Liquid Scintillation Counting of Polar Molecules: Effects of pH on Water Association, Adsorption to Glass and Elution from Paper

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When weak β-emission from some polar molecules in aqueous solution was measured by liquid scintillation counting, unstable count rates were found.[1] The decrease of count rates with time may lead to great problems, especially in double labelling experiments. This "progressive" quenching was thought to be due to association of water to the polar groups of the radioactive molecules.[1] In preliminary experiments we found that liquid scintillation counting of adenosine monophosphate (5′-AMP) was accompanied by such "progressive" quenching. We also reported that the addition of acid could abolish the reduction of count rates with time.[2] We supposed that this effect of acid addition was caused by repression of dissociation of ionizing groups, decreasing the possibility of progressive association of water to the molecule. Changing of pH in order to diminish the polarity of a molecule could be a simple way to prevent "progressive" quenching for other molecules too, if the principle was generally applicable.

We therefore wanted to investigate the "progressive" quenching of various molecules in aqueous solution and study how pH-changes in both acid and basic direction influenced the count rates. For this purpose one small molecule (histamine) and one macromolecule (polylysine) with basic dissociable groups and a similar pair of molecules with acidic dissociable groups (5′-AMP and RNA) were examined. The molecules were chosen to assure clearly defined ionizable groups. In some experiments DNA was used instead of RNA to minimize the uncertainty of base hydrolysis of the acid macromolecules. [^{3}H]Histamine, enzymically prepared from [^{3}H]histidine ([3-^{3}H]-L-histidine, New England Nuclear Corporation, Boston, Mass.) with subsequent chromatographic purification,[3] was a generous gift from Dr. Sten Jacobsen, Department of Pharmacology, University of Oslo. [^{14}C]-5′-AMP ([8-^{14}C]-adenosine-5′-monophosphate), [^{3}H]-5′-AMP ([2-^{3}H]-adenosine-5′monophosphate) and [^{3}H]polylysine (Sigma Chemical Company, m.w. 70 000–200 000, mean m.w. 139 000) were obtained from the Radiochemical Centre, Amersham, U.K. ^{14}C-Labelled rat liver total RNA was obtained as described previously.[1] ^{3}H-Labelled DNA was extracted from *E. coli* by the method of Marmur [4] after labelling with [^{3}H]thymidine.

PPO (5 g), bis-MSB (0.5 g), naphthalene (80 g), 2-methoxyethanol (400 ml) and toluene (600 ml) were used as scintillation liquid. The spectrometer, a Packard Tri-Carb model 3365 fitted with bi-alkali photomultiplier tubes, was operated at 10 °C. The channels were adjusted so that chemical quenching reduced the tritium and the ^{14}C counts to the same extent. No adjustments of amplification or discriminator settings were done during the count period.

Count rates in aqueous solution were assayed by adding 50 μl of an aqueous solution of the radioactive material to 15 ml precooled scintillation liquid. After shaking, 450 μl of water, or a 0.5 M solution of HCl, NaOH or NaCl was added, and the counting started immediately. The normalized count rates reported are fractions of the initial count rates obtained. Initially, buffers of pH values slightly above or below the pK values of the radioactive molecules were used. However, when the scintillation liquid was added, the pH changed so much that only 0.5 M HCl or 0.5 M NaOH could assure pH values to the acidic or basic side of the pK values.

In some experiments known amounts of the radioactive material were applied and dried on 3×4 cm^2 of Whatman No. 1 paper. The paper was cut to pieces and shaken in 0.5 ml of one of the above solutions for 5 min at room temperature. Scintillation liquid (15 ml) was then added, and the shaking repeated. After cooling in the dark, the vials were shaken once more for 1 min before counting. These counts given in per cent of counts obtained from the respective radioactive compounds added directly to the scintillation liquid, were taken as an expression of the elution from the paper. This was checked by recounting of the solution after removal of the paper.

The results of the stability assays are depicted in Fig. 1. In the presence of H_2O or NaCl the count rates of 5′-AMP, RNA and polylysine declined markedly with time, whereas histamine count rates were only slightly reduced. Amino groups are not strongly polar. Therefore one might expect small effects on count rates in amine-containing compounds. The molecules containing phosphate groups showed greater instability in count rates than the others. Preliminary experiments using different polar groups seem to show a decreasing tendency towards instability in the following order: Phosphate groups, sulfate groups, carboxyl groups and amino groups. The drop in count rates was more pronounced for the macromolecules than for the small ones. This is probably a reflection of the secondary structure of the macromolecules in aqueous environ-

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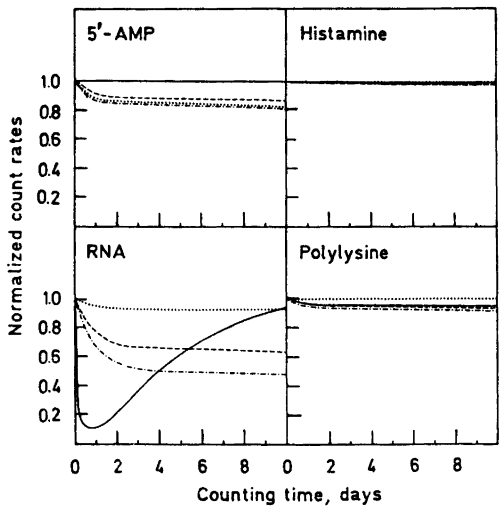

Fig. 1. Normalized count rates of AMP, histamine, RNA, and polylysine in different solutions as functions of time. Each substance was dissolved in 50 µl water and added to 15 ml scintillation liquid. Water (- - -), 0.5 M NaCl (— · —), 0.5 M HCl (—), or 0.5 M NaOH (···; 450 µl) was added, and the counting started.

ment where the polar groups, turning outwards, constitute a larger part of the molecular surface. The external standard count rates remained the same during the whole observation period, excluding the possibility of general quenching of the scintillation liquid. Usually, the falling count rates could not be attributed to adsorption by the vial walls, because the decline proceeded at the same rate both in glass and in polyethylene vials, and because count rates close to the originals could be obtained after transfer of the contents from one vial to another. In this case the mechanical shaking probably reversed the "progressive" quenching.[1] Occasionally, however, the count rates of AMP or histamine fell below the usual (data not shown). This was shown to be caused by adsorption to the vial walls, and was experienced more often when using new glass vials.

Addition of HCl to the AMP-vials stabilized the count rates while NaOH did not. Addition of NaOH to the polylysine vials stabilized the recordings while HCl was ineffective. These results were in accordance with the general repression-of-dissociation-hypothesis. The results obtained with NaOH for the histamine-vials did fit too, but the slight improvement also obtained with HCl in these vials is incompatible with the hypothesis. RNA was precipitated by acid and count rates dropped markedly. This was followed by a slow redissolution (if hydrolysis can be excluded) of the precipitate in the rather hydrophobic milieu. The final result — after 9 days — in RNA-vials with acid was thus in agreement with the hypothesis, but of little practical importance due to the precipitation. The addition of NaOH to RNA-vials stabilized the count rates which is in disagreement with the hypothesis. Base hydrolysis of the macromolecule could be excluded as identical results were achieved with DNA. Thus it seems probable that count rates may be stabilized by repression of the dissociation of the radioactive molecules in some instances, but the principle does not appear to be general.

Table 1. Per cent elution of AMP and histamine from Whatman No. 1 paper.[a]

Eluent	AMP	Histamine
H_2O	70 ± 2	64 ± 1
NaCl, 0.5 M	70 ± 2	76 ± 2
HCl, 0.5 M	87 ± 2	72 ± 2
NaOH, 0.5 M	55 ± 2	95 ± 2

[a] Mean of 6 parallels ± SEM in per cent of radioactivity applied.

Other advantages were obtained by the addition of acid to AMP-vials and base to histamine-vials as the adsorption to vials walls (see above) was abolished. However, we never observed adsorption in the presence of the salt solution either, so this might be attributable to the ionic strength of the solution. Elution from paper (Table 1), tried only for smaller molecules, as they are the ones usually chromatographed, was enhanced when the dissociation of polar groups was repressed. Thus the elution of AMP was increased to 87 % by HCl and of histamine to 95 % by NaOH. The lesser polarity of histamine may explain the differences between AMP and histamine.

When difficulties are encountered in liquid scintillation counting with water association to polar groups, adsorption to glass vials or incomplete elution, repression of dissociation of polar groups seems to be worth considering, when problems due to precipitation can be omitted. We have found comparable results with other biological molecules such as orotic acid, cystine and cyclic AMP.

Acknowledgement. This work was supported by grants from the Norwegian Research Council for Science and the Humanities.

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Received January 7, 1977.