also be found in the inner corneal layer (Figure 3). In most cases the lens retained a connection with the inner cell layer of the cornea by its capsule, which could be observed even 188 days after lentectomy (Figure 5). The reconstitution of the lens from the epithelium could be observed between 57 and 60 days and in some cases at an even earlier stage. In cases where the lens was removed completely, no evidence of lens regeneration from the dorsal margin of the iris could be found in this series of experiments, not even 6 months after the operation.

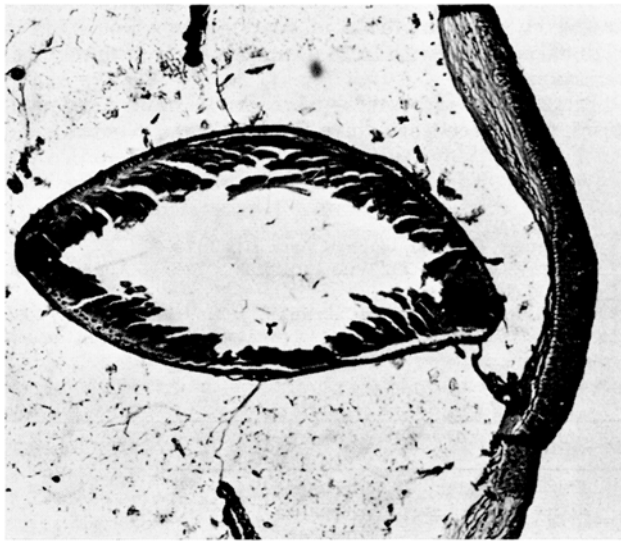

Fig. 5. 188 days after lens removal. The lens still retains connection with the inner corneal layer by the capsule. × 59.

The present results show that in *X. laevis*, as in other anurans according to reports so far, the complete removal of the lens is difficult. Where there was lens formation, it developed from the fragments of lens epithelium which were retained within the capsule, as has been suggested in the vacuoles (OKADA[9,10]).

Zusammenfassung. Es wurde die Regeneration der Augenlinse erwachsener *Xenopus laevis* nachuntersucht. Eine vollständige Entfernung der Linse gelang in wenigen Fällen. Die Neuentwicklung der Linse wurde nur von Linsenepithelresten, die nach der Lentektomie in der Kapsel zurückgeblieben waren, induziert.

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PRO EXPERIMENTIS

A Sensitive Method for the Detection of Racemization in Peptide Synthesis

A few years ago we reported that diastereoisomeres of DL/DL dipeptides can be separated by paper[1] or thin layer chromatography[2]. This phenomenon allows one to evaluate the extent of racemization which occurs during introduction or removal of different protecting groups[3] used in peptide chemistry, or during the formation of the peptide bond[4].

In the latter case, phenylalanine or valine, *N*-protected by formyl or trifluoroacetyl residues were coupled with tertiary esters of phenylalanine or valine with the help of methods to be examined. The amount of the diastereoisomere D–L was estimated in free dipeptides.

Unfortunately such amino protecting groups (chosen due to the lack of other more appriopriate ones) are rather 'unnatural' and therefore inadequate. They can considerably diminish racemization and sometimes they can even protect the acylating acid against racemization[5].

Their removal by alkali or acid leads to partial hydrolysis of the peptide bond, and when its diastereoisomeres have the same Rf as the component amino-acids, such dipeptides cannot be used as test for the detection of racemization.

A perfect means for the protection of the amino group of the acylating amino acid would be incontestably acylamino acids or acyl peptides themselves. Unfortunately up to now only dipeptides could be resolved chromatographically into their diastereoisomeres.

To solve this problem we suggest: (a) to protect the amino group of the acylating amino acid with suitably *N*-protected amino acids or peptides, (b) to couple the peptide with tertiary butyl esters of L-amino acids with the help of the method to be examined, (c) to degrade the free peptide with the EDMAN method, and (d) to estimate the amount of diastereoisomere D–L in the dipeptide obtained.

After the synthesis of the peptide, the unreacted substrates must be removed from the reaction mixture by repeatedly washing out (under chromatographic control) in order to avoid the appearance of additional spots which

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could interfer with the detection of diastereoisomeres by overlapping.

To prevent possible partial separation of diastereoisomeres, only crude product must be used.

The protecting groups are removed with HBr-acetic acid. The thioureide is formed according to SJÖQUIST[6] and the salt of the phenylthiocarbamyl peptide (15–20 mg) so obtained cyclized upon treatement with 0.5 ml of 0.6 *N* HCl in dry acetic acid at room temperature within 30 min. After removal of the solvent and the excess of HCl under reduced pressure, the residue containing the dipeptide hydrochloride is washed out with ethyl acetate in order to remove the thiohydantoine derivatives formed.

By using this procedure, the amino acids composing the dipeptide retain their configuration and the dipeptide is not hydrolysed.

The Table shows protecting groups which have been used in our experiments, but theoretically every amino acid could be suitable except those which do not undergo the EDMAN degradation. This method permits us, therefore, to study the influence of penultimate amino acids or peptides on the extent of racemization of the acylating amino acid during the formation of the activating species or during their aminolysis.

Phenylalanine and valine have been used both as acylating and as acylated amino acids. Also here almost any dipeptide can serve as model, provided their diastereoisomeres have sufficiently different Rf.

The sensitivity of our models upon racemization was tested by using methods of coupling which have been claimed to occur with little racemization or with no racemization at all.

DETERMANN and WIELAND[7] report, using an ANDERSON test[8], that racemization can be completely avoided when

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No. of experiment	Synthesis of model peptides	Kind of activation	Reaction conditions				Yield %	Racemization %
			Solvent	Formation of the active intermediate	Action of the nucleophilic component	Literature		
1	Z-Val-Phe-OH + HCl.H-Phe-OBu^t	ClCOOEt	$CHCl_3$	30 min at – 15°C 2 eq. NEt_3	18 h, 18°C	[13]	76	100
2	Z-gly-Phe-OH + HCl.H-Phe-OBu^t	ClCOOEt	THF, H_2O	3 min at – 15°C 2 eq. NEt_3	till 20°C for 25 min	[7]	71	13
3	Z-gly-Phe-OH + H-Phe-OBu^t	ClCOOEt	THF	40 sec at – 15° 1 eq. NEt_3	till 20°C for 25 min	–	43	3
4	Z-gly-Phe-OH + H-Phe-OBu^t	ClCOOEt	THF	'inverse method' 40 sec at – 15°C NEt_3	till 20°C for 25 min	[9]	48	3
5	Z-gly-Phe-OH + H-Phe-OBu^t	ClCOOBu^i	THF	4 min at – 15°C, *N*-methylmorpholine	1 min at – 15°C till 20°C for 25 min	[10]	80	undetectable
6a	Z-gly-Phe-OH + HCl.H-Val-OBu^t	ClCOOEt	$CHCl_3$	30 min at – 15°C NEt_3 (2 eq.)	18 h, 18°C	[13]	61	100
6b	Z-gly-Phe-Val-OH + HCl.H-Phe-OBu^t	ClCOOEt	THF, H_2O	1.5 min at – 15°C NEt_3 (2 eq.)	18 h, 18°C	–	65	5
7a	Z-gly-Phe-OH + HOSu[a]	ClCOOEt	THF	3 min at – 15°C NEt_3	18 h, 18°C	–	89	50
7b	Z-gly-Phe-OSu + H-Phe-OBu^t	-OSu	THF	–	2 h, 18°C	[14]	87	
8	Z-gly-Phe-OH + H-Phe-OBu^t	DCCI	THF	13 h at – 5°C; 4 h, 18°C		[15]	86	20
9a	Z-gly-Phe-OH + HOQ[b]	DCCI	THF	13 h at – 5°C; 4 h, 18°C		[12]	87	80
9b	Z-gly-Phe-OQ + H-Phe-OBu^t	-OQ	THF	–	20 h, 18°C	[12]	92	
10a	Z-gly-Phe-OH + $BrCH_2CN$	–	AcOEt	18 h, 18°C	–	[4]	76	undetectable
10b	Z-gly-Phe-OCH_2CN + H-Phe-OBu^t	$-OCH_2CN$	THF	–	20 h, 18°C 2 eq. AcOH	[4]	75	
11a	Z-gly-Val-OH + $BrCH_2CN$	–	AcOEt	18 h, 18°C	–	[4]	65	undetectable
11b	Z-gly-Val-OCH_2CN + H-Phe-OBu^t	$-OCH_2CN$	THF	–	20 h, 18°C 2 eq. AcOH	[4]	80	
12	Z-gly-Val-N_3 + H-Phe-OBu^t	azide				[16]	43	undetectable
13	BOC-gly-Val-N_3 + H-Phe-OBu^t	azide				[16]	42	undetectable

[a] -OSu = *N*-hydroxysuccinimide residue. [b] -OQ = 8-hydroxyquinoline residue.

the time for the formation of mixed anhydride with ethyl chloroformate was shortened to less than 10 min. Our experiments made under the same conditions (experiment 2) and under conditions that are known to diminish racemization (experiment 3) could not entirely confirm this statement.

The 'inverse method'[9] leads on our model also to racemization. The procedure of ANDERSON[10] performed with *N*-methylmorpholine and not with NEt_3 gave, however, peptides with retained configuration (experiment 5).

The –OSu[11] and the 8-OQ[12] esters of *N*-protected dipeptides undergo aminolysis without being racemized. Such esters cannot be obtained in an optically pure form when they are prepared directly through reactive intermediates (experiments 7 and 9). The esterification of *N*-protected dipeptides with bromoacetonitril[4], however, gives optically homogenous cyanomethylesters which undergo aminolysis without racemization (experiments 10 and 11).

No loss of optical activity has been found when the acid azide method was used (experiments 12 and 13).

By the stepwise elongation of the peptide chain from the carboxyl end, the extent of racemization of each newly formed peptide bond (experiments 6a and 6b) can be controlled in a microscale by the presently proposed method.

Zusammenfassung. Empfindliche Methode zur Bestimmung der Racemisierung im Laufe der Peptidsynthese: *N*-Acylaminosäure oder Peptide werden als Schutz der Aminogruppe der acylierenden Säure benutzt.

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Bestimmung der Transferrintypen bei Rindern durch vertikale Polyacrylamidgel-Elektrophorese

Bisher wurde die Untersuchung der Transferrintypen auf Polyacrylamid durch Disk-Elektrophorese beschrieben[1]. Der Vorteil dieser Methode gegenüber der Stärkegelelektrophorese liegt darin, dass das synthetische Polyacrylamidgel chemisch besser definiert ist, teilweise aber auch eine bessere Auftrennung der Proteine stattfindet. Der Nachteil der Disk-Elektrophorese liegt in der schwierigen Vergleichbarkeit der Proben untereinander.

Um diesen Nachteil auszuschalten, verwenden wir das Verfahren der Disk-Elektrophorese in einer Apparatur, die ein direktes Vergleichen der Serumtransferrintypen untereinander und mit einem Standard erlaubt.

Methodik. Als Elektrophoreseapparatur wurde eine Vertikalelektrophorese nach RAYMOND[2] (E-C Apparatus Corporation, Philadelphia), als Puffer für die Elektrodenkammern ein *Tris*-Glycinpuffer (6,0 g *Tris*, Glycin 28,8 g/l) pH 8,5 verwendet. Das Gesamtgel besteht aus 3 verschiedenen Gelen, das unterste zum leitenden Abdichten der unteren Elektrodenkammer gegen den Pufferraum, das mittlere zum Auftrennen der Probe und das oberste zum Vortrennen der Probe.

Für diese Gele sind folgende Lösungen notwendig: (A) Acrylamid (Schuchardt) 150 g, *Bis* (N,N'-Methylen-*bis* acrylamid) 2 g, *Tris* 45,7 g, 1*N* HCl 60 ml, gelöst in 500 ml H_2O. (B) Acrylamid 25 g, *Bis* 3 g, *Tris* 8 g, 1*N* HCl 60 ml, gelöst in 500 ml H_2O. (C) Ammoniumpersulfat 1,4 g in 100 ml H_2O.

12,5 mlA werden mit dem gleichen Volumen Wasser, 0,4 ml TMED (N,N,N',N'-Tetramethyläthylendiamin, Fa. Fluka) und 0,5 ml C vermischt. Diese Lösung wird blasenfrei in die schräg gestellte Apparatur gegossen.

Nach dem Auspolymerisieren dieser Mischung (10 bis 15 min) wird die mittlere Gellösung bestehend aus 25 ml A, 25 ml H_2O, 0,8 ml TMED, 1 ml C und 0,16 ml Tertiärbutylaminoäthylacrylat (Rohm und Haas, Philadelphia) in die vertikal gestellte Apparatur eingebracht.

Der das Gel enthaltende Spalt wird bis 1 cm unter die Höhe der inneren Kühlplatte mit dieser Lösung angefüllt. In den freibleibenden Raum wird vorsichtig Wasser eingebracht, ohne dass eine Vermischung mit der Gellösung stattfindet. Dadurch wird der direkte Kontakt mit Luft vermieden und eine glatte Oberfläche der Gelschicht erreicht. Polymerisationsdauer 20–25 min.

Aus der nun waagrecht gelegten Apparatur wird das Wasser möglichst vollständig entfernt, Wasserreste auf der Geloberfläche können ein Anpolymerisieren des obersten Gels an das mittlere verhindern. Die Lösung für das oberste Gel wird in die Apparatur gegossen (25 ml B, 25 ml H_2O, 0,8 ml TMED, 1 ml C). In diese Flüssigkeit wird der Probenteiler eingesetzt. Nach dem Auspolymerisieren des Gels (20–25 min) wird das an der Rückseite des Probenteilers befindliche Gel entfernt, der Apparat senkrecht gestellt und die Kammern mit Puffer gefüllt. Der Probenteiler kann dann vorsichtig herausgezogen werden.

Die Proben können mittels einer Mikropipette in die vorgeformten Schlitze gebracht werden, das Serum ist

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