FORMATION OF FLAVOUR PRECURSORS BY THE AMP PATHWAY IN CHICKEN MEAT

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Abstract

Ribose, a key flavour precursor in chicken meat, is believed to derive from the ATP breakdown pathway. This paper reports an investigation of this pathway. AMP, IMP, inosine and adenosine were added to chicken crude extract that had been dialysed against either phosphate or citrate buffer. Analyses were conducted to determine the quantities of key metabolites. These studies suggest that a number of pathways may contribute to the breakdown of AMP and its metabolites. Phosphate ions play a key role in determining the balance between these pathways.

Introduction

The volatile compounds responsible for meat flavours and odours develop during cooking by complex reactions between natural components in raw meat. In chicken, ribose has previously been shown to be of particular importance for flavour generation, with a much greater effect than ribose-5-phosphate or thiamine, when the natural concentrations of these compounds are taken into account (1). The generally accepted route of formation of ribose is via the breakdown of ATP to AMP and inosine to give ribose and hypoxanthine (2), as illustrated by the heavy arrows in (Figure 1). However, many other related pathways are known in living organisms and the enzymes classified (Figure 1). While information is available on these individual enzyme pathways, few studies have investigated the whole enzyme system. Amongst the few reports of these pathways in meat is that of Lee and Newbold (3) who presented evidence, using enzyme inhibitors, that, in ox muscle (*longissimus dorsi*), IMP was degraded to hypoxanthine by a pathway involving steps (d) and (f) rather than (d) and (e) (Figure 1). However, sugar concentrations were not reported.

Both IMP and inosine are present in chicken meat postmortem and only a small proportion appears to be converted to ribose (4). For optimum flavour generation it would be desirable to increase this conversion. Phosphate ions are important cofactors or products of several of these pathways (Figure 1) and it was expected that their presence or absence would inhibit some pathways and promote others.

This study was designed to test the effect of the presence or absence of key metabolites on the formation of sugars and nucleotides by the AMP breakdown pathway in a simple extract intended to mimic the whole chicken enzyme system.

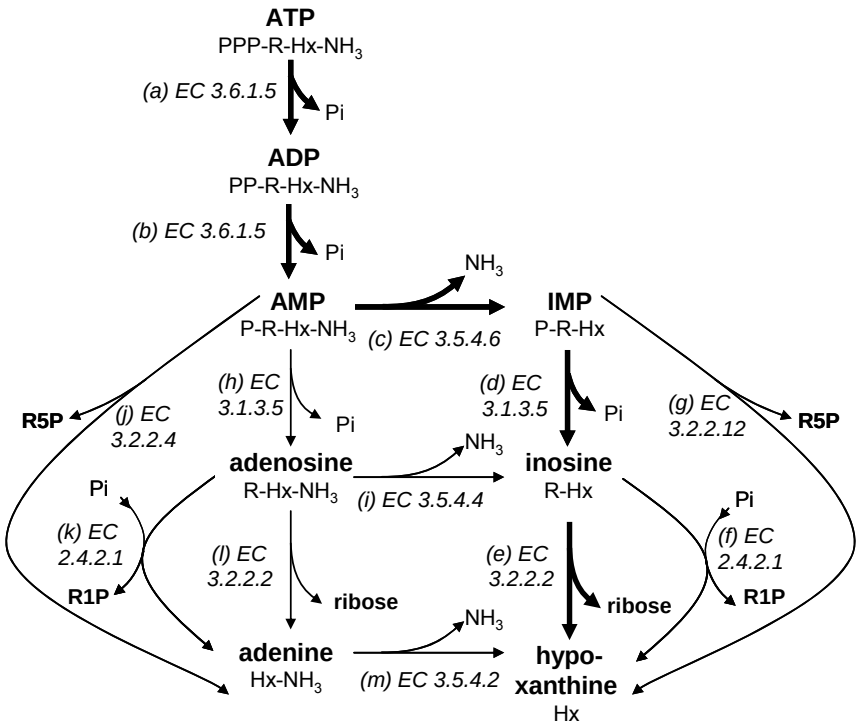


Figure 1. *Known ATP breakdown pathways that may occur in chicken meat.*

Experimental

Chicken breast meat without skin (10 kg, pH 5.98) was minced and mixed thoroughly, before taking a sample (750g). This sample was homogenized with 1500 ml KCl (0.16 M) for 3 minutes at 4 °C and the mixture centrifuged at 14,000 g at 4 °C for 30 minutes (Beckman centrifuge, UK). Two aliquots of the crude extract supernatant (650 ml) were dialysed against 10 liters of either phosphate or citrate buffer (0.1 M, pH 6.0). Phosphate buffer is one of the major buffering constituents of meat, and was used at a concentration to simulate the ionic strength and pH of meat, while citrate buffer was chosen to give the same ionic strength and pH, but an absence of phosphate ions. The dialyzed crude extracts (450 ml) were supplemented with a vitamin, co-factor and mineral solution (50 ml) to supply any essential cofactors that would have been lost through dialysis. Its composition was based on that of several commercial assay media (Sigma, Poole, UK) and comprised: riboflavin (0.1), thiamine (0.1), biotin (0.001), niacin (0.2), para-aminobenzoic acid (0.2), panthanoic acid (Ca^{2+}) (0.1), pyridoxine HCl (0.2), pyridoxamine (0.08), folic acid (0.02), cobalamine (0.1), NADP (765.0), NAD (3.0), FMN (3.0), FAD (3.0) (mg L^{-1} of mixture added); $MgCl_2$ $4H_2O$ (0.1), $MgNO_3$ (0.1) (mM of mixture added).

Two experiments were conducted. In Experiment 1, solutions of IMP, AMP, inosine and adenosine (6 µmol in 1 mL HPLC grade water) were added individually to 5 ml of dialyzed crude extract (against either phosphate or citrate buffer) in triplicate and incubated at 37 ºC for 0, 5 or 18 hours. In Experiment 2, crude extract dialyzed against citrate buffer was used. IMP or inosine (20 µmol or 6 µmol, respectively, in 1 mL HPLC grade water) was added, with or without phosphate (600 µmol, in 1 mL HPLC grade water) to 5 ml of dialyzed crude extract to give a final volume of 7 mL. Samples were prepared in triplicate and incubated at 37 ºC for 0 or 5 hours. All samples were treated with perchloric acid (15% v/v) and neutralised with KOH (6 M). Extracts were analysed by reverse phase HPLC (5) for nucleotides and

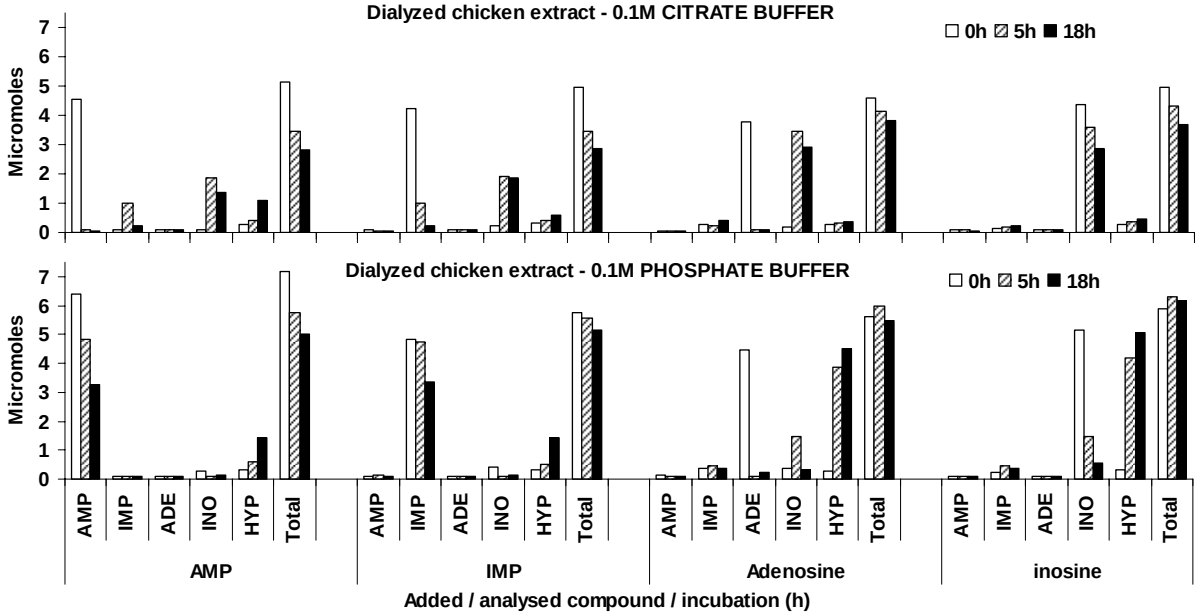
related compounds (Experiment 1) and by GC-MS, for sugars and sugar phosphates (Experiment 2), using a method adapted from that of Le Blanc and Ball (6).

Results and Discussion

Figures 2 and 3 show the effect of adding selected AMP metabolites into dialyzed chicken crude extract on the quantities of nucleoside-related compounds and sugars. Figure 2 shows that there was no large change in metabolite concentrations between 5 and 18h incubation, as would be consistent with exponential growth of microorganisms. Furthermore, the residual sugars in dialyzed (or non-dialyzed) crude extract, to which no metabolites had been added, did not show any decrease between 0 and 5 hours (data not shown). This gives confidence that the effects observed are largely due to the action of enzymes endogenous to the chicken muscle rather than due to microbial growth.

The simple extraction process and the inclusion of a range of minerals and cofactors aimed to ensure that the enzymes in this system remained active after extraction and dialysis. The conversion of metabolites observed in dialysed and undialysed extracts data not shown) showed that the enzyme systems remained active. The different breakdown steps will be discussed in reverse order, as the later steps have a clear effect on the progress of those that precede them.

Inosine breakdown. The breakdown of inosine to form hypoxanthine is evidently very dependent on the presence of phosphate (Figure 2), with little breakdown occurring in citrate buffer. This suggests that the generally accepted pathway (e) may not be the most important pathway for the breakdown of inosine and that pathway (f), requiring phosphate and giving ribose-1-phosphate, may be more important. This agrees with the results reported by Lee and Newbold (3). Ribose-1-phosphate was sought using GC-MS; it was not detected but appeared to be labile under analysis, so its absence cannot be assumed.



AMP = adenosine 5'-monophosphate; IMP = inosine 5'-monophosphate; ADE = adenosine; INO = inosine; HYP = hypoxanthine

Figure 2. *Effect of adding compounds (6 μmol) to crude extract of chicken, dialyzed against citrate or phosphate buffer, on AMP metabolites.*

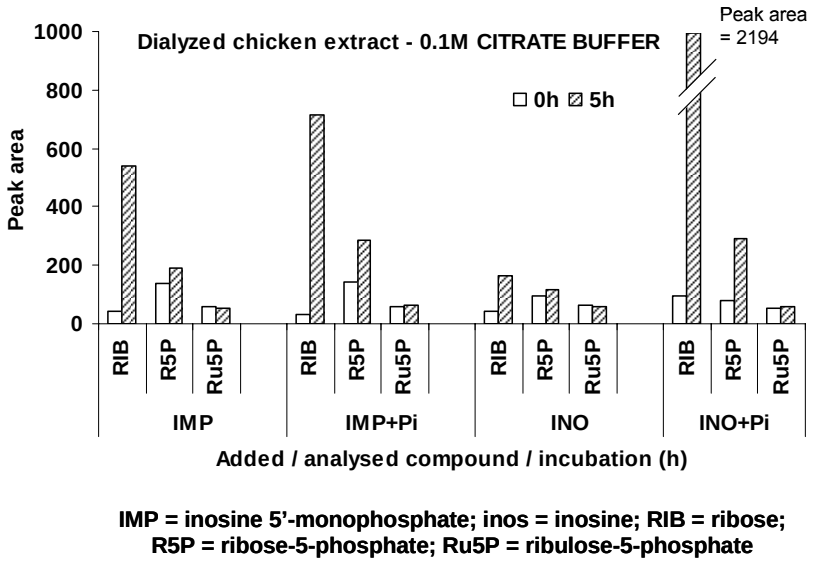


Figure 3. *Effect of adding IMP (20 μmol) or inosine (6 μmol), with and without phosphate (Pi), to crude extract of chicken, dialyzed against citrate buffer, on sugars.*

Unexpectedly, both ribose and ribose-5-phosphate were increased with phosphate (Figure 3). The very large increase in ribose with added phosphate is difficult to explain as neither its breakdown from inosine nor its formation from ribose-1-phosphate would be expected to be enhanced by phosphate. The ribose-5-phosphate might be formed either by a reversal of pathway (d) to form IMP in the presence of phosphate (7), followed by pathway (g) to give ribose-5-phosphate and hypoxanthine, or by pathway (f) to give hypoxanthine and ribose-1-phosphate, which may then be converted to ribose-5-phosphate (8). Figure 2 indicates that only small quantities of IMP were formed on addition of inosine in the presence of phosphate (by a reversal of (d)), which agrees with the findings of Lee and Newbold (3). Thus, pathway (f) may be the more important breakdown route for inosine, despite the absence, as yet, of direct evidence for this.

IMP breakdown. The dephosphorylation of IMP to inosine (d) is inhibited, at least partially, by phosphate (Figure 2). However, again, the presence of phosphate caused more ribose and ribose-5-phosphate to be detected (Figure 3). The additional ribose-5-phosphate may be formed by (g), with IMP giving ribose-5-phosphate and hypoxanthine, or by the alternative pathway suggested for inosine breakdown. As expected, ribulose-5-phosphate, almost certainly formed by the pentose phosphate pathway, was unaffected in the presence or absence of phosphate.

Adenosine breakdown. Adenosine breaks down very readily to give inosine, presumably by pathway (i) in (Figure 1). This conversion is rapid, with addition of adenosine and inosine giving identical products at 5 and 18 hours. However, there is no direct evidence in the data reported in (Figure 2) that adenosine is formed from AMP by pathway (h), though it is possible that this could be due to rapid onward breakdown of adenosine to inosine. From the almost complete loss of added adenosine to inosine (in citrate) and hypoxanthine (in phosphate), it is likely that any adenosine present in chicken meat would be rapidly broken down to inosine.

AMP breakdown. In citrate buffer, AMP breaks down to give a small amount of IMP together with inosine and hypoxanthine (Figure 2). These pathways would appear to be fast. In contrast, in phosphate buffer, little of the AMP breaks down and

little IMP or inosine is detected. Nevertheless, hypoxanthine is formed. This may reflect the incomplete inhibition of pathways (h) / (d) by phosphate followed by rapid breakdown of inosine (and adenosine) to give hypoxanthine.

Role of phosphate. Dusek *et al.* (9) reported free phosphates as ca. 4500 mg "free P_2O_5"/kg in chicken breast. It may be calculated from this that the concentration of phosphates in the aqueous component of chicken (assuming a water content of 75%) is ca. 0.08M. These concentrations are only a little lower than in the phosphate buffer used in Experiments 1 and 2 (0.1 M). Thus, in chicken meat, it is likely that phosphate enhances the breakdown of inosine more than it inhibits its formation.

Conclusion

Evidence is provided that, whilst most of the accepted AMP breakdown pathways occur, additional pathways may also contribute to the breakdown of AMP and its metabolites to give ribose and related flavour precursors. Phosphate ions play a key role in determining the balance of activities of these pathways and of the products formed.

References

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