsahujících vaječný bílek. Byly testovány jak standardní vzorky, tak i kompletní vaječné bílky. V práci jsou rovněž diskutovány možnosti a oblasti použití metody. Principem stanovení je HPLC na chelátotvorném sorbentu nasyceném měďnatými ionty. Vzorky jsou nanášeny při pH 7,5 a eluovány isokraticky pufrem obsahujícím 1,75 mM imidazolu. Tyto podmínky byly shledány jako optimální pro dobré oddělení ovomukoidu od ostatních bílkovin vaječného bílku a pro dosažení maximální reprodukovatelnosti.

Klíčová slova: ovomukoid; vaječný bílek; analytické metody; IMAC; HPLC

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