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APPENDIX

Preparation of *N*-Succinimidyl 3-(4-Hydroxyphenyl)propionate

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To circumvent the 'iodination damage' sometimes observed when ^{125}I is introduced into proteins and peptides by direct substitution, we envisaged a procedure in which the iodination step would be carried out in the absence of protein, the label first being introduced into a moiety which would then, in a separate step, be attached to the protein; a procedure which may be called labelling by conjugation, as distinct from labelling by substitution.

The reagent used as the carrier of the ^{125}I should be readily iodinated, preferably under the standard conditions in current use for the direct iodination of proteins; the labelled reagent should be readily isolated from the iodination mixture; it should react directly and selectively with groups commonly occurring in proteins without the need for separate chemical activation and with minimal manipulation; and the bond so formed should be chemically stable.

N-Succinimidyl 3-(4-hydroxyphenyl)propionate was selected as a reagent meeting these requirements. As it is a phenol it can be iodinated under the same conditions as the tyrosine in proteins, the iodinated reagent can be simply isolated by extraction and it will react in aqueous solution with the terminal or side-chain amino groups present in most proteins and peptides to form amide bonds.

The reagent was prepared by condensation of 3-(4-hydroxyphenyl)propionic acid with *N*-hydroxysuccinimide by using dicyclohexylcarbodi-imide, by the general procedure of Anderson *et al.* (1964).

Experimental

Preparation of N-succinimidyl 3-(4-hydroxyphenyl)-propionate

3-(4-Hydroxyphenyl)propionic acid (1.661g; 10mmol) and *N*-hydroxysuccinimide (1.151g; 10mmol) [both from Fluka A. G., Buchs, Switzerland, recrystallized from ethyl acetate by addition of di-2-propyl ether] in tetrahydrofuran (7ml) were treated at −18°C with dicyclohexylcarbodi-imide (2.475g; 12mmol). The mixture was stirred at −18°C for 2h, kept at room temperature for 10h and treated with acetic acid (0.12ml) to destroy excess of carbodi-imide. After 1h more the mixture was diluted with ethyl acetate (10ml), the dicyclohexylurea was filtered

off (2.63g), washed with ethyl acetate and the combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was recrystallized from ethyl acetate (20ml) by addition of light petroleum (b.p. 45–60°C) (10ml) to afford 2.51g (95%) of the ester, m.p. 120–122°C, R_F 0.59 [t.l.c. on silica gel in ethyl acetate–methanol (3:1, v/v)], still containing a small amount of the acid (R_F 0.49). This material is suitable for use in the iodination and coupling reactions. Further crystallization from propan-2-ol (20ml) by addition of water (60ml) at, 0°C gave, after drying [10h at 13.3Pa (0.1 Torr)] 0.91g (36%) of chromatographically homogeneous material, m.p. 129°C (Found: C, 59.4; H, 5.3; N, 5.2; $C_{13}H_{13}NO_5$ requires C, 59.3; H, 5.0; N, 5.3%).

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