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Design and Construction of the Automatic Peptide Library Synthesizer

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An automatic peptide library synthesizer capable of randomizing 20 building blocks in 20 reaction vessels is described. The design is based on uniform bead distribution by gravity sedimentation after thorough mixing by inert gas bubbling and stirring. The instrument can be alternatively used as a multiple peptide synthesizer since it can deliver any reagent to any reaction vessel.

The synthesis of peptide libraries based on the principle of solid-phase coupling of individual components in separate reaction vessels and subsequent mixing of the particles of the solid carrier (the "Split Synthesis method") has been well described (1–3). Manual synthesis of the libraries is simple but routine, and the possibility of careless operator error is significant.

Automation of this synthetic procedure is clearly desirable for increasing productivity. Two other systems for automated library production have been described (4, 5). Both utilize a robot arm for mixing and redistribution of the resin. We have automated library synthesis in the simplest fashion, using gravity for uniform distribution of solid-phase particles.

MACHINE CONSTRUCTION

The instrument APLS1 (Automatic Peptide Library Synthesizer 1) is shown in Fig. 1. Random distribution of beads is achieved by sedimentation in randomization fluid (dimethylformamide) after thorough mixing by inert gas bubbling. The apparatus comprises several interacting parts shown in Fig. 1, the functions of which can be followed on the scheme in Fig. 2.

The reactor box, at the top part of the machine, contains the reactor (RE) with 20 glass vessels (CLn n = 1–20) for condensation of 20 different amino acids; a dosing head, which can be turned and shifted up and down, that delivers the solutions of 20 amino acids and 3 reagents through Teflon capillaries equipped with optoelectronic sensors of liquid flow (OSAn n = 1–20 and OSRn n = 1–3); and a motor (M2) used for stirring the reactor content during the randomization step and for distribution of washing solvent. The remaining components of reaction chamber are: a positioning device for the dosing head comprising the stepper motor (SM1), a position sensor (OSP), and a pneumatic piston (PC1); closing valves for the glass reaction compartments (VOn n = 1–20); and manifold (MN). In the bottom of the reactor box are pneumatic and hydraulic connections for mixing and randomizing gas and valves (VT1–VT7) used for gas introduction to the reaction vessels and for the removal of reactants and solvents.

In the bottom left part of the reactor box are a waste solvent vessel (W1) and a randomization solution vessel (WR). A gear pump for washing solvent delivery (GP1) and a filtration vessel (F) are located on the left side of the reactor. On the right side of the reactor are two reduction valves (PR4 and PR5) for adjustment of operating gas pressure for stirring and randomization steps.

The bottom part of the instrument is the box with amino acid solution storage vessels together with pressurizing and depressurizing three-way valves. This box also contains reducing valves (PR2 and PR3). An IBM PC compatible computer for instrument operation is placed on the top part of this box.

On the back wall of both boxes power sources for electronic circuits, electromagnetic solenoid valves, a vacuum pump, and some plumbing are located. The instrument requires a source of compressed inert gas (min. 20 psi) attached to an exhaust fan. An external vacuum source (25 Torr) may be used as an alternative to an internal vacuum pump.

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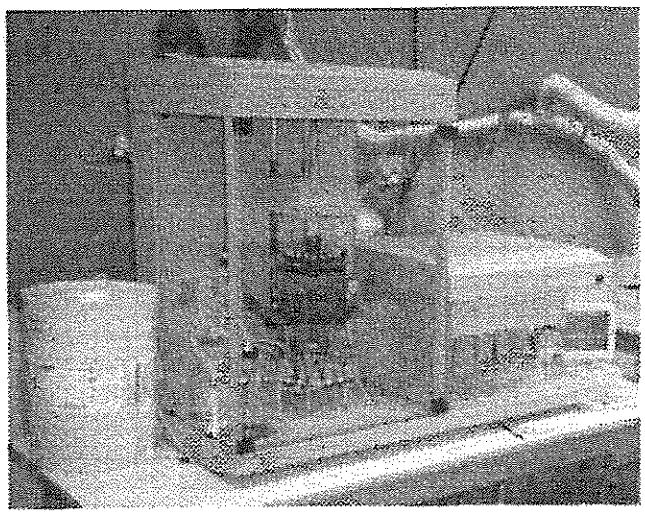

FIG. 1. Automatic peptide library synthesizer APLS1.

DESCRIPTION OF FUNCTION

The reactor (RE) construction is the subject of a patent application and is depicted in detail in Fig. 3. It is composed of an upper glass tube (1) of internal diameter 160 mm, an upper connecting section (2) with a middle cone (3), 20 glass chambers (CLn n = 1–20) (4) of internal diameter 16 mm and volume 12 ml, a lower connecting piece (5), 20 electromagnetic valves (VOn n = 1–20), and a connecting manifold (MN). The middle cone (3) of the upper connecting piece (2) has a shape similar to a kitchen juice extractor with 20 indented segments that have a slope of 42° and terminate at the lip of each glass chamber (4).

The glass chambers (CLn) are equipped with glass frits (6) and are sealed by Teflon O-rings (7). Electromagnetic valves closing the chambers (VOn) are placed under lower connectors. It is possible to stir (bubble N_2) or empty any chamber independently of the others. All liquid lines from the glass chambers are connected in manifold MN for simultaneous application (through valve VT5) of vacuum during the emptying of vessels (to W1) or the addition of compressed inert gas for stirring.

In an attempt to best simplify the solution volume measurement and delivery, we have developed a new principle of pressurizing and depressurizing storage bottles with amino acid solutions (An n = 1–20) and reagents (Rn n = 1–3), which is the subject of another patent application. Each storage bottle (An and Rn) is closed by a stopper, and a dosing tubing is connected to a particular position on the rotatory dosing head. Another tube is connected with the three-way valve VAn (n = 1–20) or VRn (n = 1–3). The three-way valve in the inactivated stage connects the space above the liquid surface in the bottle with atmospheric pressure. For dosing, the three-way valve is switched and the liquid in the bottle is pressurized by pressure P3 (3 PSI) regulated by reductive valve RP3. Liquid flows through the dosing tube to a position on the dosing head and its presence is detected by an optoelectronic sensor (OSAn or OSRn). From this point, the time needed for delivery of the necessary volume of the solution is measured. Dosing is finished by switching the three-way valve VAn or VRn to the original position, which depressurizes the liquid in the bottle. Since outlets

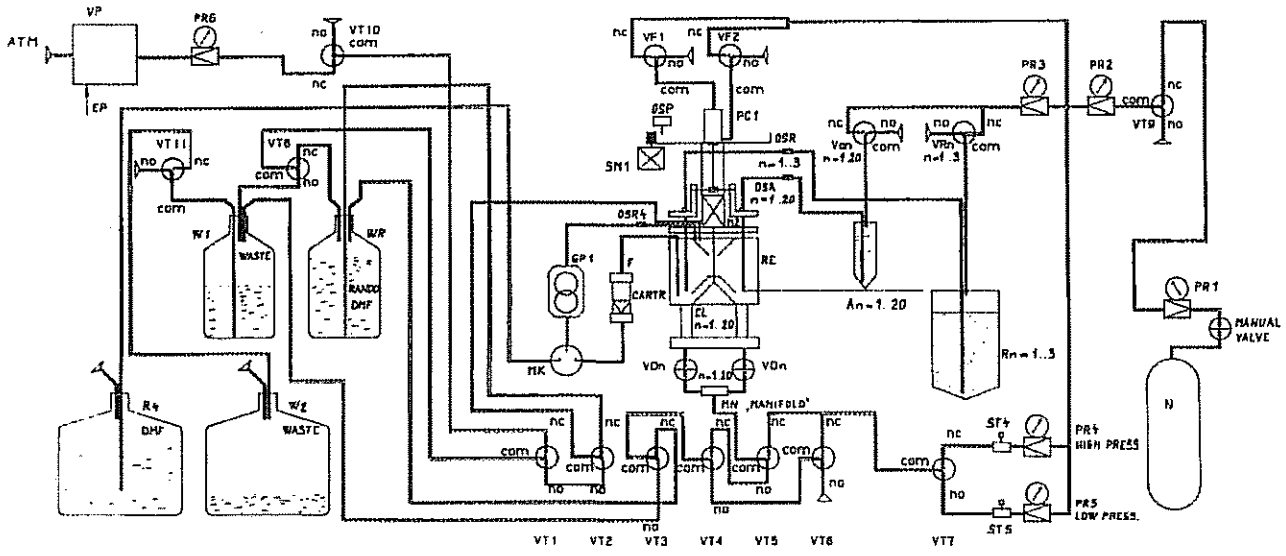

FIG. 2. Functional scheme of the instrument. Abbreviations are explained in the text.

of all dosing tubings are 15 cm higher than the liquid in the storage bottles, liquid in the tube is sucked back to the storage bottle by hydrostatic pressure.

The dosing system consists of: a dosing head, constructed from the ring (8), in which 23 stainless steel tubings (9) are fixed; a leading ring (10); pneumatic piston PC1 for vertical movement of dosing head and its actuating valves VF1 and VF2; a positioning mechanism (optoelectronic sensor of the zero position OSP, stepper motor SM1 with mechanical transmission) for turning the dosing head such that any amino acid (An) or reagent (Rn) can be delivered to any reaction chamber (CLn). The dosing head is equipped with a mechanical stirrer (11) and an inlet for washing solvent (12). Dosing tubes are threaded through the stainless steel tubes (9) and extend about 10 mm over the ends. When the dosing head is in the delivery position, i.e., when it is in the lower position, outlets of the tubes for amino acid solutions point to the middle of the glass reactor chambers (CLn) (4). The optoelectronic sensors of liquid flow OSAn (n = 1–20) and OSRn (n = 1–3) are placed just before the entrance of the tubing to the delivery (dosing) head. Pressurizing and depressurizing three-way valves (VAn and VRn) for particular vessels containing amino acids (An) and reagents (Rn) are placed just above each vessel.

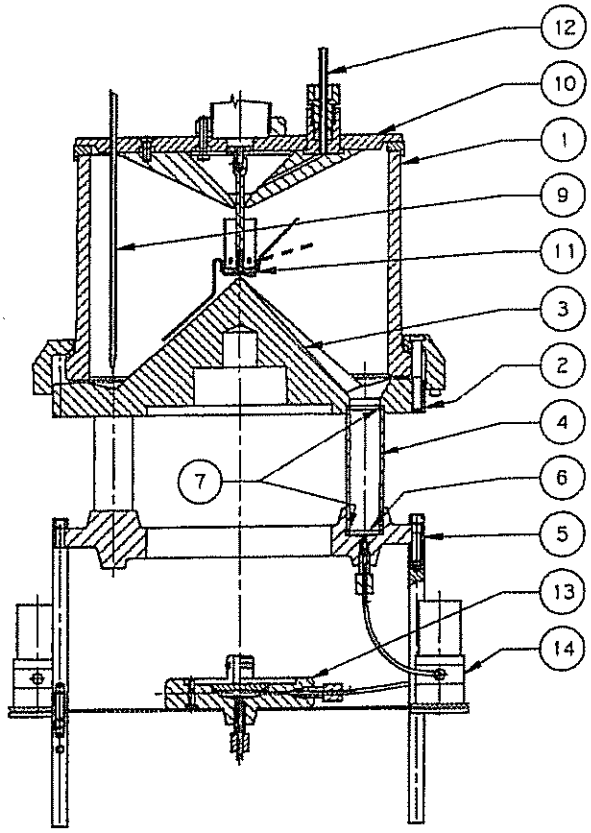

FIG. 3. Reactor assembly.

Mixing in each reaction chamber is realized by a mild stream of inert gas introduced from below each chamber. The necessary overpressure P5 (4 PSI) of inert gas is regulated by the reducing valve PR5, and gas flow is regulated by the regulator ST5. After activation of valve VT5, the manifold (MN) is pressurized and subsequent stepwise opening of the valves (VOn) stir each chamber.

Mixing the contents of the reactor during the randomization step or during removal of the solid support after the synthesis is analogous to that of each individual chamber; the only difference is the higher gas pressure P4 (10 PSI) set by valve PR4 and regulator ST4. Activation of valve VT7 leads the pressure P4 to valve VT5. After all valves VOn and valve VT5 are opened, the gas enters all reaction chambers.

Removal of used reagents and washing solvents to the "first" waste bottle is realized as follows. The vacuum pump is activated and the waste bottle W1 is evacuated through valve VT1. After valve VT4 is activated, liquid is removed from the reaction chambers through valves VOn. The randomization liquid is removed analogously. Valve VT8 evacuates container WR and liquid is lead through valves VOn (all), VT3, and VT4.

The washing solvent is pumped from container R4 that is placed outside of the instrument. The gear pump GP1 feeds the liquid into the middle of the dosing head, where it flows freely onto the rotating blades of the stirrer (11) above the dividing cone (3). Liquid is sprayed onto the sides of the reactor (1) and onto the dividing cone (3) and is divided equally into reaction chambers CLn (4).

The first filling of the reactor with randomization liquid may be performed in the same way as that with washing solvent (e.g., DMF). For subsequent randomizations the liquid is pumped from the container WR. Valves VT3, VT7, VT6, and (after pressurization of container WR) VT2 are activated and the contents of vessel WR are transported into the reactor.

Emptying of the "first" waste container is realized by subsequent activation of valves VT8, VT7, and VT6 (pressurization of vessel W1) and opening of valve VT11. In this arrangement the contents of container W1 are moved to the "final" waste container W2 that is located outside of the instrument.

Removal of the solid carrier from the reactor after synthesis is completed is achieved with the suspension dilution technique. The tubing connected with the top of glass filtration chamber F is placed into the reactor. Valve MK is manually turned to connect the filtration chamber with gear pump GP1. The reactor is filled with randomization liquid, and pump GP1 then pumps the liquid through the filtration chamber back to the reactor through the middle of the dosing head (as in the case of the washing step). After 15 min of pumping, more than 99.9% of the solid particles are retained in the filtration chamber.

The above-described processes are computer controlled. The computer connection was designed to be compatible

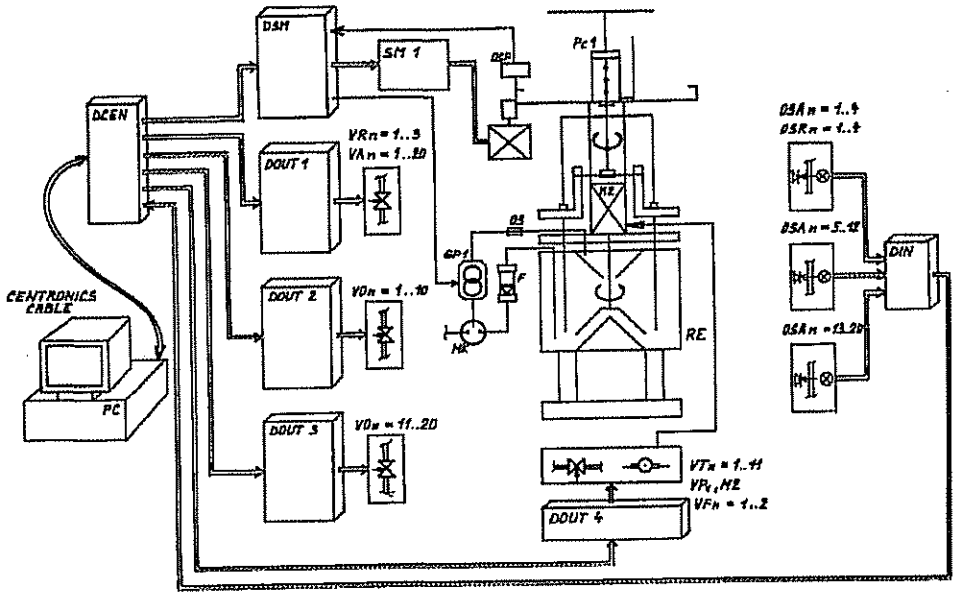

FIG. 4. Electronic block scheme of the instrument.

with any standard IBM PC type computer. This was the reason for the selection of an otherwise uncommon method of instrument control (CENTRONICS), which is normally used for the connection of printers. A simplified block diagram is given in Fig. 4.

The board DCEN contains combinatory logic circuits that decode computer-originated numerical signals arriving via a standard CENTRONICS cable. This cable uses the usual wiring for this type of connection. The voltage values are also standard. The difference is only in the protocol used for the connection. The DCEN board

TABLE 1

Basic Commands of the Metalanguage for APLS-1

Command	Comments
DISP1/[v]/⟨CR⟩	Delivery from container 1 (BASE)
DISP2/[v]/⟨CR⟩	Delivery from container 2 (CREA)
DISP3/[v]/⟨CR⟩	Delivery from container 3 (BLUE)
DISP4/[v]/⟨CR⟩	Delivery from container 4 (WASH)
DISPx/[v]/[ACG...]⟨CR⟩	Delivery of amino acid solutions
RADMF/I|O/⟨CR⟩	Randomization liquid (DMF) (I = in, O = out)
DRAIN/S|A|I/t/⟨CR⟩	Move liquid to the "first" waste
WASTE/t/⟨CR⟩	Transfer to waste
STIRR/H|L|O/⟨CR⟩	Stirrer (High, Low, Off)
MIX/S|A|I|F/t/⟨CR⟩	Mixing by inert gas (S = single—one chamber at a time, A = all chambers simultaneously, I = according to initialization command consecutively, F = one at a time, but with higher gas pressure)
INIT/[.....---...]⟨CR⟩	Initialization of reaction chamber (the chamber with "-" mark is not initialized
STOP⟨CR⟩	Interruption of synthesis
WAIT/t/⟨CR⟩	Waiting for t seconds
@ [text]⟨CR⟩	any comment

Parameters used: t time in seconds; v volume in milliliters

TABLE 2

Initialization of the Synthesis

Command	Comments
INIT/	
DRAIN/S/20/	Drain all reactor chambers
RDMF/I	Introduce randomization liquid
STIRR/H	Stirr at high speed
MIX/A/30	Bubble gas through all chambers
STIRR/O	Stop the stirrer
WAIT/20	Wait 20 seconds
RDMF/O	Transfer randomization liquid into container RW
DRAIN/S/15	Drain all chambers separately
STIRR/H	Start of the first washing
DISP4/20	Deliver washing solvent (DMF)
STIRR/O	
DRAIN/A/20	
STIRR/H	Start of the second washing
DISP4/20	
STIRR/O	
DRAIN/A/20	
STIRR/H	Start of the third washing
DISP4/20	
STIRR/O	
DRAIN/A/20	

TABLE 3

Example of One Step of Library Synthesis

Command	Comment
INIT	Instrument initialization deprotection
DISP1/10	Delivery of piperidine solution
MIX/S/5	
WAIT/60	
MIX/S/5	
WAIT/60	
MIX/S/5	
DRAIN/S/20	
	Washing
STIRR/H	
DISP4/10	Delivery of washing solvent
STIRR/O	
MIX/S/5	
DRAIN/A/20	
.	
.	
.	
.	
	Coupling
DISP3/8	Delivery of an indicator
DRAIN/S/10	Solvent removal
DISPx/25/AAADEFG-HIKKKLMNPQRST	Delivery of amino acids (alanine is delivered to the first three chambers and lysine is delivered to the chambers 10–12, i.e., library will be enriched in alanine and lysine and it will not contain cysteine, valine, tyrosine, and tryptophan)
DISP2/12	Delivery of condensation reagent (DIC)
MIX/S/10	The first mixing
WAIT/300	
MIX/S/10	
.	
.	
.	
.	
WAIT/300	
	Washing after coupling
DRAIN/S/20	
STIRR/L	
DISP4/15	
STIRR/O	
MIX/A/15	
DRAIN/A/20	
.	
.	
.	
.	
	Randomization
DRAIN/S/20/	Drain all reactor chambers
RDMF/I	Introduce randomization liquid
STIRR/H	Stirr at high speed
MIX/A/30	Bubble gas through all chambers
STIRR/O	Stop the stirrer
WAIT/20	Wait 20 seconds
RDMF/O	Transfer randomization liquid into container RW
DRAIN/S/15	Drain all chambers separately
STIRR/H	Washing the walls of reactor
DISP4/20	Deliver washing solvent (DMF)
STIRR/O	
DRAIN/A/20	
STIRR/H	Second washing
DISP4/20	
STIRR/O	
DRAIN/A/20	
.	
.	
.	
.	
DRAIN/A/20	
WASTE	Transfer of liquid from W1 to waste

translates the sent values of digital outputs to the registers, the outputs of which are already directly connected with the boards DSM, DOUT1, DOUT2, DOUT3, DOUT4, which contain the actuating microvalves and stepper motors. The signals coming from the optoelectronic sensors for liquid presence generated by the board DIN are treated similarly. After their demultiplexing by the board DCEN the signals are sent to the computer.

The board DSM contains actuators with electronic current regulation of the particular phases of stepper motor SM1. It also contains a two-bit input and output gate, which is used for the control of gear pump GP1 and for the scanning of the signal from the optoelectronic sensor for zero position of the dosing head OSP.

Microvalves VR (n = 1–3) and VA (n = 1–20) are controlled by the board DOUT1, microvalves VO (n = 1–10) by the board DOUT2, microvalves VO (n = 11–20) by the board DOUT3, and valves VT (n = 1–11), VF (n = 1 and 2), the output socket for eventual external vacuum pump VP1, and the direct-current motor for the stirrer M2 are controlled by the board DOUT4.

Output signals from optosensors OSA (n = 1–20) and OSR (n = 1–4) evaluating the presence of the liquid require standardization, and they are processed to level TTL by the board DIN. The board DIN contains analog circuits of the towed comparator type, which can eliminate long-lasting changes of capillary transparency and technical parameters of the optoelectronic components used.

Software for this equipment was designed based on the experience gained during the construction of several automatic peptide synthesizers (6, 7). Since a new task—randomization—was added to the synthetic process, the possibility for the operator to modify even the complicated technological operations was added to the program.

A specific language (metalanguage) of interpretation was created. This language contains commands that clearly identify particular technological operations. The synthetic program is an arrangement of these commands together with numerical or logical values for their parameters. Simple editing with the possibility of creating macro commands with repetition allows for very effective programming.

The function of the program is illustrated below. The comments describe actions of the particular actuators of the equipment in various stages of the synthesis. Basic commands are given in Table 1.

As an example of the real synthesis, we describe here the introductory operations and condensation of amino acids in one position. Synthesis of a library with fixed positions might be slightly different.

At the beginning of the synthesis all valves and other components, including the dosing head, are in the inactive position. The given amount of the solid carrier (1 to 20 g) suspended in the appropriate solvent is introduced into the reactor and the liquid is drained (DRAIN/A/t). Homogeneous distribution of solid support to the single re-

action chambers (CLn) is performed by a randomization step: the reactor is filled with randomization solvent (DMF, command RDMF/I) until the top of the dividing cone is reached (3). The stirrer (11) is activated (low speed, command STIR/L) and inert gas (pressure P4) is introduced into the reaction chambers (4) (command MIX/I/t, MIX/A,t). Sedimentation (WAIT/t) follows through stirring of the resin. The randomization solvent is transferred to container WR, the walls of the reactor and dividing cone are washed (STIR/H, DISP4/t, STIR/O), and washing solvent is disposed (DRAIN/S/t). Table 2 illustrates the described program.

The cycle in which amino acids are attached starts with addition of the deprotecting reagent (piperidine in DMF, command DISP1/t), which cleaves Fmoc protecting groups. During deprotection the reaction chambers are mixed one at a time (MIX/S/t). After removal of the piperidine solution (DRAIN/S/t), the washing solvent is introduced (STIR/H, DISP4/t, STIR/O), resin in the chambers is mixed (MIX/S/t) by inert gas (pressure P5), and liquid is drained to "first" waste (DRAIN/A/t). This step is repeated several times. After washing, the indicator (bromophenol blue) solution (8) is delivered (DISP3/t) and the chambers are drained (DRAIN/A/t). Delivery of amino acid solutions to the single chambers (DISPx/t) is followed by the delivery of coupling reagent (DISP2/t). During condensation the chambers are mixed intermittently (MIX/S/t). After coupling is finished (resin in all chambers should be decolorized), the solutions are removed to waste W1 and resin is washed as above. The last step in the synthesis of libraries is randomization. This step is preformed in the same way as described above for synthesis initialization. An example of one cycle of synthesis is given in Table 3.

All software was written in TURBO PASCAL version 6.0 and runs under MS DOS 5.0.

CONCLUSION

The described automatic peptide library synthesizer was successfully applied to the syntheses of peptide libraries on different types of solid-phase supports. The system is effective and more efficient than manual synthesis.

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