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Letter to the Editor

Coupling of mono- and polyamino ligands to solid phases by nucleophilic attack on coupling groups that may be spontaneously hydrolysed

Sir,

A number of methods exist for coupling ligands of the form R–NH_2 to solid phases (e.g. refs. 1–8). Many of these rely on one or many nucleophilic amino groups on the ligand reacting with 'activated' coupling groups on the solid phase, while at the same time the 'activated' coupling groups may be degraded in an alternative reaction with water. We remark that the efficiency of coupling in terms of (amount of ligand coupled)/(amount of ligand available in solution) depends critically on the number of amino groups per ligand molecule and propose a possible explanation.

Such reactions are often (e.g. refs. 1 and 8) tested in one or both of the following circumstances: (A) a polyamino ligand such as a protein, where the proportion of available ligand coupled is of significance to the author; (B) a monoamino low-molecular-mass ligand in plentiful supply where the prime consideration is the number of ligand molecules that may be attached to a fixed amount of solid phase. This, however, gives little idea of the performance that may be expected in the situation where a large proportion of a scarce monoamino ligand must be coupled, and this may be very poor even if the method gives good results by criteria A and B mentioned above. For example, while tresyl chloride-activated 'Dynospheres' in our laboratory will couple as much as 50% of presented immunoglobulin, and may be capable of coupling 5 μmol/ml glycine if glycine is presented at a concentration of 500 m*M*, they will only couple around 1% of presented [^{14}C]glycine or [^{3}H]glucosamine under comparable conditions.

It may be that the following account, no doubt oversimplified, explains this observation. Suppose that the concentrations of water, 'activated' coupling

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group and ligand-based amino groups are denoted by W, C and N, respectively, and that the corresponding concentrations at the beginning and end of reaction are W_0 and W_1, C_0 and C_1 and N_0 and N_1, respectively; moreover that the second-order rate constants for the competing reactions

coupling group + water → wasted coupling group

and

coupling group + ligand amino → coupled ligand

are A and B, respectively. Then the following differential equations predict the outcome of the reaction:

$$dW/dt = -AWC$$

$$dN/dt = -BNC$$

$$dC/dt = -BNC - AWC$$

hence

$$dN/dW = BN/AW$$

and therefore

$$\ln(N_1/N_0) = B/A \ln(W_1/W_0)$$

or

$$(N_1/N_0) = (W_1/W_0)^{B/A}$$

Now, if the number of coupling groups and the amount of water are held constant, then $W_1/W_0 \geq (W_0 - C_0)/W_0$ and, hence, the fraction of ligand amino groups coupled $\leq K = 1 - [(W_0 - C_0)/W_0]^{B/A}$ giving a theoretical upper limit to the fraction of presented amino groups coupled to the gel. Now let n be the number of amino groups on each ligand molecule that are available for coupling. Then if a fraction K of available ligand amino groups is coupled, then the expected fraction of ligand molecules coupled will be $1 - (1-K)^n$. For high n values this fraction will be very much larger than for low n values; thus, if $K = 0.01$, the upper limiting fraction coupled for monoamino ligands will be 1%, while for human immunoglobulin G with an estimated 100 lysine amino groups on its surface [9], the upper limiting fraction coupled will be 63%.

This account of course assumes that the presence of n amino groups dispersed on the surface of a ligand makes the rate of reaction of these amino groups fully n times as fast, which is likely to be an overestimate of the real increase in reaction rate. However, it illustrates that testing coupling methods using only methods A and B referred to above may fail to demonstrate real differences in usefulness of two methods due to different B/A rate-constant ratios and hence different values of K. In particular, the cyanogen bromide

coupling method [7] is in our hands capable of coupling at least 30% of presented monoamino ligand, an advantage which has kept it in use in spite of the superior stability of the bonds formed by other methods [1–6].

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