IN VITRO NUCLEAR MEDICINE

Attenuation of I-125 Radiation by Chloroform and Other Dense Solvents, and Its Relevance to Radioimmunoassays: Concise Communication

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The counting efficiency of iodine-125 in well-type scintillation counters is reduced when the labeled compound is dissolved or suspended in chlorinated hydrocarbons or potassium iodide solution. The reduction is probably caused by absorption of the weak gamma and x-rays of I-125 by the halogen atoms in the solvent or solute molecules. This phenomenon may introduce artifacts into procedures involving radioiodinated compounds and organic solvents and KI, but it could also be useful in differentiating bound from free labeled ligands by their differential solubility (and attenuation) in dense solvents. The reduction in counting efficiency can be overcome by evaporation of the solvent, or by the use of emitters with higher energy radiation.

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There is widespread use of I-125-labeled compounds in clinical and experimental laboratories. Counting efficiency of the gamma and x-radiation from I-125 is high. However, we observed lowering of the counting efficiency of I-125-labeled compounds when they were dissolved in chloroform. This report shows that several chlorine-containing solvents and 2 *M* KI have that effect. The implications of these observations are discussed.

MATERIALS AND METHODS

Carbon tetrachloride, 2-chloropropane, potassium iodide, (Des-Asp[1])-angiotensin II,* and I-125 were purchased commercially.

(Des-asp[1])-angiotensin II was iodinated and purified according to methods outlined in previous publications (*1,2*). This peptide was chosen for the experiments because it is relatively soluble in organic solvents, a property that was under study when we noted the phenomenon reported here (*3*). Labeled peptide was stored at −70°C in 0.05 *M* sodium phosphate buffer, pH 7.4.

Experiments were performed in glass tubes measuring 0.8 × 7.5 cm. To prevent adsorption of labeled polypeptides, the tubes were filled with 0.5% aqueous solution of bovine serum albumin for 1 hr at room temperature, rinsed with water, and dried in a warm oven. Labeled peptide was dissolved in methanol. Iodine-125-labeled des-aspartyl[1]-angiotensin II, 0.02 μCi in 20–200 pg, was added to each tube in a volume of 0.05 ml, and the tubes were counted. One milliliter of solvent was added to each tube, and the tubes were counted again. They were placed in a 50°C water bath and the solvent was evaporated with a stream of nitrogen. Tubes were then counted again. Where the effects of 2 *M* KI were tested, tubes were counted only twice, before and after the addition of KI. Radioactivity was measured in gamma scintillation counting systems. Windows were set to bracket 20–40 keV. The effect of geometry was tested by varying the volume of a nonattenuating solvent (methanol) between 0.05 and 1.0 ml. No difference was observed.

To calculate a parameter (attenuation coefficient, A.C.) that would predict effects of solvents on radiation, the following reasoning was used. "Radiation traversing a layer of substance is reduced in intensity by a constant fraction, μ, per centimeter. After penetrating to a depth

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TABLE 1. EFFECTS OF SOLVENTS ON COUNTING EFFICIENCY OF IODINE-125, AND CALCULATED ATTENUATION COEFFICIENTS

Solvent	Reduction in cpm (%)	Attenuation coefficient for 27.5 keV x-ray (cm^{-1})
Methanol	0.0	0.293
2-Chloropropane	21.5	1.351
2 *M* potassium iodide	37.9	2.995
Methylene chloride	45.8	3.530
Chloroform	49.3	4.161
Carbon tetrachloride	50.5	4.608

x, the intensity is $I = I_0e^{-\mu x}$, where I_0 is the intensity at the surface. μ/ρ is the mass absorption coefficient, where ρ is the density of the material (*4*)." The value of μ depends on the absorbing substance and the energy of the radiation being absorbed. Gamma counters detect four x-rays and one gamma from I-125 decay. The last column of Table 1 is based on the interaction between solvents and the most prevalent radiation, the 27.5-keV x-ray (*5*). The density of individual elements (C, H, O, Cl, and I) in each solvent was calculated based on the solvent's composition, formula weight, and density. The mass absorption coefficients (μ/ρ) for electromagnetic radiation at 27.5 keV for the five elements involved were estimated from values for other energies obtained from the literature (*4*). In the case of KI, mass absorption for I only was used. Values of μ for individual elements in each solvent were calculated based on μ/ρ values and the density (ρ) of each element within a given solvent. The resultant A.C. was calculated equal to $\mu_C + \mu_H + \mu_O + \mu_{Cl}$ or μ_I.

To test the feasibility of using solvent attenuation as a tool in competitive binding assays, we studied the reaction of a lipophilic peptide with antibody in the presence and absence of chloroform. The heptapeptide I-125-des-aspartyl[1]-angiotensin II has been shown to enter the organic phase of a chloroform/water system when cardiolipin is dissolved in the organic phase (*3*). The peptide is also bound avidly by most antibodies induced by immunizing rabbits to angiotensin (*1*).

We incubated aqueous solutions of labeled peptide with antibody or buffer for 30 min. Aliquots of these mixtures were added to tubes containing either chloroform or cardiolipin dissolved in chloroform. The tubes were then shaken vigorously at room temperature for 5 min. When the phases had separated, the tubes containing both phases were counted in a well counter.

The extent of peptide-antibody interaction was measured after shaking by passing an aliquot of the aqueous phase over a small gel-filtration column. The contribution of chloroform to the diminution of counts was corroborated by counting the organic layer separately, then removing the chloroform by evaporation and counting the residue.

We constructed a standard curve by adding increments of unlabeled des-aspartyl[1]-angiotensin II to some tubes. Attenuation was measured directly, then calculated as a proportion of the maximum attenuation. This was compared with the proportion of radioactivity bound to antibody, determined by gel filtration of an aliquot of the aqueous phase.

RESULTS

As shown in Table 1, chlorine-containing solvents and potassium iodide lowered the counting efficiency of I-125. The effects of solvent are presented as a percentage of the radioactivity counted before the addition of solvent. A comparison of the radioactivity counted in the tubes before solvent was added with that counted after all solvent was evaporated showed that losses of radioactive material were 5% or less. The results were similar with the three different well counters. The figures in the last column of Table 1 were calculated as described above.

The results of the antibody-binding experiments are summarized in Figs. 1 and 2. Despite the presence of a chloroform layer, antibody bound 65% of the labeled peptide, as shown by gel filtration of the aqueous phase. The two-phase system, containing pure chloroform and an aqueous solution of labeled peptide, showed no attenuation of radioactivity (first bar of Fig. 1).

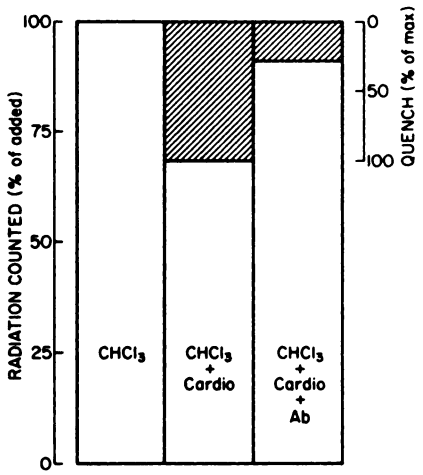

FIG. 1. Protection by antibody of I-125 des-aspartyl[1]-angiotensin II from attenuation by cardiolipin-chloroform. Labeled peptide was dissolved in aqueous buffer, layered over chloroform, and shaken vigorously. First bar shows radioactivity recorded after shaking. Second bar shows radioactivity recorded when cardiolipin was present in chloroform phase. Third bar shows radioactivity recorded when cardiolipin was present in chloroform phase and antibody to angiotensin was present in aqueous phase. Decrement shown in second bar is attributed to partitioning of labeled peptide into chloroform when cardiolipin is present. Partitioning can be prevented partially by binding of peptide to antibody in aqueous phase.

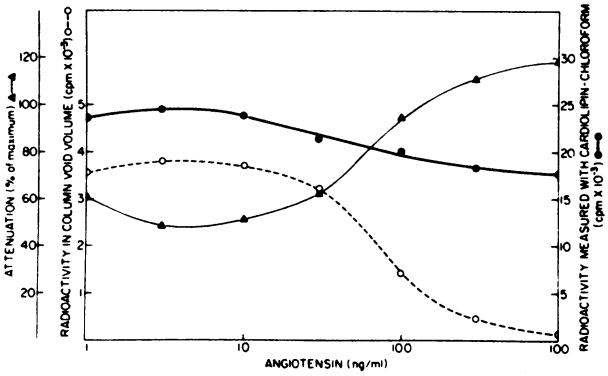

FIG. 2. Reversal of attenuation by unlabeled peptide. Experiment was performed as described in text and in legend to Fig. 1, except that increments of unlabeled peptide were added to aqueous phase of some tubes before antibody. Radiation recorded from tubes after shaking with chloroform-cardiolipin is shown as closed circles. Attenuation is calculated as percentage of maximum (closed triangles). Proportion of radioactive peptide bound to antibody was measured independently by passing aliquot of aqueous phase over gel-filtration column (open circles, dashed line).

When cardiolipin was dissolved in the chloroform, with no antibody present in the aqueous phase, 78% of the peptide was partitioned into the chloroform phase. This resulted in attenuation of 31.6% of the total radioactivity in the tube (second bar of Fig. 1). Antibody in the aqueous phase kept some of the labeled peptide in the aqueous phase, and prevented 71% of the attenuation that would otherwise have occurred in the absence of antibody (third bar of Fig. 1). When unlabeled peptide was also added, attenuation was increased in proportion to the amount of peptide (Fig. 2). Presumably the unlabeled peptide displaced radioactivity from the antibody, subjecting it to solubilization and attenuation by cardiolipin-chloroform.

DISCUSSION

The observed decrease in counting efficiency for iodine-125 dissolved in solvents and KI can be partially explained by consideration of the mass absorption coefficients of the solvents. As shown in Table 1, the observed absorption of radiation was greatest for those solvents with the highest attenuation coefficients (A.C.), where $e^{-A.C.}$ is the predicted fraction of 27.5-keV x-rays that survive after traveling through 1 cm of a given solvent. Predicting the precise effect that each solvent would have on counting efficiency is difficult, primarily because of the complex geometry of counting. However, the rank order of effects matches the rank order of A.C. Most of the attenuation of the radiation from I-125 is probably caused by the presence of chlorine or iodine, which comprise a significant fraction of the molecular weight of the solvent.

Users of I-125 and other gamma-emitting nuclides should be aware that chlorine-containing solvents and compounds that contain even heavier atoms may cause a significant reduction of counting efficiency. This caveat may become more significant as gamma emitters are adapted to assays of compounds soluble in organic solvents, such as steroids, drugs, and small peptides. It would apply much less to emitters such as iodine-131, whose radiations have higher energies.

Our data show that it is feasible to use attenuation of radiation by solvent in competitive binding assays. A suitably labeled antigen would be one that is soluble in chloroform or other dense solvents when free in solution but not when bound to antibody or other macromolecules. A suitable solvent would be one that dissolves the antigen but does not disrupt the antigen-antibody reaction. The amount of free antigen could be determined by measuring the reduction in counting efficiency when the solvent is added. This approach would eliminate the need for a procedure that physically separates bound from free antigen.

FOOTNOTE

* Bachem, Inc., Torrance, CA.

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