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ELECTROCHEMICAL REMOVAL OF PROTECTING GROUPS OF INTEREST IN PEPTIDE SYNTHESIS

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A study has been made of the electrochemical cleavage of the benzyloxycarbonyl group (Z) in a series of amino-acids and peptides in DMF/Bu_4NBF_4 solutions. For most of these compounds, the cyclic voltammograms show two well-defined reduction peaks and, when controlled potential electrolyses were carried out at a potential just after the first peak, toluene was identified as a product in a yield above 90%. Hence, the first reduction process is associated with the cleavage of the Z group. Several possibilities are suggested for the origin of the second peak and the importance of controlling the acidity of the medium throughout the electrolysis is also shown.

Introduction

Peptide synthesis is carried out by amino-acid coupling and in this process some strategic and tactical problems have to be considered. The success of coupling reactions depends on the activation of certain groups in the molecules which are often unreactive; on the other hand, some other groups in the molecule may be too reactive and could participate in unwanted reactions. It is, then, obvious that throughout

the synthesis of large molecules, activation and protection steps will alternate. Another strategic problem concerns deprotection of groups either selectively during the sequence or simultaneously at the end of the synthesis.

Figure 1 shows some examples of fully protected peptides together with their abreviated forms. In principle all the protecting groups exemplified can be cleaved selectively or simultaneously by electrochemical methods; the same methods can be applied to the formation of peptide bonds or the S-S links in cystine type systems (figure 1 c)).

It is intended to investigate some of these procedures of peptide synthesis and the work described here is particularly related to the cleavage of protecting groups.

The electrochemical removal of protecting groups is considered to be advantageous but it has not yet been thoroughly studied or developed into convenient laboratory procedures although some valuable contributions can be found in the literature [2-5] The advantages of the electrochemical methods can be summarized as: a) mild conditions for cleavage; this is not always achieved mith conventional methods where degradation of the molecules competes with the desired cleavage; b) high degree of selectivity which can be promoted by appropriate substitution within the protecting groups; c) uniform experimental technique; the cells and equipment are the same for the removal of the different protecting groups; d) by correct choice of the potential, instead of selective cleavage, the simultaneous removal of all groups at the end of the synthesis is possible, in a single step.

Table 1 shows examples of protecting groups used

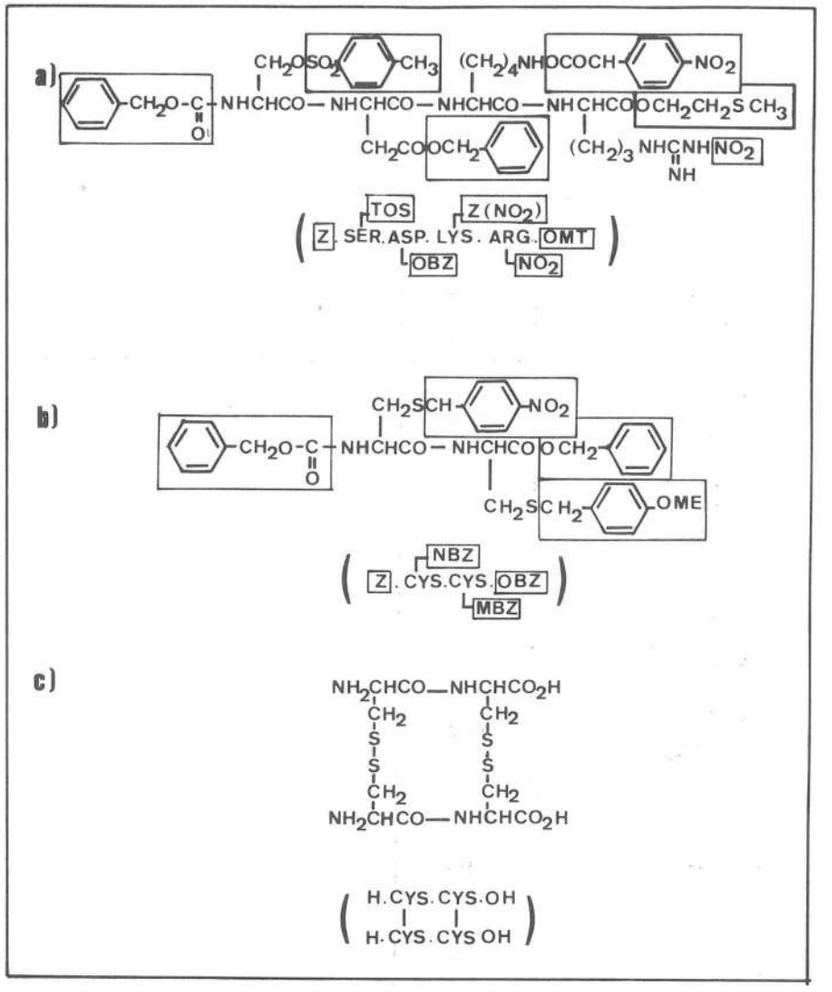
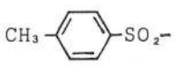
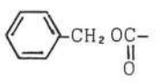
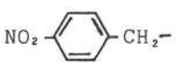
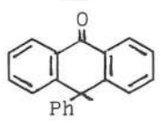
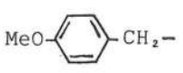


FIGURE 1 - a) Fully protected tetrapeptide; b) Fully protected dipeptide; c) Unprotected cyclic tetrapeptide. The protecting groups are framed and the abreviated forms of the peptides are in brackets.

in synthesis and their average cleavage potentials. They will obviously vary slightly according to the type of function (-OH, $-NH_2$, -SH, etc) those groups are protecting.

TABLE 1 - Cleavage potentials of some common protecting groups

PROTECTING GROUPS	Reaction type	CLEAVAGE POTENTIALS(V vs. SCE)
	red	-2.2
	red	-2.7
	red	-1.0
	red	-1.3
	ox	+1.6
	ox	+0.8
$CH_3SCH_2CH_2-$	ox	+2.3

These are only a few examples that show the wide region of possible cleavage potentials, from very positive to very negative values.

It is the objective of this programme of work to make a systematic and detailed study of the electrochemical cleavage of the groups most commonly used in synthesis. So far, a kinetic and mechanistic study of the removal of the tosyl group in esters, amides and phenols has been reported[7,8].

The present paper reports some results obtained with the benzyloxycarbonyl group (Z) which is considered to be of great importance in peptide synthesis. It is intended to get a better understanding of the reaction mechanism and to define the conditions for high yield electrolysis. For this investigation nine different amino-acids and peptides were prepared, each one of them with an amine group protected by the Z group and the carboxyl function in the ester form. The overall electrode reaction should be, using compound I in table 2 as the example,

$$C_6H_5CH_2OCNHCH_2CO_2CH_2C_6H_5 + 2e \xrightarrow{H^+} C_6H_5CH_3 + CO_2 + NH_2CH_2CO_2CH_2C_6H_5 \quad (1)$$

Experimental

The electrochemical techniques used were cyclic voltammetry and coulometry. The equipment consisted of a Hi-Tek model DT 2102 potentiostat, a Hi-Tek model PPR1 wave form generator, a Houston 2000 X-Y recorder and a laboratory built integrator. The cyclic voltammetric experiments were carried out in a three electrode cell, where the working electrode was a vitreous carbon disc, the secondary electrode a platinum spiral and the reference electrode the saturated calomel electrode (SCE) which was in a separate compartment linked to the working electrode compartment by

a Luggin capillary. The carbon disc used as the working electrode was polished with alumina, washed and dried for each cyclic voltammogram. Coulometry experiments and preparative electrolyses were carried out in a three compartment cell. The cathode compartment was separated from the anode one by a glass frit. The reference electrode was the SCE placed in another compartment and was linked to the working electrode by a Luggin capillary. The secondary electrode was a large platinum grid and the working electrode was either a large mercury pool or an amalgamated copper grid. Typical examples of the cells used are sketched in figure 2.

Solutions were always de-aerated for several minutes before recording cyclic voltammograms and also during electrolysis. The solvent used in all cases was dimethylformamide which was purified by vacuum distillation from calcium hydride. The supporting electrolyte was Bu_4NBF_4 prepared by mixing aqueous solutions of Bu_4NHSO_4 and $NaBF_4$ followed by recrystallization of the product from water. The amino-acids and peptides were prepared using reported procedures.

Product analysis was carried out either directly from the catholyte solution by GLC or by extraction procedures.

Results and discussion

Table 2 shows a list of the compounds studied having their amine group protected by the Z group and the carboxyl function in the ester form. In two of these compounds (III and V) the amine function protected by the Z group is secondary. Figure 3 a) shows a cyclic voltammogram typical for most of the compounds studied. There are two well-formed,

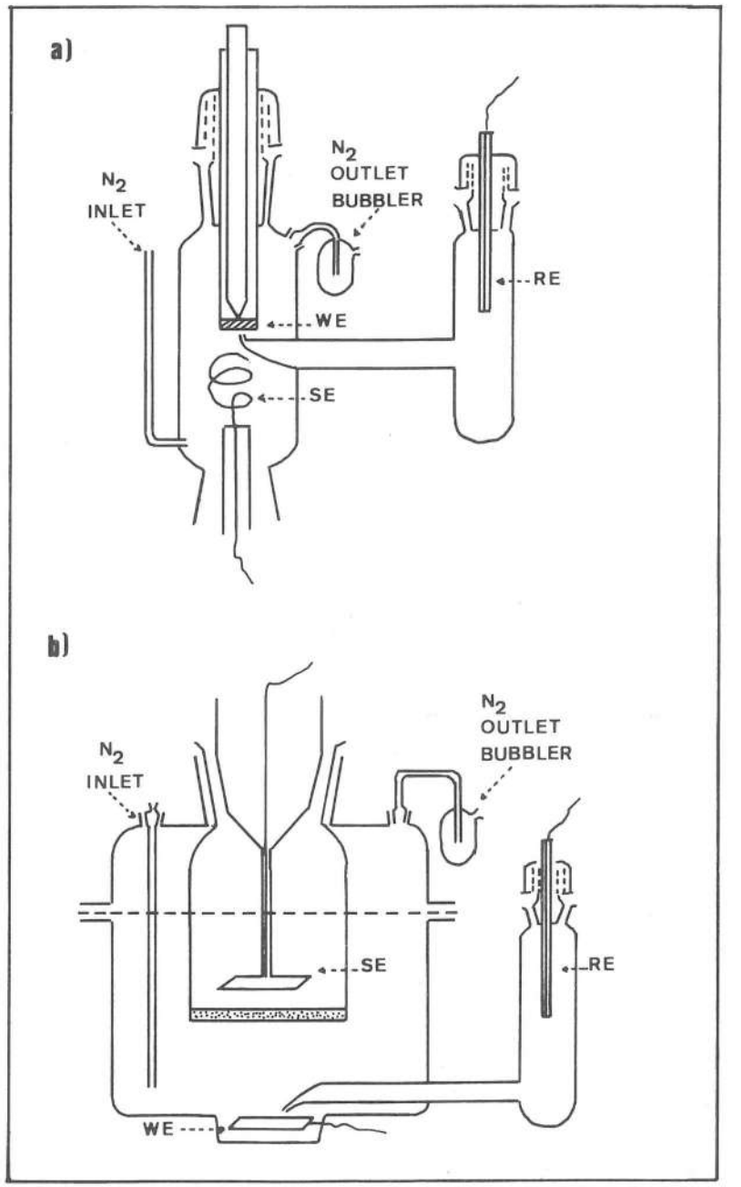


FIGURE 2 - Sketch of the cells used a) for cyclic voltammetry and b) for preparative electrolyses.

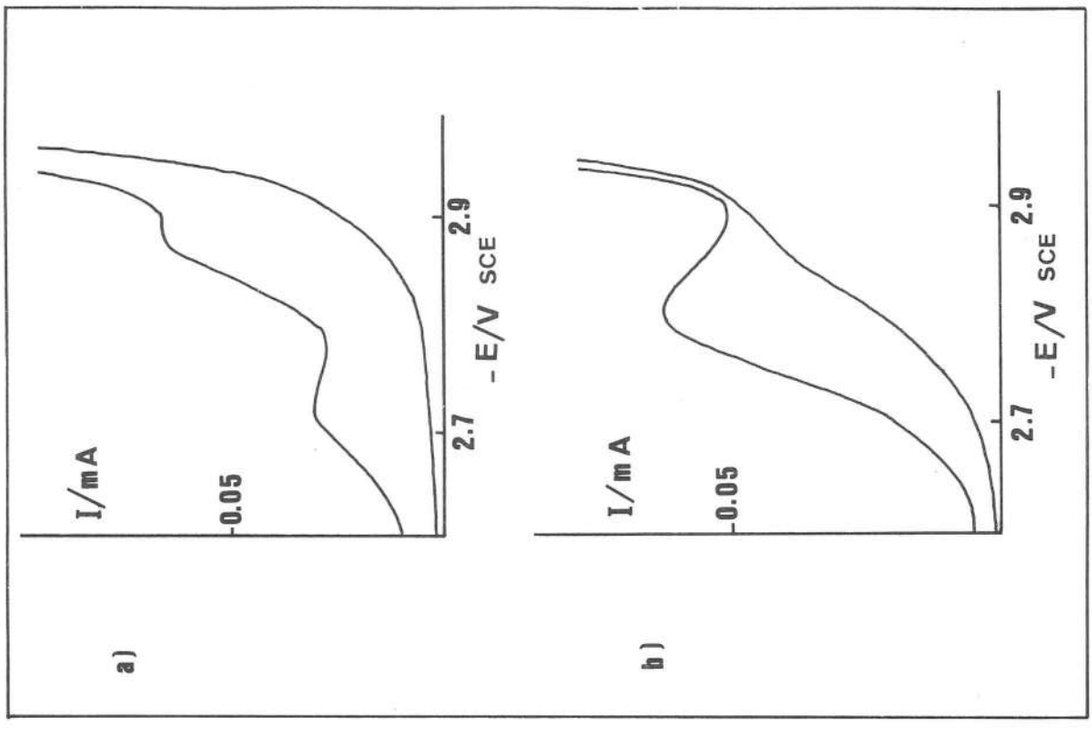


FIGURE 3 - a) Cyclic voltammogram for a solution of 0.005M Z-Leu$_2$-OMe in 0.1M TBAB/DMF at a sweep rate of 0.1Vs^{-1} at a vitreous carbon electrode of 0.05 cm^2 of area; b) Cyclic voltammogram for a solution of 0.005M Z-Pro-OBu^t at the same conditions as in a).

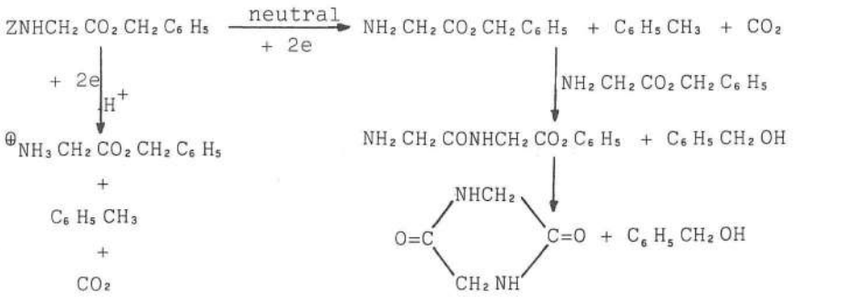
T A B L E 2 - Electrochemical data for the amino-acids and peptides studied

Nº	COMPOUND	Ref. for preparation	PEAK 1			PEAK 2
			$-E_p$/V	$E_p-E_{p/2}$ mV	$I_p/v^{1/2}$ c	$-E_p$/V
I	$ZNHCH_2CO_2CH_2C_6H_5$	12	2.74	69	28	2.88
II	$ZNHCHCO_2CH_3$ $CH_2OC(CH_3)_3$	14	2.79	64	31	2.93
III	$ZN-CH\ CO_2C(CH_3)_3$	10	2.85	65	83	-
IV	$ZNHCHCONHCH_2CO_2C_2H_5$ $CH_2C_6H_5$	13	2.77	60	24	2.90
V	$ZN-CHCONHCH_2CO_2C(CH_3)_3$	14	2.85	70	73	-
VI	$ZNHCHCONHCHCO_2C_2H_5$ $CH_2CH(CH_3)_2$ CH_3	14	2.79	64	28	2.90
VII	$ZNHCHCONHCHCO_2CH_3$ $CH_2CH(CH_3)_2$ $CH_2CH(CH_3)_2$	11	2.78	62	25	2.91
VIII	$ZNHCH_2CONHCH_2CONHCH_2CO_2C(CH_3)_3$	9	2.71	61	16	2.82
IX	$Z-(NHCHCONHCHCO)_2-OCH_3$ $CH_2CH(CH_3)_2$ $CH_2CH(CH_3)_2$	14	2.75	56	22	2.91

Note: potentials referred to SCE ; v= 0.1 v/s

totally irreversible reduction peaks of approximately equal height, quite close to the negative potential limit of the solvent. Only compounds III and V show a single peak as shown in figure 3 b). The cyclic voltammetric data obtained with these nine compounds is presented in table 2. $I_p/c.v^{1/2}$ is independent of v, that is, the reaction corresponding to the first peak is diffusion controlled. For compounds III and V this parameter is considerably higher but this does not necessarily indicate a major change in the mechanism. It is more reasonable to consider that the addition of an electron is more difficult when the amine function protected by the Z group is secondary and this causes the two peaks to coalesce.

It is common practice to carry out the chemical cleavage of the Z group in the presence of at least 1 mole of acid/mole of peptide to protonate the amine group which is formed. This is to prevent the unwanted reaction (intra- or inter-molecular, depending on size) of the type



(2)

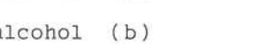
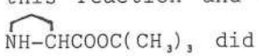
Moreover, it can be seen that the cathodic cleavage of the Z group, as shown in equation (1) consumes $2H^+$/molecule of peptide. Hence the cathodic reduction should perhaps be carried out in the presence of $3H^+$/molecule of peptide to ensure the formation of the protonated amine. In the absence of added proton, it is also to be expected that the catholyte will become basic during the electrolysis and this leads to the danger of racemization of the peptide. On the other hand, the addition of too much acid will surely lead to proton reaching the electrode surface and to consequent hydrogen evolution. Therefore, it was also considered essential to investigate the cyclic voltammetry of the protected compounds in the presence of added acid.

With equimolar concentration of added HCl, compounds VII and VIII gave cyclic voltammograms where the first reduction peak was little affected by the presence of the proton donor, but the second reduction peak was diminished in height (typically by 70% at v=0.05 V s^{-1}). Compound III again showed only a single peak but in the presence of equimolar HCl, the peak current was almost halved. It is, then, clear that even this small amount of proton is sufficient to diminish considerably the importance of the second reduction process. Unfortunately, cyclic voltammograms for solutions containing higher HCl concentrations were complex and already showed evidence for hydrogen evolution at less negative potentials than those essential for the reduction of the protected peptides.

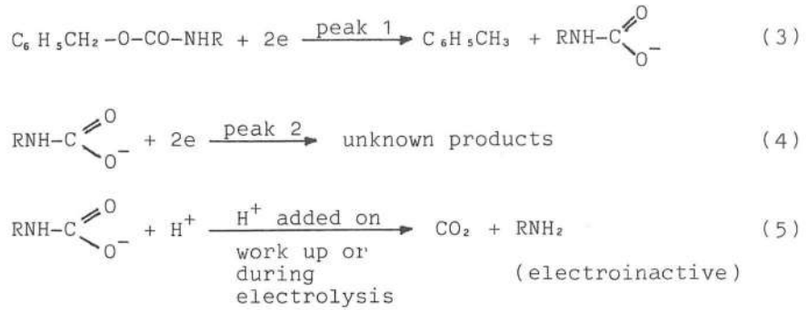
Controlled potential electrolyses have been carried out for compounds III, VII and IX in DMF/Bu_4NBF_4 (without added H^+) at potentials just negative to the first reduction

peaks. In all cases, toluene was indentified as a product with a yield above 90% and hence it is certain that the first reduction precess is associated with the cleavage of the Z group. The analysis of the peptide residue is more complex and has not yet been completed; from the cleavage of compound III, however, it is clear that a substantial yield (< 40%) of the expected amine is isolated, although in these conditions, t-butanol is also identified in the catholyte. The mechanism for its formation is the subject of further studies.

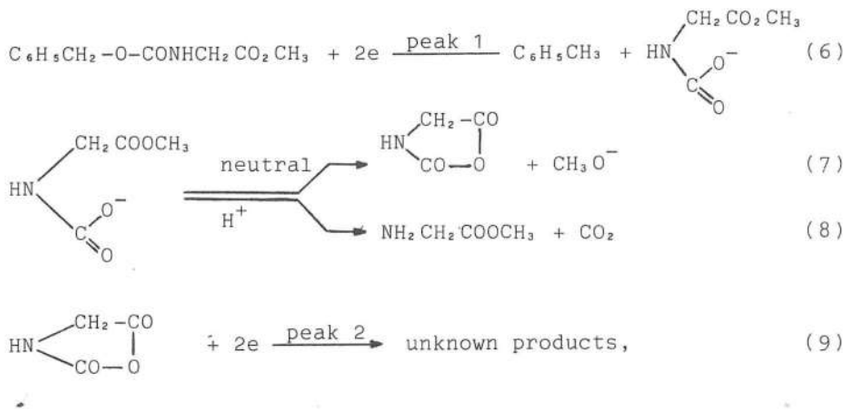
Several possibilities for the origin of the second peak have been considered.

(i) That the second peak is due to the cathodic cleavage of the ester function to give free amino-acid and alcohol. This explanation does not seem to be correct since (a) electrolyses of compound VII at the potentials of the first and second peaks lead to similar yield of alcohol (b) a cyclic voltammogram for  did not show a peak at this potential or indeed until beyond -3.0 V; (c) there seems no reason why the addition of proton should inhibit this reaction and the addition of HCl to the solution of  did not change the reduction behaviour.

(ii) If the intermediate urethane anion, X^- is stable in the absence of proton but rapidly decarboxylated by acid, the following mechanism would explain the experimental observations.



(iii) A third possibility would involve an eecee mechanism where the chemical step is prevented by proton, e.g.



where steps (6) and (8) are essentially the same as (3) and (5) above but written in different ways.

All these possibilities are the subjects of further studies. The control of the acidity of the medium throughout the electrolysis is considered to be of dominant importance and several techniques to achieve this goal are also under investigation.

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O EFEITO DE TUNEL NA JUNÇÃO SEMICONDUTOR-ELECTRÓLITO

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A model is presented of the space charge layer formed in semiconductors deposited as a thin layer on an insulator substrate.

The variations in charge transfer coefficients is studied for tin oxide electrodes.

The anodic current density arising from a tunnel effect is calculated by a simplified expression, which considers the variation in barrier width with applied potential.

I Introdução

A junção semicondutor-electrólito tem sido extensivamente estudada [1-6], mas nada tem sido referido quando o semicondutor é formado por uma película fina depositada sobre um substrato isolador como o vidro ou plástico, e o contacto eléctrico é obtido do mesmo lado da interface acima da zona de separação ar-líquido-semicondutor, em vez de ter lugar na face oposta, como normalmente é referido.

Em semicondutores "altamente dopados" as camadas espaciais de carga, desenvolvidas quando o eléctrodo é mergulhado no electrólito e submetido a potenciais de polarização, podem ser suficientemente delgadas de modo a que alguma barreira de potencial, eventualmente formada, possa ser facilmente vencida pelos transportadores de carga, por efeito de túnel.

Memming e Möllers [7], com algumas aproximações, calcularam o valor da espessura da barreira x(E), a partir da equação de Poisson e, por um processo mais ou menos moroso, obtiveram, por aplicação da teoria de e-