95. Solubilization of Peptides in Non-polar Organic Solvents by the Addition of Inorganic Salts[1]): Facts and Implications

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Dedicated to Prof. *Gerhard Schröder* on the occasion of his 60th birthday

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The solubility of open-chain peptide derivatives (12 examples) in non-polar, ether-type organic solvents may be greatly increased by addition of salts (LiCl, LiBr, LiI, $LiBF_4$, $LiClO_4$, NaI, $MgBr_2$, $CaBr_2$, $ZnCl_2$) or of titanates ($Ti(OEt)_4$, $Ti(OCHMe_2)_4$). Examples are reported *(Tables 2–6)* in which this solubilizing effect leads to peptide concentrations more than one-hundred-fold those in the absence of salt (*cf.* Boc-Ala-Gly-Gly-Gly-OH in THF from 2 $g \cdot l^{-1}$ to $\geqslant 300\ g \cdot l^{-1}$ with 6 equiv. of LiCl). ^{1}H-NMR Spectra of one of these solutions are reported *(Fig. 1)*. There are no indications for epimerizations of stereogenic centres on the peptide backbone. Possible applications of these solutions in peptide chemistry are discussed.

Introduction. – Solubility is often a limiting factor in syntheses and reactions of peptides [2]. Even during polymer-supported assembly of amino acids to peptides, 'aggregation' of the growing chains may be a limiting factor [3]. In the course of our work on C-alkylations of Li enolates of glycine units within oligopeptides [1], we noticed a dramatic effect of LiCl upon the solubility of certain peptides in tetrahydrofuran (THF). This has led to an extensive investigation of the phenomenon, preliminary results of which are described herein, together with a discussion of their possible significance for peptide chemistry in general.

Results and Discussion. – As representative examples, we chose the mostly open-chain tri-, tetra-, and pentapeptides **1**–**11**. They are either commercially available[3]) or were supplied to us by companies[4]). The solubilization of the nonapeptide **12** was studied by another group[5]). As non-polar organic solvent, we tested most extensively THF, but also Et_2O, dioxane, dimethoxyethane (glyme), polyethylene glycol 200 (PEG) were used, as well as the more polar MeCN, *N*,*N*-dimethyl formamide (DMF), and *N*,*N*-dimethylpropyleneurea (DMPU)[6]).

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[1]) Partially mentioned in a recent review [1].

[2]) Diploma thesis (1987) and part of the projected Ph. D. thesis of *A.T.*, ETH, Zürich.

[3]) Peptides **1**, **3**, **5**, **6**, and **7** from *Bachem Feinchemikalien AG* (Bubendorf); **2** and **10** from *Senn, Chemicals AG* (Dielsdorf).

[4]) Peptides **4**, **9**, and **11** from *Cilag AG* (Schaffhausen); **8** from *Sandoz AG* (Basel).

[5]) *P. Lansbury* and *K. Halverson* (Massachusetts Institue of Technology, USA) kindly conveyed the data of **12** to *D. S.* during a visit in March 1989 and agreed with their consideration in the present paper.

[6]) DMPU (= *3,4,5,6-tetrahydro-1,3-dimethylpyrimidin-2(1H)-one*) is a non-mutagenic substitute of hexamethylphosphoric triamide (HMPA) [4].

Z-Ile-Gly-Gly-OH **1**

Boc-Gly-Gly-Nva-OH **2**

Z-Gly-Gly-Nva-OH **3**

$HCl \cdot H_2N$-Asp(OBzl)-Val-Tyr-OBzl **4**

Z-Gly-Gly-Gly-Gly-OH **5**

H_2N-Ala-Gly-Gly-Gly-OH **6**

Boc-Ala-Gly-Gly-Gly-OH **7**

Z-Thr-Asn-Thr-Gly-OH **8**

$HCl \cdot H_2N$-Lys(Z)-Asp(OBzl)-Val-Tyr-OBzl **9**

H_2N-Gly-Gly-Gyl-Gyl-Ala-OH **10**

Z-Arg-Lys(Z)-Asp(OBzl)-Val-Tyr-OBzl · HCl **11**

H_2N-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-OH **12**

The inorganic salts included in our study so far are LiCl, LiBr, LiI, $LiBF_4$, $LiClO_4$, NaI, $MgBr_2$, $CaBr_2$, and $ZnCl_2$ (see *Table 1* for their solubilities); furthermore, we used $Ti(OEt)_4$ and $Ti(OCHMe_2)_4$[7]). All components were carefully dried for the solubilization experiments[8]).

Aliquots of the peptide and the salt were combined with the desired solvent and stirred under Ar at room temperature[9]). If, after certain periods of time, no dissolution had taken place, additional amounts of solvent were injected. We left the mixtures for 20 min to 24 h to reach solution. The peptide concentrations thus obtained are the lower

Table 1. *Solubilities of Selected Inorganic Salts in Some of the Solvents Used to Solubilize Peptides.* The values given were determined by the same techniques as those for the peptides in *Tables 2–7* (we are sure that there are more accurate values in the literature!). The liquid titanates $Ti(OEt)_4$ and $Ti(OCHMe_2)_4$ are miscible with THF.

Salt	Solvent	Concentration [mg · ml^{-1}]	Temp. [°]
LiCl	THF	48	r.t.
	THF	30	–78
LiBr	THF	450	r.t.
LiI	THF	330	r.t.
$LiBF_4$	THF	560	r.t.
$LiClO_4$	THF	270	r.t.
	Glyme	55	r.t.
NaI	THF	37	r.t.
$CaBr_2$	THF	10	r.t.
$ZnCl_2$	THF	345	r.t.

[7]) Ph_3B was also tested and showed no solubilizing effect on **4** and **7** in THF.

[8]) It is our experience that 1–3 equiv. of H_2O per mol peptide may be present without 'ruining' the solubilizing effect described herein. The use of peptide solutions for carrying out certain reactions (*cf.* deprotonation by strong bases [1]) requires rigorous exclusion of H_2O.

[9]) In some cases (*e.g.* with LiBr, $LiClO_4$), we noticed that the process was exothermic.

values of the ranges given in *Tables 2–6*. Removal of the solvent *in vacuo* led to finely divided solid residues, sometimes fluffy powders, sometimes solidified foams containing, besides the peptide and the salt, variable amounts of solvent. Redissolution of these residues required much less solvent and usually occurred instantaneously. The peptide concentrations realized in this second solution step were sometimes enormous and might reach the 50% mark. They are given as upper values of the ranges in *Tables 2–6*. In these solutions, the peptide is solubilized by the inorganic salt and *vice versa*: compare the solubilities of the salts alone *(Table 1)* with their concentrations in the peptide-containing solutions *(Tables 2–6)*.

Some of the highly concentrated solutions[10]) were stirred or stored at room temperature for up to three months to make sure that they were not unstable or metastable supersaturated solutions (melts of solvated salt-peptide complexes!). In other cases, we noticed that excess inorganic salt slowly separated, with the peptide remaining in solution[11]). The amount of peptide in solution can be determined by NMR spectroscopy[12]) (see concentration values without ranges in *Tables 2–6*). With some titanate-mediated solutions (*e.g.* of **3**), we observed interesting effects: cloudiness developed after 0.5–1.5 h and slowly disappeared after 1–2 days of stirring at room temperature. While a stirred solution of the tripeptide hydrochloride **4** (240 mg·ml^{-1}) with 3.1 equiv. of $ZnCl_2$ in THF stayed clear for several days, the Z-protected Ile-Gly-Gly-OH (**1**) went into solution with an initial concentration of > 170 mg·ml^{-1} under the same conditions but partially separated with a deposit after 30 min, leaving a 90 mg·ml^{-1} concentration of the peptide dissolved. Some of the solutions containing peptide and LiCl in THF (*e.g.* of **3** and **7**) were cooled all the way down to dry-ice temperature without precipitation; the undoubtedly oversaturated solutions thus obtained were viscous but could still be stirred.

It is clear that the *rate of dissolution* is a decisive factor in preparing these highly concentrated peptide solutions. Thus, we assume that in those cases in which we failed to obtain solutions (peptides and peptide derivatives[13])[14]) **5**, **6**, **8**, and **10**, *Table 6*), this rate

Table 2. *Solubilization of Z-Ile-Gly-Gly-OH* (**1**) *at Room Temperature*

Added metal derivative		Solvent	Concentration of peptide [mg·ml^{-1}]
	mol-equiv.		
none		THF	23 (3.8 at −78°)
LiCl	2.8	THF	⩾ 340
LiCl	5.7	THF	100 to ⩾ 180
$ZnCl_2$	2.9	THF	90
$Ti(OCHMe_2)_4$	3.0	THF	⩾ 190
LiCl	3.0	AN	1.2

[10]) *E.g.* THF solutions of **3** with 3 equiv. of LiCl, of **4** with 6 equiv. of LiCl, of **11** with 6 equiv. of LiCl, of **3** with 3 equiv. of $LiClO_4$, of **4** with 2.9 equiv. of $LiClO_4$, and of **3** with 3.3 equiv. of NaI.

[11]) For instance, $MgBr_2$ or NaI from the solution of **4** in THF (see *Table 4*).

[12]) The solution was evaporated, the residue dissolved in a deuterated solvent (CD_3OD or D_2O), and an aliquot of MeCN added as a standard.

[13]) With the exception of **8**, these non-solubilizable tetra- and pentapeptide derivatives contain mainly glycine units and/or are unprotected at either end *(Table 6)*.

[14]) Attempts with the following protein enzymes also failed: pepsin from hog stomach, lipases from hog pancreas, from porcine pancreas, and from *Candida cylindracea*. With and without LiCl, there were at most 1–2 mg·ml^{-1} in solution (by filtering and weighing the filtrate residue). So far, we have not employed 'suspensions' of these enzymes in LiX-containing THF for transformations *à la Klibanov* [5].

Table 3. *Solubilization of Z-Gly-Gly-Nva-OH* (**3**) *at Room Temperature*

Added metal derivative	mol-equiv.	Solvent	Concentration of peptide [mg·ml^{-1}]
none		THF	27 (5.1 at −78°)
LiCl	3.0	THF	200 to ⩾ 500
LiCl	6.0	THF	50 to ⩾ 130
LiCl	6.9	THF	20 to ⩾ 125
LiBr	3.6	THF	80 to ⩾ 470
LiBr	6.0	THF	> 50
LiI	2.9	THF	310 to ⩾ 420
$LiClO_4$	3.0	THF	200 to ⩾ 340
$LiBF_4$	3.0	THF	130 to ⩾ 360
NaI	3.3	THF	50 to ⩾ 80
$MgBr_2$	2.0	THF	⩾ 90
$CaBr_2$	1.9	THF	34
$Ti(OEt)_4$	3.0[a])	THF	260 to ⩾ 510[a])
$Ti(OCHMe_2)_4$	3.0	THF	70 to ⩾ 440[b])
none		Dioxane	28
LiCl	3.1	Dioxane	20
none		Glyme	10
$LiClO_4$	3.2	Glyme	> 145

[a]) Evaporating the soln. and redissolving the residue gave a soln. containing *ca.* 400 mg·ml^{-1} peptide with 1.5–3.0 equiv. of the titanate (by NMR), due to the volatility of the titanate.

[b]) Supersaturated solns. containing up to 1 g·ml^{-1} could be obtained, but became heterogeneous while being stirred for some time.

Table 4. *Solubilization of H_2N-Asp(OBzl)-Val-Tyr-OBzl·HCl* (**4**) *at Room Temperature*

Added metal derivative	mol-equiv.	Solvent	Concentration of peptide [mg·ml^{-1}]
none		THF	1.5
LiCl	2.9	THF	170 to ⩾ 510
LiCl	6.0	THF	100 to ⩾ 170
$LiClO_4$	2.9	THF	100 to ⩾ 520
NaI	3.0	THF	9 to ⩾ 140
$MgBr_2 \cdot OEt_2$	3.0	THF	60 to ⩾ 150[a])
$MgBr_2$[b])	2.6	THF	50 to ⩾ 100
$CaBr_2$	2.2	THF	15
$ZnCl_2$	3.1	THF	240 to ⩾ 390
$Ti(OCHMe_2)_4$	3.1	THF	270 to 540[c])
$MgBr_2 \cdot OEt_2$	6.0	Et_2O	oily deposit
none		Dioxane	2.3
LiCl	3.5	Dioxane	12
none		Glyme	0.45
LiCl	5.0	Glyme	> 27
LiCl	2.8	MeCN	4.5

[a]) From a concentrated soln., some $MgBr_2$-THF complex crystallized slowly and redissolved upon warming. The crystalline deposit did not contain any peptide (by NMR).

[b]) Prepared from Mg and $Br(CH_2)_2Br$ in THF (crystallized in beautiful needles); the sample used for this and other experiments contained $MgBr_2$ and THF in a 1:1.5 ratio.

[c]) After 0.3–1.5 h, these solns. turned turbid. The titanate generated a bright yellow soln. of **4**, not observed with other peptides.

Table 5. *Solubilization of Boc-Ala-Gly-Gly-Gly-OH* (7) *at Room Temperature*

Added metal derivative		Solvent	Concentration of peptide [mg · ml^{-1}]
	mol-equiv.		
none		THF	2.0 (0.8 at −78°)
LiCl	3.3	THF	120 to ⩾ 160
LiCl	5.9	THF	50 to ⩾ 300
$MgBr_2 \cdot OEt_2$	6.0	THF	< 2
$ZnCl_2$	3.0	THF	5

Table 6. *Solubilization Experiments with Other Peptides in THF at Room Temperature*

Peptide	Added metal derivative		Concentration of peptide [mg · ml^{-1}]
	mol-equiv.		
2	none		27
	LiCl	6.5	70 to ⩾ 100
	LiBr	2.9	80 to ⩾ 470
	LiBr	5.9	45 to ⩾ 270
5	none		< 0.8
	LiCl	6.9	< 0.35
	LiBr	6.7	< 0.5
	LiI	3.4	< 0.1
6	none		< 0.3
	LiCl	8.8	< 0.1
8	none		0.7
	LiCl	2.8	< 0.1
9	none		3.8
	LiCl	3.1	300 to ⩾ 380
	LiCl	6.8	120 to ⩾ 360
10	none		< 0.4
	LiCl	5.8	< 0.1
	LiCl	31	< 0.1
11	none		0.7
	LiCl	3.5	135 to ⩾ 190
	LiCl	6.0	135 to ⩾ 230
12[a])	LiBr	20	⩾ 100[b])

[a]) See also *Footnote 5*.

[b]) According to the investigators[5]), this peptide is insoluble in DMF, DMSO, CF_3CH_2OH, $CHCl_3$, and H_2O.

may just be too small. Of the solvents used besides THF so far, glyme looks most promising – on the other hand, DMPU[6]) may be a solvent superior to DMF in peptide chemistry without added salts (*Table 7*).

While the great solubilizing effect of salts and other polar metal derivatives on peptides in non-polar organic media such as THF is unprecedented, there are many related effects described in the literature: *i*) Natural and unnatural cyclic peptides have been shown to form complexes with alkali and alkaline-earth ions in the crystalline state[15]) and in solution [9–12]. From the NMR spectra, it was concluded that the ions

[15]) For reviews on the structures of crown-ether-type complexes of peptides with alkali and alkaline-earth metal ions, see *Dobler*'s monograph [6]. Comprehensive lists of the crystal structures of organic Li [7] and higher alkali-ion [8] derivatives have been compiled by *v.R. Schleyer* and co-workers.

Table 7. *Solubilities of Some Peptides in DMPU*[6]) *at Room Temperature and in the* Absence *of Added Salts.* Comparison with DMF and PEG.

Peptide	Solvent	Temp. [°]	Concentration of peptide [mg · ml^{-1}]
1	DMPU[a])	r.t.	> 350
	DMF	r.t.	> 350
4	DMPU[b])	r.t.	⩾ 410
	DMF	r.t.	⩾ 200
	PEG[c])	r.t.	⩾ 120
5	DMPU	90[d])	⩾ 20
	DMF	90[e])	⩾ 15
7	DMPU	r.t.	> 200
	DMF	r.t.	> 200
	PEG[f])	r.t.	⩾ 30
8	DMPU	r.t.	> 120
10	DMPU	90	< 0.1
11	PEG[g])	90	⩾ 25

[a]) Adding DMPU to a mixture of **1** and 2.7 equiv. of LiCl led to a highly viscous gel which could not be redissolved by adding more DMPU.

[b]) In the presence of 2.9 equiv. of LiCl, we could prepare a soln. of **4** containing *only* 70 mg · ml^{-1}.

[c]) No change in solubility was observed in the presence of 3.3 equiv. of $LiClO_4$.

[d]) The mixture was heated for *ca.* 30 s. During this time, it became homogeneous. After cooling to r.t., the soln. could be stirred for at least one week without a deposit being formed.

[e]) The mixture was heated for *ca.* 2 min. During this time, it became homogeneous. After cooling to r.t., precipitation took place within 10 min.

[f]) The solubility increased to ⩾ 40 mg · ml^{-1} in the presence of 3.1 equiv. of LiBr.

[g]) Upon stirring the soln. over the weekend at r.t., the peptide precipitated at least partially. A soln. of *ca.* 25 mg · ml^{-1} peptide in PEG/3.0 equiv. of LiBr, prepared by warming, stayed homogeneous even after prolonged stirring at r.t.

influence the conformational equilibria. Indeed, the changes of the ^{1}H-NMR spectra in (D_8)THF of the open-chain Boc-protected tripeptide **2** *(Fig. 1)*, brought about by the addition of up to 20 equiv. of LiCl, suggest that discrete complexes are formed which equilibrate more or less slowly on the NMR time scale. Some of the open-chain peptides have been recovered unchanged from LiX-containing THF solutions [1]; thus, no epimerization of stereogenic centres on the peptide backbone appears to take place under these conditions. *ii*) Alkali halides and simple peptides may crystallize from aqueous solutions as anhydrous 1:1 complexes ('*Pfeiffer*'sche Neutralsalzverbindungen der Polypeptide und Eiweisskörper' [14] [15])[16]). This indicates that the solvation energy of the salt and peptide in H_2O is outweighed by the ionic interactions between M^+ and carboxylate or amide groups in the crystals containing fully separated counterions[17]). *iii*) Finally, it has been reported that a protective protein antigen can be extracted from cells of *Bordella pertussis* by 0.75–2.25M aqueous LiBr, with the LiBr exerting a stabilizing effect on the protein at room temperature and at pH 6.0–8.5 as compared to NaCl, suggesting specific interactions of Li^+ with the protein [17].

[16]) For X-ray structures, see [16] and drawings in Fig. 23 of [1] and in [7] [8].

[17]) In the LiBr complex of ^+H_3N-Gly-Gly-Gly-O^-, the NH_3^+ and COO^- groups form a channel-like arrangement, with the Li^+ ions tetrahedrally surrounded by three carboxylate and one amide O-atom, and the Br^- ions 'stuffed' in between near the glycine moieties in the middle of the tripeptide [16].

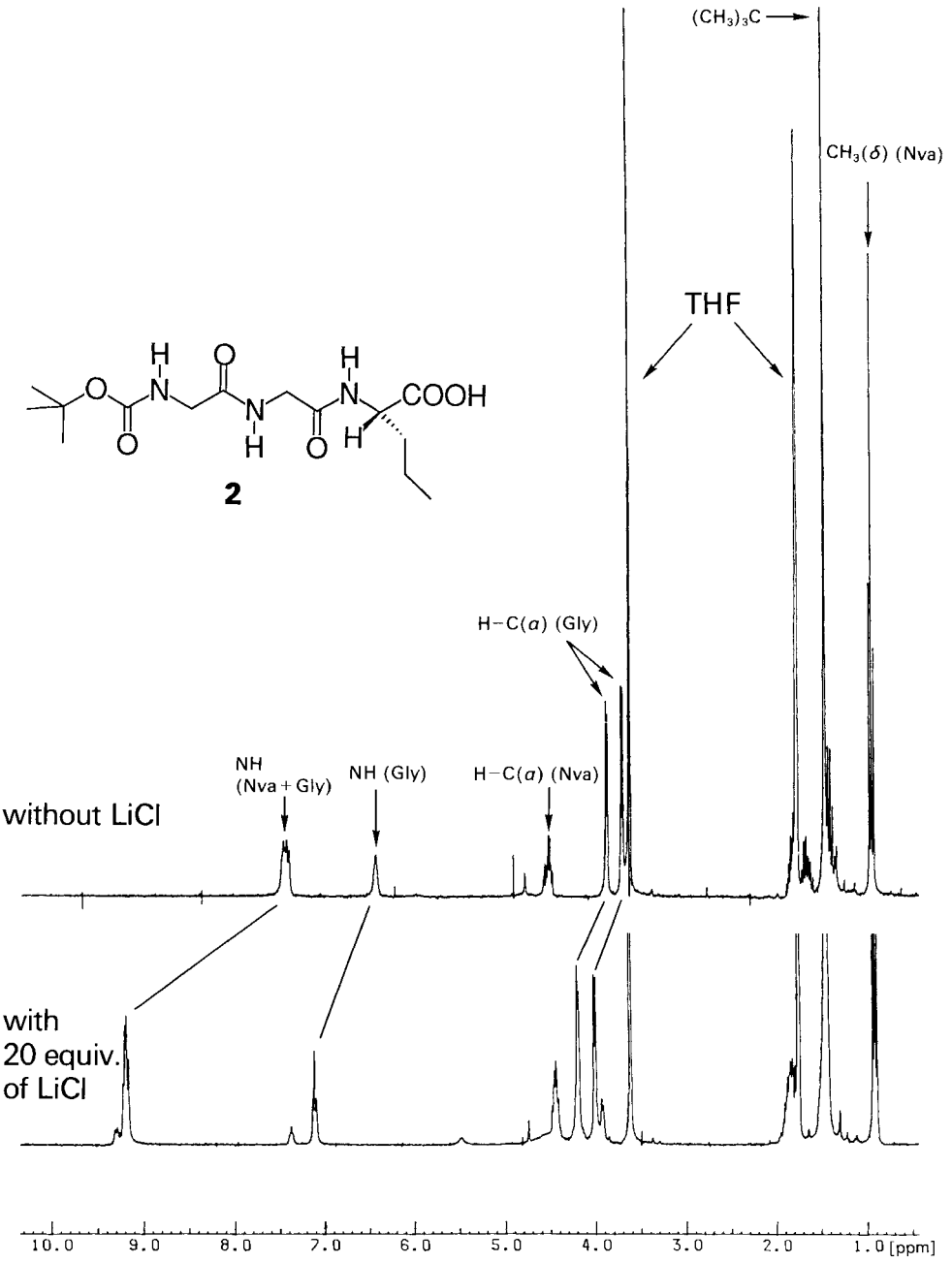

Fig. 1. *Effect of LiCl on the ^{1}H-NMR spectrum of Boc-Gly-Gly-Nva-OH* (**2**) *in (D_8)THF at room temperature.* Similar pronounced shifts are caused by $MgBr_2$ and $ZnCl_2$ (NH signals of **2** down to 10 and 8 ppm, resp.). The ^{7}Li-NMR spectrum of LiCl in the presence and absence of **2** consists of a *singlet* at 0.487 and 0.507 ppm, respectively (rel. to LiCl in D_2O; the total range of ^{7}Li-NMR is *ca.* 5–6 ppm) [13]. The N*H* signals of other peptides are similarly shifted from their normal position at *ca.* 6.5–8 ppm downfield to 7.5–9.5 ppm.

In addition to such specific interactions between amides[18]) or peptides and alkali/alkaline-earth ions, there are numerous other effects which come to mind in connection with our discovery. Even when confining ourselves to LiX effects, we can allude to only a few of them here. The addition of salts to a non-polar medium such as THF will certainly increase its polarity ('salt effect')[19]). Thus, the outcome of many reactions in organic chemistry is subject to influenes by Li salts (*Wittig* reaction [20], conversions of Li-enolates [1] and other enol derivatives [21], certain *Diels-Alder* reactions [22], deprotonations and subsequent reactions of CH-acidic compounds by tertiary amines [23–27], and other proton-transfer processes [28]), the detailed mechanisms being mostly unknown. Li salts can be used for *Lewis*-acid-mediated rearrangements [29]. Solutions of LiCl in *N,N*-dimethylacetamide are used as unique[20]) media for poly(1,4-benzamide) ($X(CO-C_6H_4-NH)_nH$) [30] and for cellulose [31], and the solutions of Li salts in molten acetamide have intriguing properties [32]. Otherwise insoluble polylithiated species can be solubilized by LiX addition [1] [33][21]).

Implications and Outlook. – The salt-mediated solubilization of peptides may turn out to be most useful. For the first time, rather concentrated solutions of certain notoriously insoluble oligopeptides in aprotic, non-polar and non-nucleophilic solvents can be obtained. These might be used for carrying out reactions, *e.g.* peptide couplings (liquid and solid phase), glucosidations, additions of peptide-bound XH groups (NH_2, OH, SH) to C=C bonds, base-catalyzed [23–27] reactions of glycine or sarcosine CH_2 groups with electrophiles [1], removal of serine or threonine side chains by *retro*-aldol cleavage, oxidations of serine or threonine OH groups to aldehyde or ketone C=O groups and subsequent conversions, electrolytic transformations, deprotections, detachement of a peptide from a support, *etc.*, under conditions not applicable in protic or nucleophilic solvents[22]). It is also conceivable that salt-containing solvent systems can be used for analytical and preparative separations and purifications of peptide mixtures by column or thin-layer chromatography (normal or reverse-phase SiO_2, Al_2O_3, *Sephadex*)[23]). Polyethylene-glycol- and salt-containing solutions of peptides should be tested for the preparation of matrices for fast-atom-bombardement (FAB) and plasma-desorption (PD) mass spectroscopy[24]). Some of these exciting prospects are now subjected to experimental test in our own and in other laboratories.

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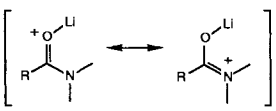
[18]) The complexation of Li^+ with the amide-carbonyl O-atoms leads to increased barriers of rotation around the $RCO-NR_2$ bond [18], see also [9–12]:



[19]) See the classical books by *Szwarc* [19] and the numerous original investigations cited therein.

[20]) 'Attempts to develop other lithium salt solvents (... for cellulose) by replacing the chloride anion were not successful. Apparently, the only other organic solvent that can be used in place of *N,N*-dimethylacetamide is *N*-methylpyrrolidinone' [30].

[21]) *Cf.* the solubilization and chemical modification of polyamides by treatment with NaH in DMSO and addition of electrophiles [34].

[22]) The nucleophilicity of the counterion X^- in MX_n could be chosen such that it is compatible with the desired reaction.

[23]) The solutions of salts and simple, inexpensive peptides in ethers are candidates as chiral solvents for certain asymmetric syntheses.

[24]) *Cf.* the 'spiking' of sample matrices for FAB mass spectroscopy with alkali salts [35].

The complexation of metal ions by peptides, causing their respective solubilization as reported here, is an interaction of profound importance in biochemistry and medicine. The alkali ions Na^+ and K^+, and the alkaline-earth ions Mg^{2+}, Ca^{2+}, and Zn^{2+} are involved in fundamental reactions of life. The Li^+ ion has strong effects on the central nervous system and is used for long-term treatment of manic depression [36–38]. The transport of these and other ions through cell membranes takes place *via* so-called ion channels ('passive' transport) or by their being entrapped in lipophilic complexes ('active' transport). Both mechanism may involve proteins or peptides, the conformation of which may

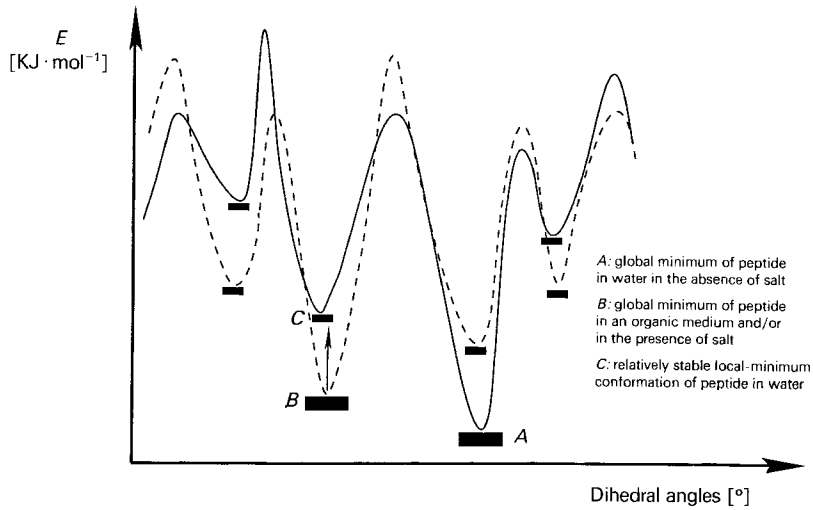


Fig. 2. *Potentials of conformations of a peptide* (solid curve) *and of its complex with metal ion(s)* (dotted curve). The global minima *A* and *B* with and without salt differ (*cf.* [6–12]), and so do the local minima (solvent changes, *i.e.* the environment in which the peptide is embedded, have similar effects). A local-minimum (metastable) conformation *C* of the peptide may be generated by switching media.

be altered to adopt the host ions. A most spectacular, recently disclosed example is the 'polymorphism' in gramicidin[25]) transmembrane channels [39–41]. As indicated in *Fig. 2*, it is conceivable that an ion complex of a peptide in an organic solvent, when brought into H_2O, and thus freed of its 'charged companion', might fall into a local-minimum peptide conformation which is flanked by high enough barriers to survive for some time. This would be a means of generating metastable conformations of peptides with properties different from those of the 'normal' peptide.

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[25]) Gramicidin is an alternating D–L pentadecapeptide containing only the hydrophobic amino acids Ala, Val, Leu, and Trp.

Some Typical Solubilization Experiments. – *Solubilization of Z-Ile-Gly-Gly-OH* (**1**) *in THF in the Presence of 2.9 Equiv. of $ZnCl_2$.* To 99.1 mg of **1**[26]) and 103.7 mg (2.9 equiv.) of $ZnCl_2$[27]) under Ar, 0.3 ml of THF[28]) were added. After stirring for 1 h at r.t., another 0.3 ml of THF were added to the heterogeneous mixture, and 1 h later a homogeneous soln. was formed. The mixture became heterogeneous again ½ h later. Another 0.2 ml of THF were added, and stirring was continued for another 24 h. From the still heterogeneous mixture, 0.7 ml were withdrawn with a syringe fitted with a membrane filter (0.45 μm), evaporated, and dried for 2 h at $5 \cdot 10^{-2}$ Torr. The residue (207.6 mg) was dissolved in 2 ml of CD_3OD, and MeCN (12.9 mg) was added as an internal standard for the ^{1}H-NMR. Integration of the NMR signals (7.30 (*s*, arom. H of **1**); 1.85 (*m*, 4 H of THF); 2.00 (*s*, MeCN)) indicated a content of 63.9 mg of **1** and 44.9 mg of THF (solubility: 90 mg peptide/ml THF). The peptide to $ZnCl_2$ ratio was *ca.* 1:4.

Solubilization of Z-Gly-Gly-Nva-OH (**3**) *in THF in the Presence of 3.0 Equiv. of $Ti(OEt)_4$.* To 102.8 mg of **3**[26]) and 191.1 mg (3 equiv.) of $Ti(OEt)_4$[29]) under Ar, 0.4 ml of THF[28]) were added. After 10 min, the heterogeneous mixture had become homogeneous (solubility: ⩾ 260 mg peptide/ml THF). After stirring for 3 days, the still homogeneous soln. was evaporated and dried for 3 h at $5 \cdot 10^{-2}$ Torr. The foamy residue (280.7 mg) could be dissolved by adding 0.2 ml of THF (solubility: ⩾ 510 mg peptide/ml THF)[30]). This soln. was again evaporated and the residue dried for 20 h at $5 \cdot 10^{-2}$ Torr. A soln. of this residue in CD_3OD was investigated by ^{1}H-NMR. The signal integration (7.40 (*s*, arom. H of **3**); 3.70 (*q*, $J = 6$, CH_2 of $Ti(OEt)_4$)) gave a ratio of 1.6 equiv. of $Ti(OEt)_4$ per equiv. of **3**, only traces of THF were detected by this analysis.

Solubilization of Boc-Ala-Gly-Gly-Gly-OH (**7**) *in THF in the Presence of 5.9 Equiv. of LiCl.* To 62 mg of **7**[26]) and 90 mg (5.9 equiv.) of LiCl[27]) under Ar, 0.5 ml of THF[28]) were added. After 2 h stirring at r.t., the mixture was still heterogeneous. Addition of 0.75 ml of THF and stirring for 14 h led to a homogeneous soln. (solubility: 50 mg peptide/ml THF). This soln. was evaporated and dried for 4 h at $5 \cdot 10^{-2}$ Torr. The foamy residue (204.4 mg) contains, in addition to the peptide and LiCl, 52 mg (0.06 ml) of THF. To this residue were added 0.15 ml of THF which led to a homogeneous, viscous soln. within 30 s. The soln. contained a total of 0.21 ml of THF (solubility: ⩾ 300 mg peptide/ml THF). During stirring of this soln. for 2 weeks and allowing to stand at r.t. for 5.5 months, no precipitation was observed.

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[26]) Dried at 80° and $5 \cdot 10^{-2}$ Torr for 4 h.
[27]) Dried at 160° and $5 \cdot 10^{-2}$ Torr for 5 h.
[28]) Distilled over K under Ar.
[29]) Distilled at 110–115°/$1 \cdot 10^{-1}$ Torr.
[30]) Obviously, some $Ti(OEt)_4$ was removed during the period of drying *in vacuo*. The solubility value given was calculated by assuming that there was no THF left in the residue (*cf.* the NMR measurement described below). Less than the 0.2 ml of THF used might have been sufficient for solubilization.

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