Case Study of ARIZ-85C Application to Isolation of the Binding of Target Proteins

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ABSTRACT

It was required to extract the bindings of Prp19p and other proteins from a lipid droplet for research of obesity repression. Extraction of some specific bindings could be done through dissociation of the other protein bindings. SDS was adopted as a dissociating agent but it also dissociated Prp19p bindings. That contradiction was surmounted with application of ARIZ-85C, part 1. The solution idea was proved to be useful to lift out Prp19p bindings out of a lipid droplet and sufficiently effective for the following further research.

1) Background

The case of this paper followed the earlier biochemical research[1]. The project of this case was started just after Siyoung Cho and her colleagues found that Prp19p is important for further research of repression of obesity and finished at the end of 2006 before the publication of the earlier research. For the readers, the brief explanation of the earlier work is given below.

Repression of obesity is the main target of pharmaceutical industry for business in these days. Amore Pacific, one of the leading companies in that area of Korea, has found that research on lipid droplet must be the gate to conquer obesity.

Lipid droplets are subcellular organelles considered as a major energy depot by storing neutral lipids. Therefore, the biogenesis of a lipid droplet is central to whole body energy homeostasis. Besides, lipid droplets seem to have important roles in lipid trafficking in adipocytes, cell signaling and several important human diseases. It is thus important to understand the mechanism of deposition and mobilization of cellular neutral lipids. It is expected that a set of proteins involved in lipid droplet biogenesis could be composed of effective targets for the regulation of the whole body energy homeostasis. Lipid droplets are surrounded by a phospholipid monolayer into which many proteins are embedded. Some of these droplet-associated proteins are reported to play important roles in the functions of the lipid droplets. As one of them, Prp19p is an integral component of the heteromeric protein complex(the NineTeen Complex, or NTC) in nucleus and it is essential for the structural integrity of NTC and its subsequent activation of the splicesome. Researchers identified Prp19p as a member of proteins associated with lipid droplets. Downregulation of Prp19p expression with RNA interference in 3T3-L1 cells repressed lipid droplet formation with the reduction in the level of expression of perilipin and S3-12. Prp19p or Prp19-interacting proteins during droplet biogenesis in adipocytes may be considered as another class of potential targets for attacking obesity and obesity-related problem.

In order to research how to control Prop19p-interacting proteins, it has to be possible to isolate the bindings of Prp19p and other proteins. Isolation of target proteins requires dissociation of the bindings of the other proteins in an adipocyte. In general, the recommendable dissociating agent is Sodium Dodecyl Sulfate(SDS).

This paper is related to the problem that there was a big trouble to use SDS as a dissociating agent. This paper says about TRIZ consultation from 15th of September to 17th of November, 2006. The result of this case was proved to be effective and then has been used for further research.

2) Reasons that TRIZ was introduced

Probably, the researchers would have been searching for different dissociating agents as an alternative to SDS ‘through trial and error method’ if TRIZ had not been introduced. The way to find proper biochemical agents could be one of the best examples of trial and error method. That requires

lots of time and very often embarrasses researchers. The researchers wanted more speedy and effective procedures for solutions. TRIZ was introduced not as the one and only way to solve the problem situation but as a more effective way to overcome the trouble. The management of Amore Pacific R&D Center intended to check the effectiveness of TRIZ through the project of this problem.

3) Problem Identification from the Initial Problem Situation

Just like the general solving process of a certain problem, the first step of this case was 'identification of the problem'. We hardly guide identification of IFR correctly at the very starting point of a project. Generally, we cannot confirm what IFR could be until the correct contradiction or the correct function relationship is formulated. For correct formulation of contradictions and function relationship, we need several preliminary steps.

The first step is the identification of what we want. We found that it is very effective for problem solving to re-identify what we want with ENV model of OTSM-TRIZ[2,3]. ENV model helps us to abstract the essence of the problem which is unlikely to be gotten through a sentence of several words. ENV results from a feed-back process along the problem solving process. After ENV identification of 'what we want', we move on the next step. If we find any needs to re-identifying 'what we want' during the next several steps, we go back to the 'ENV' of 'what we want'. These steps are our recommendation for real TRIZ application. Table 1 shows the final ENV model of this case.

Element	Name of feature	Value of feature	
		Current	Desired
Other cell components	position	With Prp19p + proteins	Away from Prp19p + proteins

Table 1 ENV model for identification of the project goal

Through ENV model of the goal, we could abstract the general ways to isolate the target, 'Prp19p-combined protein'. Actually, we had no information about what proteins combine with Prp19p in a lipid droplet and then we had no choice but to dissociate cell components by SDS. SDS was the only detergent we had found which could complete the required dissociation to all cell components. This situation made us select 'co-immunoprecipitation with SDS' as the way to separate the target protein bindings.

4) Multi-Screen Thinking Based on OTSM-TRIZ

According to OTSM-TRIZ, we identified the phase of our problem through multi-screen thinking[2,4]. Multi-screen thinking based on OTSM-TRIZ viewpoint enabled us to identify which phase should be addressed among several phases of the whole co-immunoprecipitation process.

As shown in Fig.1, several agents like Phosphate Buffered Saline (PBS), Nonidet P 40 (NP 40), etc are used for the process. 'During reaction with SDS' phase seemed to cause the main problem. SDS could be effective to separate all protein bindings except the bindings with Prp19P. However, if we adopt SDS to the amount enough to dissociate the other cell components, the target protein combination, or Prp19p-Proteins like Prp19p combined with soluble N-ethylmaleimide-sensitive fusion (NSF) protein attachment protein(SNAP)-ap, might be also dissociated to be useless samples for our research. We needed the combination of Prop19p and other not-identified proteins.

For a more clarified image of the problem, we moved on to 'Function Analysis' of the problematic phase.

Function Analysis

	Time -2 :before introduction of SDS	Time -1: during reaction with SDS	Time 0: SDS introduction + mixing	Time 1 : detecting (Anti-body introduction)
(Components of) Supersystem & Environment	other protein, H2O NP40, sodium deoxy cholate, PBS	other protein, H2O NP40, sodium deoxy cholate, PBS	other protein, H2O NP40, sodium deoxy cholate, PBS	Anti-body : 2 types
System	Prp19p+SNAPap+lipid droplet : binded	???	Prp19p, SNAPap, lipid droplet (all of them are dissociated)	
Subsystems	Prp19p, SNAPap, lipid droplet	???	Prp19p, SNAPap,lipid droplet	

Fig. 1 problem phase identification according to multi-screen thinking based on OTSM

5) Function Analysis

The 'Function Analysis[4]' was done for the identified problem phase. This result of 'Function Analysis' would be used for part 1 step 2 of ARIZ-85C. Function analysis on chemical/biochemical systems should be supported by interpretation of the chemical interactions in terms of physical changes[5]. We tried to identify the interactions among entities in a cell when SDS introduced. Actually, we had no technically precise information about the real phenomena. We built the model focusing only the target entities and relying on our knowledge and imagination. You could not use this model when you are analyzing the phenomenon in a cell. However, at least, this model could be a good reference. The goal of function analysis is not a solution idea list but a problem list for applying TRIZ thinking ways to the problem situation.

Fig. 2 shows the function analysis of 'during reaction with SDS' when the concentration of SDS is higher than 4 %.

SDS, concentration higher than 4 % is enough to dissociate all bindings among proteins.

According to the result of function analysis, we got the main problem list.

(1) harmful function problem : the function 'to dissociate the binding of Prp19p & proteins(in Fig. 2, we specified the proteins as SNAPap) delivered by SDS

(2) insufficient/excessive useful function problem : there is no such a problem when the concentration of SDS is over 4 %.

(3) contradiction problem : the physical contradiction of the concentration of SDS is related to the technical contradiction between the dissociation of the bindings of other proteins and the undamaged bindings of Prp19p.

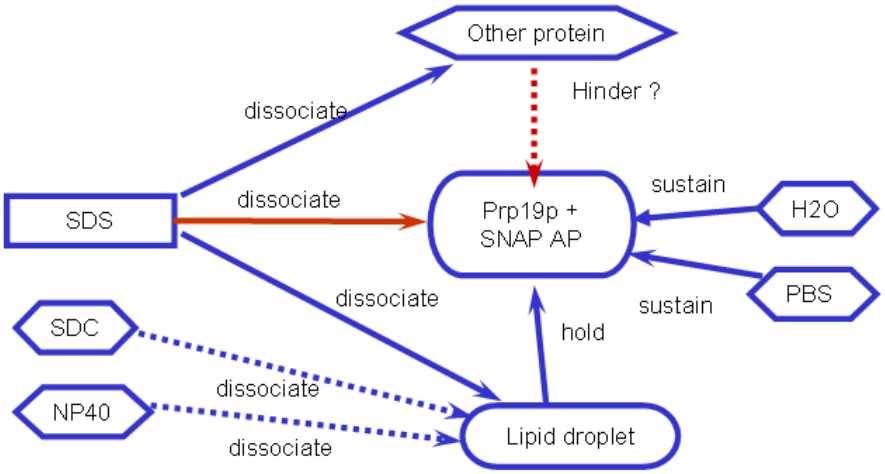

Fig. 2 function Analysis of the identified problem phase

6) Contradiction

Even though we have little information on the state of the inside of the dissociated cell, we could formulate the initial contradiction for ARIZ-85C application.

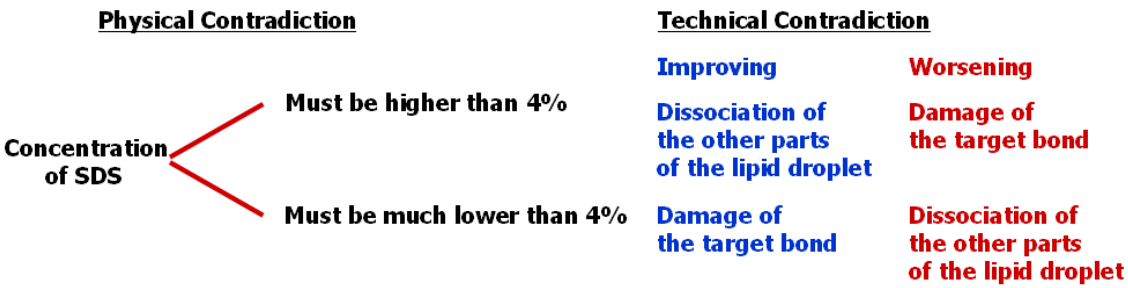

Fig. 3 key contradiction resulted from the function analysis

For more clarified description of the problem situation, the physical contradiction was formulated in

ENV model of OTSM-TRIZ. 'Element' was SDS, 'Name of feature' was 'concentration', and 'Value of feature' was 'higher than 4% v.s much lower than 4%'. If we had been able to solve this contradiction through 'separation in time, space, and system scale', the ENV expression must have been very helpful.

7) Application of ARIZ-85C Part 1

If we had gotten enough information on the problematic phenomenon, we might have applied ARIZ-85C to the contradiction obtained from the function analysis. Biochemical/chemical problems usually seem to accompany shortage of knowledge on the problem situation. You usually find yourself in case that only a kind of discovery could show us how to solve the problem. In that case, you don't know what should be done for solutions.

Based on the experiences of problem solving with OTSM-TRIZ[6], we found it that only the part 1 of ARIZ-85C could clarify to reduce the searching area for problem solving, or to achieve 'the key task of problem solving' in OTSM-TRIZ terms. Actually, Hongyul Yoon, TRIZ consultant ran ARIZ part 1 as a tacit procedure for problem solving. It is not necessary to run ARIZ explicitly for application of it. If the consultant is familiar with ARIZ, it is enough for him to guide the team members along the procedure of ARIZ. Therefore, the following explanation according to ARIZ is just for convenience of the readers.

① ARIZ-85C 1.1

This step was done based on the contradiction presented in Fig.3.

The system for isolation of Prp19p bindings includes SDS, Prp19p, and the other proteins in a lipid droplet.

Technical contradiction-1: If SDS concentrated higher than 4 % is applied, the other protein bindings are completely dissociated but the target protein bindings with Prp19p get dissociated too.

Technical contradiction-2: If SDS diluted much lower than 4 % is applied, the target protein bindings with Prp19p are kept undamaged but the other protein bindings are not completely dissociated.

It is necessary to dissociate the other protein bindings completely without damage of Prp19p bindings by introducing minimal change.

② ARIZ-85C 1.2

ARIZ-85C 1.2 resulted from the model of function analysis ; 'tool' is 'SDS', 'product' is 'Prp19p bindings' and 'other parts of the lipid droplet'

③ ARIZ-85C 1.3

The models required by ARIZ-85C 1.3 are shown in Fig.4.

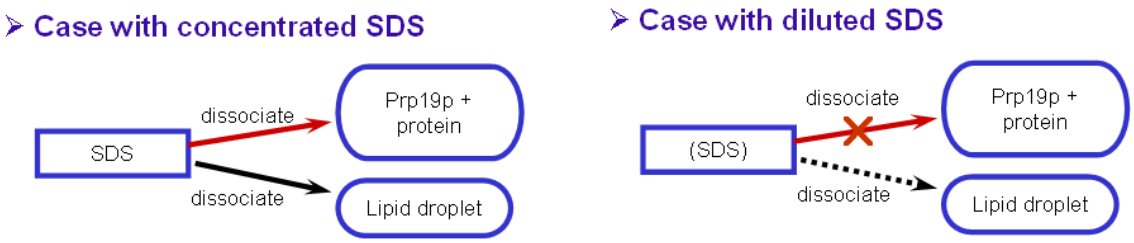

Fig. 4 diagram for ARIZ-85C 1.3 acquired from function analysis

④ ARIZ-85C 1.4

We faced the limitation of time for this project. 'case with diluted SDS' meant that we should develop new ways to dissociate proteins, which usually requires too much time for experiments. Researchers as field experts couldn't follow the solving line of 'case with diluted SDS'.

The situation made us select 'case with concentrated SDS' at ARIZ-85C 1.4 even though the original advice of Altshuller must have recommended 'case with diluted SDS'.

⑤ ARIZ-85C 1.5

ARIZ-85C 1.5 produced the extreme case of what we selected ; SDS concentrated extremely higher than 4% and complete damaged Prp19p bindings.

⑥ ARIZ-85C 1.6

- The conflicting pair ; SDS concentrated extremely higher than 4% as 'tool',
  Prp19p bindings as 'product'
- The intensified formulation of the conflict ; SDS concentrated extremely higher than 4% dissociates the other protein bindings in a lipid droplet but it also dissociates the target bindings of Prp19p.
- It is necessary to find an X-element, which would prevent dissociation of the target bindings of Prp19p, while keeping the property of the extremely concentrated SDS to dissociate the other protein bindings.

⑦ ARIZ-85C 1.7

ARIZ-85C 1.7 was run, which asks us to apply inventive standards of Altshuller to our exaggerated problem model. In the exaggerated condition, we are satisfied with the dissociation function of SDS. The problem is 'dissociation' of Prp19p bindings. For application of inventive standards, we interpreted the problem by 'substance-field model';

S_1 : Prp19p bindings

S_2 : SDS at very high concentration (greatly over 4%)

interaction between S_1 and S_2 : harmful action

Through this mapping thinking from the function model or ENV model to a substance-field model, we find several recommendations of rule 1-2-1~5. Rule 1-2-1 prompted an idea shown in Fig. 5.

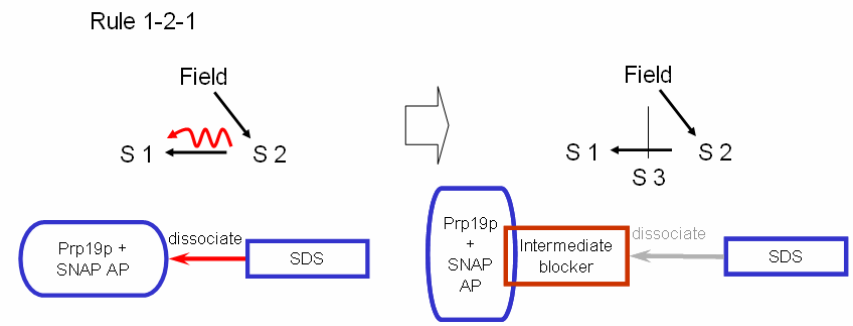

Fig. 5 idea out of ARIZ-85C 1.7

This idea says the introduction of an intermediate blocker between SDS and our target protein combination.

8) Solution Ideas and Result

Based on the basic idea of ARIZ-85C 1.7, we selected several potential intermediate blocking agents among several candidates below ;

3,3′-Dithiobis [sulfosuccinimidylpropionate] (DTSSP),

4-succinimidyloxycarbonyl-α-methyl-α-[2-pyridyldithio]toluene(SMPT),

Dimethyl 3,3'-dithiobispropionimidate•2HCl(DTBP), etc.

The test results in Fig. 6 says DSP(Dithiobis Succinimidyl Propionate) would be the best cross-linking agent for our case. Fig. 7 presents that the proteins through our new process successfully response to give us the required information for further research on how to control the proteins combined with Prp19p in lipid droplets.

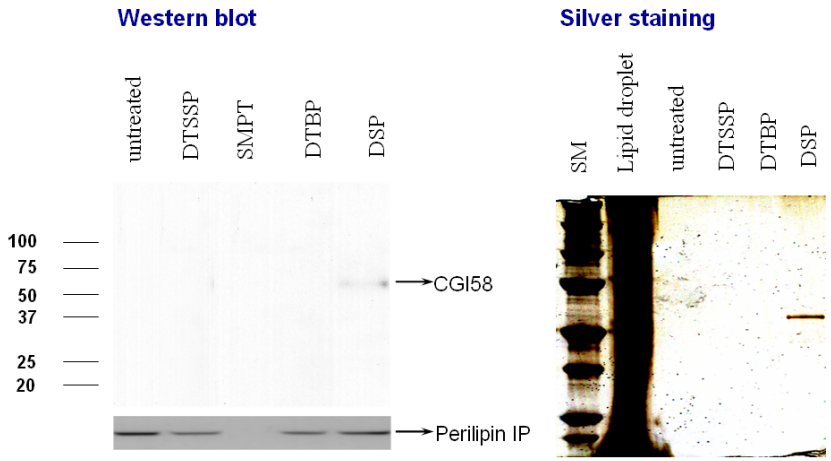

Fig. 6 test results for potential cross-linking agents

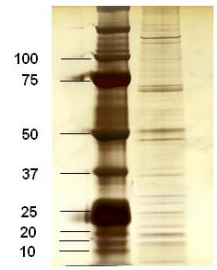

Fig. 7 gel test result of Prp19p-combined proteins after antibody reaction

9) Conclusion

Through OTSM-TRIZ approach, we got the world-first effective way to isolate Prp19p-combined proteins from lipid droplets of a human cell. ENV model and multi-screen thinking based on OTSM-TRIZ gave us the systematic thinking way to transform the non-typical problem into a kind of typical problem. Through ARIZ-85C part 1, we got the idea as a potential solution after a shorter period and less trials than usual. The developed world-first way to take out the target protein Prp19 bindings has been used for the next step of obesity repression research since the end of 2006.

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Hongyul Yoon is a TRIZ specialist (level 4, #63) certified by MATRIZ and OTSM professional (level 4, #1) certified by Nikolai Khomenko. He first met TRIZ as an engineer of LG Electronics in 1996. Since 1998, he has run a lot of trainings and problem solving projects for world best companies including LG, Samsung, POSCO, Hyundai Motors, etc. He is CEO of TRIZ Center in South Korea and keeps developing new practical application of TRIZ and OTSM in technical and non-technical fields like new market idea generation.