Waters Lab Highlights

An Internal Communication of Applications and Techniques

LAH 0412 6/89
DP/LS/RS/AA/PH

Contamination of Samples and Its Impact on the Limit of Quantitation When Using Pico-Tag® AAA

In a previous Lab Highlight[1] the factors which limit sensitivity and accuracy of chromatographic analyses of amino acids were considered. In this Lab Highlight, the issue of sample contamination, which limits compositional analysis, is discussed.

In order to determine sources and levels of contamination, the contributions from a reagent blank and a hydrolyzed blank were measured and compared to the theoretical values for a 100 ng lysozyme sample[2]. The reagent blank was obtained by derivatizing water in an empty tube and the hydrolyzed blank was obtained by hydrolyzing and derivatizing an empty tube. The results are shown in Figure 1. Note that the reagent blank contributes very little (the bars are nearly indistinguishable from the baseline), while there is a significant contribution from the hydrolyzed blank. This suggests that the contamination arises from protein or peptide, not from free amino acids. Such sample contamination can arise from many sources, including airborne particulates, dirty glassware, carryover from LC columns used for protein isolation, poor lab practices by the operator, insufficiently pure reagents, etc.

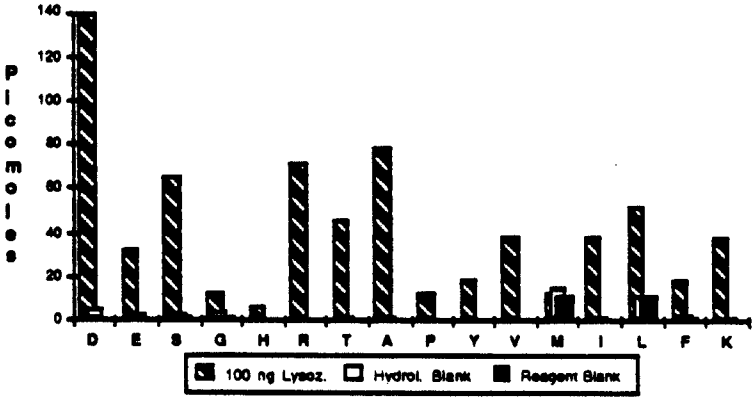

Figure 1. Analysis of amino acid content of 100 ng of hydrolyzed hen egg white lysozyme compared to that from a hydrolyzed blank. Refer to text for discussion.

Proper cleaning of the sample tubes is essential for high sensitivity work. This was verified by the comparison of hydrolyzed blanks before and after pyrolysis treatment of the tubes. Figure 2 shows that acid washing of the tubes can result in background levels of as high as 10-15 picomoles. We have even seen some tubes result in excursions as high as 20-30 picomoles. But, pyrolysis of the tubes (400-500 °C overnight), along with care in handling samples, buffers, etc., can consistently reduce background levels to below about 5 picomoles. The values shown in Figure 2 were obtained by hydrolyzing the "clean" sample tubes, and then derivatizing in the normal manner.

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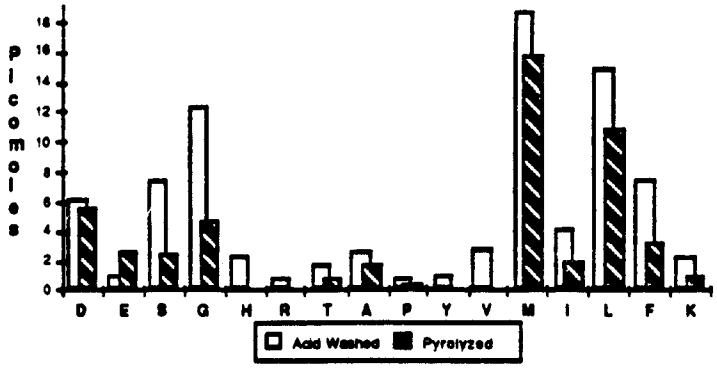

Figure 2. Amino acid analysis of acid washed and acid washed, pyrolyzed hydrolysis tubes.

The 1-5 picomole background level (which can only be achieved with great care) sets a limit on the ability to accurately determine amino acid composition on small amounts of proteins and peptides. For proteins, we have found this limit to be about 500 ng (0.5 ug). Although good values can be obtained for most residues at lower levels, poor results are frequently obtained for specific amino acids (e.g., high values for serine and glycine due to background contamination, low values for tyrosine and methionine due to hydrolytic degradation, and low yields for histidine and arginine, possibly due to the presence of metal ions). Investigations of these phenomena are on-going.

Polypeptides can be analyzed at lower levels, as shown in Figure 3 for oxidized insulin B chain. Even with as little as 100 ng of sample hydrolyzed, the composition can be unambiguously determined.

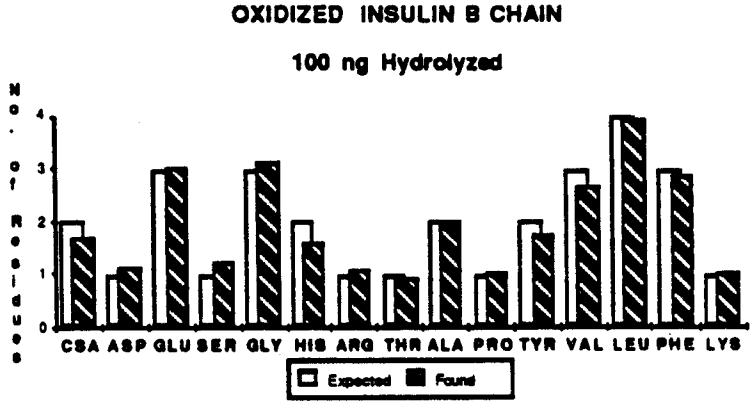

Figure 3. Amino acid analysis of 100 ng of hydrolyzed oxidized insulin B chain.

The data presented in this Lab Highlight suggests that the Pico-Tag method has more than adequate sensitivity to deal with real world samples isolated using today's technology. Other derivatives have been shown to give higher sensitivities than PTC derivatives ***for amino acid standards; however, the sensitivity issue is not with standards but with real samples.*** Simply increasing the sensitivity using newer reagents will not result in higher sensitivity since it appears that protein contamination of samples is a major limiting factor in sensitivity. When our ability to isolate and handle samples improves significantly, techniques such as microbore LC and fluorescence detection may offer real advantages. Right now the Pico-Tag method does the best job available.

References:

1) LAH 0383 10/88.
2) B. A. Bidlingmeyer, T.L. Tarvin, S. A. Cohen, "Amino Acid Analysis of Submicrogram Hydrolyzate Samples" in Methods in Protein Sequence Analysis 1986, Edited by Kenneth A. Walsh, The Humana Press, 1987.

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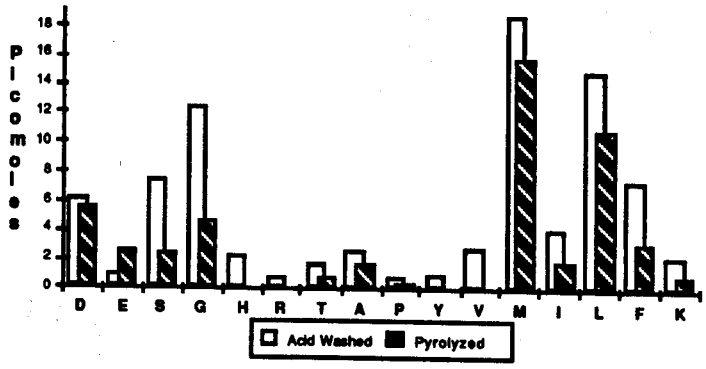

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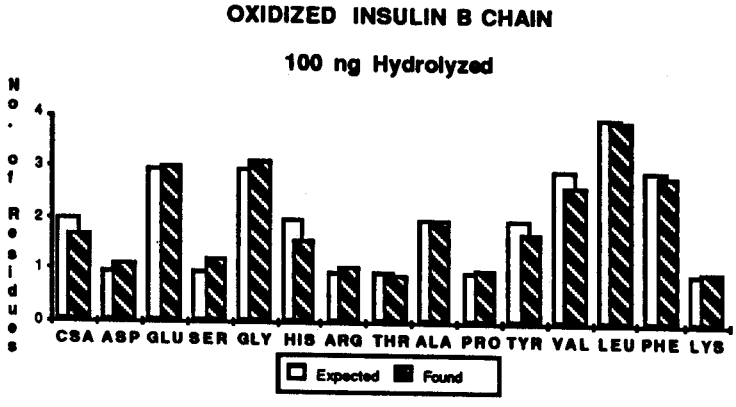

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