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CHEMICAL ISOTOPE DILUTION FOR ^{14}C AMS AND THE POTENTIAL FOR GC/AMS

RICHARD GILLESPIE*
Archaeology Department, Simon Fraser University, Burnaby
British Columbia, Canada

INTRODUCTION

The advent of accelerator-based mass spectrometry (AMS) radiocarbon dating has reduced the minimum sample size required to ca 1mg from the 1g of traditional beta counting techniques (*cf* Wölfli, Polach & Andersen, 1984). However, it is clear that even smaller samples will be necessary for some fields of endeavor, particularly environmental work where the absolute quantities are small, perhaps of the order of a few micrograms (Currie *et al,* 1985). This raises serious problems in the handling and measurement of such small amounts, so that dilution will sometimes be required. Normally, sample dilution is accomplished by adding "dead" CO_2 to the combusted sample CO_2, which requires very careful measurement of two gas pressures for the calculation of a dilution ratio. By forming a chemical derivative of a sample before combustion, gas pressure measurement is not necessary and an exact dilution ratio can be selected by judicous choice of the dilution reagent. This paper demonstrates that such a technique is possible for the AMS ^{14}C dating of derivatized amino acids.

Advantages other than smaller sample size and more convenient handling also accrue from this chemical dilution technique, most importantly in terms of chemical selectivity and specificity. A dilution reagent can be chosen to react only with a particular functional group, so that specific chemical classes can be selected for analysis. Where a sample contains several compounds of the same class, particular molecules can be isolated from the sample mixture and analyzed separately. In general, the isolation of sub-milligram amounts of organic compounds from complex mixtures is most conveniently achieved by chromatographic methods, which also allow the separation of homologous series of compounds like amino acids, fatty acids, etc. This invites the possibility of direct on-line derivitization, purification, separation, combustion, and AMS ^{14}C dating of very small, complex sample mixtures.

MATERIALS AND METHODS

Commercially available amino acid and dilution reagents were used in this preliminary study for simplicity. L-glutamic acid was selected because it is normally prepared by biologic methods and, thus, should have "modern" ^{14}C activity. The dilution reagents selected should be derived from petrochemicals with no ^{14}C. L-glutamic acid, trinitrobenzene sulfonic acid (TNBS), and Dansyl-L-glutamine (DNS-L-Gln) were purchased from Sigma Chemical Co and used without purification. The 2,4,6-trinitrophenyl derivative of L-glutamic acid (TNP-L-Glu) was prepared following the method of

* Present address: 33 Charlotte St, Leichhardt NSW 2040, Australia

Okuyama and Satake (1960), recrystallized once from methanol/dilute hydrochloric acid, melting point 168°C (literature 165–166°C). Dansyl chloride (DNS-Cl) was purchased as a 10% solution in acetone from Pierce Chemical Co and used without purification after evaporation of the solvent.

Five samples, L-Glu, DNS-Cl, TNBS, TNP-L-Glu, and DNS-L-Gln, were combusted over CuO in evacuated sealed Vycor tubes at 900°C for 2 hours, then converted to graphite using the method of Vogel *et al* (1984) and analyzed for ^{14}C on the SFU tandem accelerator mass spectrometer system at McMaster Univ, as described by Nelson *et al* (1984).

RESULTS

The ^{14}C results shown in Table 1 are expressed in terms of deviation from the modern standard (0.95 NBS oxalic acid, defined as 100% modern). The expected values were calculated assuming that the dilution reagents had zero ^{14}C and that the L-Gln had the same activity as the L-Glu. The TNP derivative adds 6 carbons to the 5 contained in glutamic acid and a molecular weight increase of 211; the DNS derivative adds 12 carbons to the 5 present in glutamine for a MW increase of 233. Thus, the original carbon is 5/11 for the TNP derivative and 5/17 for the DNS derivative. The measured value for the TNP-L-Glu derivative is in good agreement with the calculated value, indicating that such chemical isotope dilution is feasible. For more accurate work, measurements of the stable carbon isotopes for sample and dilution reagent would be used to correct the ^{14}C data. The measured values for dansyl chloride and DNS-L-Gln are not as expected, indicating that the source of the glutamine in this derivative is not the glutamic acid measured here, and that there was some contamination in the dansyl chloride. These discrepancies are to be expected in commercial products where there is no attempt at isotopic purity during manufacture. The important point is that the derivative synthesized from reagents of known activity for this experiment shows the expected dilution.

DISCUSSION

The obvious extension of this preliminary work would be to derivatize a protein hydrolysate (such as bone collagen) with one of the dilution reagents employed here, separate the individual derivatives by means of liquid chromatography, then convert each one to graphite for AMS ^{14}C analysis. Such a project would be rather tedious since there are at least 18 amino

TABLE 1
Radiocarbon measurements

Sample	Lab no.	Expected	Measured	Dilution ratio
L-Glu	RIDDL-221	>100	127.2 ± 5.8	
TNBS	RIDDL-313	0.0	0.3 ± 0.1	
DNS Cl	RIDDL-326	0.0	4.1 ± 0.1	
TNP-L-Glu	RIDDL-314	57.8	57.1 ± 1.8	5:6
DNS-L-Gln	RIDDL-315	37.4	45.5 ± 1.1	5:12

acids in collagen, a less taxing analysis could concentrate on the major constituents—glycine, alanine, glutamic acid, aspartic acid, proline, and hydroxyproline. The latter two imino or secondary amino acids have different properties which could be exploited in chemical dilution techniques: they will form the dansyl derivative but will not react with TNBS. Current methods used for isolation of the imino acids for ^{14}C dating use aggressive conditions such as hot nitrous acid (Gillespie, Hedges & Wand, 1984); by derivatizing only the primary amino acids with TNBS and using simple separation methods, the imino acids could be isolated without extensive degradation for subsequent dansylation.

Extending this idea to other kinds of sample and dilution reagent would allow many different materials to be treated in a similar manner for micro-sample dating. For example, fatty acids from soils and sediments could be converted to high molecular weight esters using "dead" long chain alcohols. These compounds (and others such as amino acids with both amino and carboxyl groups derivatized) can be separated by gas chromatography (GC) using inert gas carriers. If the GC effluent is directed into a combustion system, the CO_2 produced can be easily separated from the carrier and directed to a gas inlet ion source (Middleton, 1984) for on-line GC/AMS ^{14}C dating.

CONCLUSION

This preliminary study has demonstrated that chemical isotope dilution of reactive organic molecules may well be a useful addition to the range of micro-sample AMS ^{14}C dating techniques. Much development remains to determine the best reagents and separation methods, and the most suitable ion source for efficient production of carbon beams from an on-line system.

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